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A hitchhiker's guide: estimates of microbial biomass and microbial gene abundance in soil

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Abstract

Information on microbial biomass carbon (MBC) is crucial to assess their stocks and role for plant nutrient release in soil. Next to fumigation-extraction, molecular methods are routinely used to estimate the contribution of fungi, bacteria, and archaea to the soil microbial community. However, more information on the links between these different indices would deepen the understanding of microbial processes. The current study is based on 11 datasets, which contain MBC and MBN data obtained by fumigation-extraction and information on bacterial, archaeal, and fungal gene abundance, totalling 765 data points from agricultural, forest, and rangeland soils. Some of these datasets additionally provide information on double-stranded deoxyribonucleic acid (dsDNA) and fungal ergosterol. MBC varied around the median of 206 µg g^{-1} soil. MBN followed with a median MB-C/N ratio of 4.1. Median microbial gene abundance declined from bacteria (96×10^8) to archaea (4.4×10^8) to fungi (1.8×10^8). The median ratio of MBC/dsDNA was 15.8 and that of bacteria/dsDNA was 5.8×10^8 µg^{-1}. The relationships between MBC and dsDNA as well as between bacterial gene abundance and dsDNA were both negatively affected by soil pH and positively by clay content. The median ergosterol/MBC and fungi/ergosterol ratios were 0.20% and 4.7 (n × 10^8 µg^{-1}), respectively. The relationship between fungal gene abundance and ergosterol was negatively affected by soil pH and clay content. Our study suggests that combining fumigation-extraction with molecular tools allows more precise insights on the physiological interactions of soil microorganisms with their surrounding environment.

Keywords MBC · dsDNA · Bacteria · Archaea · Fungi · Ergosterol

Introduction

Soil microorganisms are key drivers of biogeochemical processes in soil and considered the active fraction of soil organic matter (SOM). The soil microbial biomass comprises C as well as macro- and micronutrients stored in living microorganisms (Hemkemeyer et al. 2021). Fungi and bacteria are the two dominant groups of the soil microbial biomass (Joergensen and Wichern 2008), but less abundant groups like archaea and protists are also important for soil functioning (Gattinger et al. 2002; Geisen et al. 2023). Most microorganisms in soil are dormant and do not grow as energy is limited (Jenkinson 1988; Joergensen and Wichern 2018). For this reason, the composition of the microbial groups in most soil is rather stable, comparing different soils (Joergensen and Wichern 2008) or seasons (Birgander et al. 2014). Changes in composition of the microbial groups require death and regrowth of microorganisms, decomposing their dead neighbours, which reduced the microbial biomass

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by 60–85% in comparison with the original level (Harden et al. 1993; Joergensen et al. 1990).

Microbial growth is nearly exclusively provided at the hot spots of energy availability, i.e., the rhizosphere around roots, the detritussphere around freshly incorporated plant residues and to some extent also the drilosphere around earthworm burrows (Banfield et al. 2018; Kuzyakov and Blagodatskaya 2015). Approximately 2 to 3% of soil microorganisms live in these areas where plenty of energy is available. However, most soil microorganisms inhabit areas of energy limitation and therefore starve, which results in dormancy. Nevertheless, dormant microorganisms also have important functions (Joergensen and Wichern 2018). They catabolize nutrient-containing organic components, leading to nitrogen, sulphur, and phosphorus mineralization. In addition, dormant microorganisms are an important reservoir for the maintenance of microbial biodiversity in soils.

Microbial biomass carbon (MBC) is a defined SOM fraction, which is relatively easy to quantify and to understand and can therefore be used in quantitative biogeochemical models. MBC data allow turnover calculations, which draws a quantitative relationship between MBC, microbial basal respiration, and soil organic C (SOC) (Anderson and Domsch 1989, 2010). Different approaches have been developed to estimate MBC, such as microscopic methods, substrate-induced activities, specific cell components, and fumigation methods (Jenkinson 1988; Joergensen and Wichern 2008; Kaiser et al. 1992), which all have specific advantages and disadvantages as discussed elsewhere (Martens 1995). The most common method of measuring MBC and microbial biomass nitrogen (MBN) is nowadays the fumigation-extraction (FE) method (Brookes et al. 1985; Vance et al. 1987), which gives access to the cytoplasm through cell-lysis. The advantages of this method are the clear separation between living and dead organisms, no discrimination of any microbial group, and the possibility to measure virtually all elements rendered extractable after fumigation (Khan et al. 2009; Schwalb et al. 2023a, b). The main disadvantage of the FE method is an insufficient difference in C concentration between fumigated and non-fumigated in low SOM environments, e.g., subsoils (Jörgensen et al. 2002) or desert soils (Wichern and Joergensen 2009).

Specific cell components are an important approach for measuring microbial biomass (Joergensen and Emmerling 2006; Joergensen and Wichern 2008), often with the advantage of giving additional information on major microbial groups, such as fungi, bacteria, and archaea. Cell-membrane components, such as PLFA and ergosterol (Joergensen 2022; Meyer et al. 2021), provide mainly information on the cell envelope but their quantity is affected by the cell volume and the number of organelles in eukaryotic fungi. Ergosterol is highly specific for the fungal phyla Ascomycota and Basidiomycota but has the advantage not to occur in plants or other organisms (Baldrian et al. 2013; Joergensen and Wichern 2008; Weete et al. 2010). Double-stranded deoxyribonucleic acid (dsDNA) gives information on the genomic core of a cell and is the basis for further molecular-genetic tools such as quantitative real-time polymerase chain reaction (*q*PCR), elucidating, *inter alia*, microbial diversity in soil (Hemkemeyer et al. 2014).

The main problem of utilising specific cell components for estimating soil microbial biomass is the absence of appropriate conversion values to biomass (Joergensen and Emmerling 2006). This is partly caused by their accumulation in dead organisms for a certain time (Joergensen and Wichern 2008) and their high variation in the biomass (Djajakirana et al. 1996; Jenkinson 1988). This is also true for molecular genetic tools to measure bacterial and archaeal (Stoddard et al. 2015) as well as fungal gene abundance (Baldrian et al. 2013; Heidrich and Beule 2022), which are increasingly used as routine approaches to estimate the contribution of these different groups to the soil microbial community (Meyer et al. 2021). The problem of many studies on the diversity of the soil microbiome is the determination of the relative and not of the absolute abundances by *q*PCR.

For unifying the view on soil microorganisms considering quantitative (MBC) and qualitative (microbial domains or diversity) data, the current study uses 11 datasets provided by the Sustainable Food Systems Research Centre at Rhine-Waal University of Applied Sciences in Kleve, Germany. All datasets contain MBC and MBN values obtained via FE and information on bacterial, archaeal, and fungal gene abundance. Some of these datasets additionally provide information on dsDNA and fungal ergosterol. With this set of data, we investigated the following three research questions: (1) What are the relationships between indices for total microbial biomass, i.e., MBC and dsDNA? (2) What are the relationships between dsDNA and the indices for prokaryotic microorganisms, i.e., bacterial and archaeal gene abundance? (3) What are the relationships between fungal indices, i.e., fungal gene abundance and ergosterol?

Materials and methods

Data sources

Data were acquired from 11 recent soil datasets: six derived from agricultural soils without additions: (1) **Cover crops (Germany)**: experimental soil was taken at 0–30 cm in March 2016 from two fields: Neulouisendorf (52 m asl, 51°42′16″ N, 6°18′01″ E) and Bedburg-Hau (17 m asl, 51°45′53″ N, 6°11′17″ E). In both soils, ten different cover crops and six mixtures with six plants per pot were grown for 60 d at 70% water holding capacity in a greenhouse at an average of 22 °C (Hemkemeyer et al. unpublished data).

(2) **Neulouisendorf (Germany)** soil samples were taken at 0–30 cm from 2016 until 2018 from a field experiment in Neulouisendorf (50 m asl, 51°42′07″ N, 6°18′18″ E) from two parts of the same field, which were in the crop rotational stage of carrying cover crops in 2016 and 2017, respectively. Besides fallows, there were seven species and five mixtures in 2016 and four species and two mixtures in 2017. Sampling took place in October/November of the same year and in March of the following year, with the latter differing in plots, which had either been harvested in autumn or mulched in winter (Hemkemeyer et al. unpublished data). (3) **Pfalzdorf-2019 (Germany)** soil samples, were taken at 0–30 cm from the Pfalzdorf (26 m asl, 51°42′15″ N, 6°09′41″ E) long-term intensive potato trial after six full three-year crop rotations in October 2019 (Hemkemeyer et al. 2024). (4) For **Pfalzdorf-2015 (Germany)** partly the same, partly different plots were already sampled in February 2015 at different stages in the crop rotation (Schwalb 2016, unpublished BSc thesis; partly published in Hemkemeyer et al. 2024).

(5) **DOK (Switzerland)** soil samples were collected at 0–20 cm depth in July and August 2019 from the long-term fertilization trial on arable land (Schwalb et al. 2023a), close to Therwil (307 m asl, 47°30′09.3″ N 7°32′21.6″ E), Switzerland, established in 1978 (Mäder et al. 2002). (6) **Askov (Denmark)** soil samples were taken at 0–20 cm depth in October 2019 from field B5 of the long-term experiment on arable land (Schwalb et al. 2024), established in 1894 and located at the Askov Experimental Station (63 m asl, 55°28' N, 09°07' E) in South Jutland, Denmark (Christensen et al. 2022).

Two datasets derived from non-agricultural soils: (7) **Issyk-Kul (Kyrgyzstan)** soil samples were taken in late September 2021 from natural vegetation at 0–30 cm depth around the Issyk-Kul lake (Iskakova et al. unpublished results), Kyrgyzstan, with sea buckthorn (*Hippophae rhamnoides* L.) and barberry (*Berberis vulgaris* L.) vegetation. Sampling sites were the eastern shore near Karakol (1726 m asl, 42°29′39″ N, 78°24′9″ E), the southern shore near Ton (1619 m asl, 42°33′59″ N, 78°16′49″ E), the northern shore near Korumdu (1629 m asl, 42°40′49″ N, 77°19′42″ E), and the western shore near Balykchy (1611 m asl, 42°27′35″ N, 76°14′2″ E). (8) **Jalal-Abad (Kyrgyzstan)** soil samples were collected at 0–30 and 30–60 cm depth in October 2019 close to Charbak (1000 m asl, 41°51′11″ N, 73°00′29″ E), Kyzyl-Unkur (1300 m asl, 41°23′31″, 73°03′40″ E), and Jay-Terek (1600 m asl, 41°17′16″ N, 72°53′03″ E) from natural, partly-managed walnut (*Juglans regia* L.) forests (Oskonbaeva et al. 2023).

The soils Pfalzdorf-2019, DOK, Askov, Issyk-Kul, and Jalal-Abad had been incubated at 22 °C for 14 or 28 d in the dark at 50% water holding capacity prior to analyses. Basic soil characteristics of all 11 soil datasets are presented in Table 1.

Finally, three datasets are derived from incubation experiments with agricultural soils, which received amendments in comparison with controls without amendments: (9) **Salinity (Bangladesh)** soil samples were taken after an incubation experiment with NaCl, rice (*Oryza sativa* L.) straw, and manure application treatments to investigate the salinity effects on C and N mineralization (Wichern et al. 2020). The paddy rice field soils were sampled in 2015 at 0–15 cm depth in Mymensingh (23 m asl) and Nalitabari (32 m asl), Bangladesh. (10) **Frass (Germany)** soil samples received two types of frass from black soldier fly (*Hermetia illucens*) larvae, differing in their feeding regime (Rummel et al. 2021). The arable soil was collected at Rotthalmünster, Germany (360 m asl, 48°21′39″ N, 13°11′33″ E). (11) **Microplastics**

Table 1 Soil types (IUSS Working Group WRB 2022), median contents of sand, silt, soil organic carbon (SOC), and total N as well as median soil pH of the different datasets

Dataset	Soil type	Sand	Silt (%)	Clay	Soil pH (H_2O)	SOC (mg g^{-1} soil)	Total N
Cover crops	Stagnic Luvisol	16	62	22	6.8	14.2	ND
Cover crops	Cambisol	77	13	11	7.5	22.4	2.15
Neulouisendorf	Stagnic Luvisol [a]	12	71	16	7.6	ND	ND
Neulouisendorf	Stagnic Luvisol [b]	42	45	13	7.7	ND	ND
Pfalzdorf-2019	Stagnic Luvisol	19	72	9	7.3	11.6	0.90
Pfalzdorf-2015	Stagnic Luvisol	19	72	9	7.0	16.3	ND
DOK	Stagnic Luvisol	15	70	15	6.1	13.6	1.49
Askov	Orthic Luvisol	65	25	10	6.4	10.6	0.80
Issyk-Kul	Cambisol	87	5	8	8.4	8.6	0.67
Jalal-Abad	Cambisol	5	55	40	7.7	45.6	3.15
Salinity	Fluvisol	28	48	24	7.1	ND	ND
Salinity	Cambisol	22	64	14	5.0	ND	ND
Frass	Haplic Luvisol	10	71	19	7.7	12.3	1.40
Microplastic	Fluvisol	20	26	54	6.5	ND	ND

ND Not determined; [a] field part in 2016/17; [b] field part in 2017/18

(Germany) soil samples were obtained from an incubation study with low density polyethylene and polypropylene additions, using soil samples collected at 0–15 cm depth from two arable fields in Kleve, (20 m asl, 50°08' N, 6°02' E), North Rhine-Westphalia, Germany (Blöcker et al. 2020).

Soil characteristics

Soils were sieved (< 2 mm) and air-dried before analysis. Soil pH was measured in a soil suspension with H_2O (1:5 w/v) or 0.01 M $CaCl_2$ (1:2 w/v), stirred in 5 min intervals and measured after 30 min. Soil pH-$CaCl_2$ was converted to pH-H_2O using the following equation: pH-H_2O = (pH-$CaCl_2$ + 0.373) / 0.923 according to Ahern et al. (1995). Total C and N were measured after milling by dry combustion at 900 °C using an elemental analyzer (Vario Max Cube CHN, Elementar, Langenselbold, Germany). Carbonate was measured gas-volumetrically after adding 10% HCl, using a Scheibler apparatus. SOC was calculated as the difference between total C and carbonate C.

Microbial biomass

MBC and MBN were determined in moist 20 g subsamples using the FE method (Brookes et al. 1985; Vance et al. 1987). Briefly, one 10 g portion at 50% water holding capacity was fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$. After $CHCl_3$ removal, samples were extracted with 40 ml 0.5 M K_2SO_4 for 30 min by horizontal shaking at 200 rev min^{-1} and filtered (VWR 305, particle retention 2–3 µm). The other 10 g portion of non-fumigated soil was extracted similarly. Organic C and total N in the extracts were measured after combustion at 850 °C using an automated Multi N/C 2100S analyzer (Analytic Jena, Germany). MBC was E_C/k_{EC}, where E_C = (organic C extracted from fumigated soils) − (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Joergensen 1996; Wu et al. 1990). MBN was E_N/k_{EC}, where E_N = (total N extracted from fumigated soils)—(total N extracted from non-fumigated soils) and k_{EN} = 0.54 (Brookes et al. 1985; Joergensen and Mueller 1996).

Ergosterol

The fungal cell-membrane component ergosterol was extracted for 30 min with 100 ml ethanol (96%) from a 2 g moist soil according to Djajakirana et al. (1996). Ergosterol was determined by reversed-phase HPLC (1260 Infinity, Agilent, Santa Clara, USA), using a C18 column and HPLC-grade methanol (100%) as liquid phase at a detection wavelength of 282 nm.

Double-stranded DNA

As a microbial biomass index (Bardelli et al. 2017), dsDNA was extracted from frozen soil samples using the FastDNA Spin Kit for Soil (MP Biomedicals, Santa Ana, USA) with modifications according to Hemkemeyer et al. (2014), which included bead-beating for 2 × 45 s at 6.5 m s^{-1}, an additional washing step with 5.5 M guanidine thiocyanate to remove contaminants, and re-use of the eluate for a second elution step. Subsequently, dsDNA was quantified using the intercalating-dye system QuantiFluor (Promega, Mannheim, Germany) at 485 nm excitation and 520 nm emission in the microplate reader FLUOstar Omega (BMG Labtech, Ortenberg, Germany). Considering DNA loss during the extraction procedure, obtained dsDNA content and abundances of marker genes (see below) were corrected by dividing the data by the extraction efficiency as described in Hemkemeyer et al. (2024).

Microbial gene abundance

Quantification of microbial domains/kingdoms was done via quantitative real-time PCR (*q*PCR) targeting the Internal Transcribed Spacer 1 region (ITS1) for fungal quantification and the 16S rRNA gene for quantification of bacteria and archaea using the Light Cycler 480 SYBR Green I Master and Light Cycler 480 Probes Master, respectively, in a Light Cycler 480 Instrument II (Roche Diagnostics, Mannheim, Germany). Primers were NSI1 and 58A2R for fungi (Martin and Rygiewicz 2005), BAC338F and BAC805R in combination with the probe BAC516F for bacteria, and ARC787F and ARC1059R with probe ARC915F for archaea (Yu et al. 2005). Reaction mixtures and cycling conditions have been described by Wichern et al. (2020). Cloning fragments for *q*PCR standards originated from the following species *Fusarium graminearum* (fungi), *Bacillus subtilis* (bacteria), and *Methanobacterium oryzae* (archaea) and were used in serial dilutions: 10^7 – 10^1 copies $µl^{-1}$ for fungi and archaea and 10^8 – 10^2 copies $µl^{-1}$ for bacteria. Preparation of the standards has been described by Rummel et al. (2021).

Statistical analyses

The results presented in tables and figures are expressed on an oven-dry basis (about 24 h at 105 °C). Outliers of replicated data were removed according to the test proposed by Doerffel (1984). Normality was tested by the Shapiro–Wilk test and equal variance by the Levene test. All microbial data were log-transformed to normalize the distribution. The significance of differences between the datasets was tested by the Kruskal–Wallis One-way Analysis of Variance on Ranks, followed by Dunn's pairwise multiple comparison procedure. Multiple regression models were

calculated between MBC, bacterial gene abundance, and fungal gene abundance as dependent variables and dsDNA, ergosterol, clay content, and soil pH-H_2O as independent variables. All regression models were tested for normality (Shapiro–Wilk), homogeneity of variance, absence of correlation between the residuals (Durban-Watson statistics) and absence of multi-collinearity, calculating the variance inflation factor (VIF), which never exceeded 4.0. All statistical analyses were performed using SigmaPlot 13.0 (Systat, San José, USA).

Results

MBC varied in a sixfold range around the median of 206 µg g^{-1} soil across the 11 soil datasets (Table 2). MBN followed MBC with a median MB-C/N ratio of 4.1, ranging from 3.3 to 7.3. Median microbial gene abundance declined from bacteria (96×10^8), archaea (4.4×10^8) to fungi (1.8×10^8). The range between minimum and maximum number increased in the same order from 9-, 12- to 15-fold, respectively. In contrast to the MB-C/N ratio, the median bacteria/archaea (27) and especially the bacteria/fungi gene abundance ratio (57), showed similar or even larger variation.

The median dsDNA content was 15.7 µg g^{-1} soil in the 5 datasets (Table 3). The median ratio of MBC/dsDNA ratio was 15.8 and that of bacterial gene abundance-to-dsDNA was 5.8×10^8 μg^{-1} soil. The relationships between MBC and dsDNA (Table 4) as well as between bacterial gene abundance and dsDNA were both negatively affected by soil pH and positively by clay content (Table 4). This means that the ratios of MBC and bacterial gene abundance to dsDNA declined with increasing soil pH and decreasing clay content. Both soil properties were implemented into the multiple non-linear regression analysis to improve the prediction of MBC (Fig. 1a) and bacterial gene abundance (Fig. 1b) from dsDNA.

The median ergosterol content was 0.42 µg g^{-1} soil in 7 datasets (Table 5), the median ergosterol/MBC and fungi/ergosterol ratios were 0.20% and 4.7×10^8 μg^{-1}, respectively. The non-linear regression between fungal gene abundance and ergosterol was negatively affected by soil pH and clay content (Table 4, Fig. 2). This means that the ratios of fungal gene abundance-to-ergosterol declined with increasing soil pH and clay content. The correlation coefficients of ergosterol (r = 0.22) and fungal gene abundance (r = 0.26) with dsDNA were less close than those with archaeal (r = 0.52) and bacterial gene abundance (r = 0.65).

Discussion

Total microbial biomass indices

In our study, MBC and MBN covered a quantitative range known for many soils, such as from arable soils (Kaiser et al. 1992; Nieder et al. 2008; Wardle 1998), rangeland (Joergensen 2010), and forest soils (Anderson and Joergensen 1997) under humid temperate but also under tropical climatic conditions (Joergensen 2010). MBC depends on the C input by root and shoot residues or litter fall

Table 2 Median microbial biomass C (MBC) and N (MBN), MB-C/N ratio, number of gene copies for bacteria, archaea, and fungi, ratios of bacteria/archaea and bacteria/fungi, for the total number of soil samples and separated according to 11 different datasets

Dataset	Number	MBC (µg g^{-1} soil)	MBN	MB-C/N	Bacteria ($n \times 10^8$ g^{-1} soil)	Archaea	Fungi	Bacteria/Archaea	Bacteria/Fungi
Cover crops	160	210	65	3.3 e	118	6.4	2.1	19 e	58 d
Neulouisendorf	226	175	45	3.9 d	56	1.3	1.7	40 c	34 de
Pfalzdorf-2019	28	128	25	5.1 bc	143	2.8	2.0	45 c	70 cd
Pfalzdorf-2015	39	192	27	7.3 ac	503	5.8	1.3	92 a	362 ab
DOK	32	244	58	4.4 d	134	5.0	1.7	27 cd	78 cd
Askov	31	95	24	4.2 d	121	2.0	1.4	60 ab	80 cd
Issyk-Kul	18	546	111	3.9 d	94	7.7	2.9	10 e	37 de
Jalal-Abad	57	492	85	5.2 c	136	8.4	1.1	21 de	129 bc
Salinity	124	564	81	7.0 a	113	10.0	5.8	12 e	20 e
Frass	21	254	58	4.5 d	145	1.6	4.3	92 a	36 de
Microplastic	29	335	70	4.9 abc	200	15.4	0.4	15 e	521 a
Total number	765	765	732	728	758	758	757	758	757
Median		206	53	4.1	96	4.4	1.8	27	57
25% percentile		160	36	3.5	65	1.6	1.0	17	27
75% percentile		311	70	5.4	147	8.1	3.3	44	118

Different letters within a column indicate a significant difference between medians (Dunn's pairwise multiple comparison procedure, $p < 0.05$)

Table 3 Median dsDNA content and ratios of MBC/dsDNA and dsDNA/bacterial gene copies for the total number of soil samples and separated according to 5 datasets

Dataset	Number	dsDNA ($\mu g\ g^{-1}$ soil)	MBC/dsDNA	Bacteria/dsDNA ($n \times 10^8\ \mu g^{-1}$ soil)
Cover crops	160	23.7	8.9 c	5.1 c
Neulouisendorf	189	11.9	15.8 b	4.8 c
Pfalzdorf-2015	23	23.5	7.6 c	21.4 a
Jalal-Abad	57	7.5	43.7 a	15.0 a
Salinity	124	17.2	32.8 a	7.1 b
Total number	560	560	553	558
Median		15.7	15.8	5.8
25% percentile		10.6	9.3	4.4
75% percentile		23.4	30.0	8.4

Different letters within a column indicate a significant difference between medians (Dunn's pairwise multiple comparison procedure, $p < 0.05$)

Table 4 Multiple non-linear regression analysis of microbial indices with clay and soil pH properties; all microbial data were log transformed

Dependent variable	Constant	Independent Variables	Regression Coefficients	Adjusted R^2	Number
Microbial biomass C	2.593***	dsDNA	0.413***	0.48***	552
		pH-H_2O	−0.140***		
		Clay	0.017***		
Bacterial abundance	1.197***	dsDNA	0.811***	0.50***	557
		pH-H_2O	−0.047***		
		Clay	0.009***		
Fungal abundance	2.364***	Ergosterol	0.764***	0.47***	262
		pH-H_2O	−0.222***		
		Clay	−0.011***		

*** $P < 0.001$

but also on the environmental turnover conditions, i.e., wtemperature (Conant et al. 2011; Kätterer et al. 1998; Kirschbaum 2006), soil moisture (Faust et al. 2019; Lakshmi et al. 2003; Moyano et al. 2013), soil pH (Anderson and Domsch 1993; Lauber et al. 2008; Rousk et al. 2009), and clay content (Müller and Höper 2004; Wentzel et al. 2015).

The significant relationship between MBC and dsDNA (Fig. 1a) had marked deviations in specific datasets, e.g., caused by the low dsDNA content in the clayey and carbonaceous Kyrgyzstan soils. Clay content and soil pH have strong effects on the adsorption of organic compounds (Baldock and Skjemstad 2000; Schweizer et al. 2021), particularly phosphorus (P) containing compounds (Gérard 2016) such as MBP (Brookes et al. 1982) or adenosine triphosphate (ATP) (Jenkinson 1988). Consequently, high clay content and high soil pH might have lowered the extraction efficiency of dsDNA in these soils, emphasizing the need for extraction protocols adapted to specific soil conditions (Guerra et al. 2020). However, clay content and soil pH certainly also affect composition of microbial groups and their physiological status.

Conversion of dsDNA to MBC

The median MBC/dsDNA ratio of 15.8 observed in the current study falls within the range observed in other studies (Table 6). The weighted mean of these datasets from the literature is 15, clearly above the weighted mean of 6 proposed by Joergensen and Emmerling (2006). Also, the median MBC/dsDNA ratios observed in the current five datasets, ranging from 7.6 to 43.7, are like the means presented in Table 6, ranging from 2.2 to 38. Čapek et al. (2023) stated that the lower and upper slope bounds of the linear relationship between MBC and dsDNA can be expected within 3.5 to 22. The current relationship between MBC and dsDNA was significantly affected by clay content and soil pH. These two soil properties are the dominant factors, controlling the composition of microbial groups and their physiological status.

An increasing clay content promotes bacteria (Wentzel et al. 2015) and reduces microbial maintenance requirements (Filip et al. 1972; Höper and Kleefisch 2001). As the turnover rate is the product of maintenance × yield coefficient, lower maintenance requirements slow down the microbial turnover in soil (Joergensen and Wichern 2018; van Veen

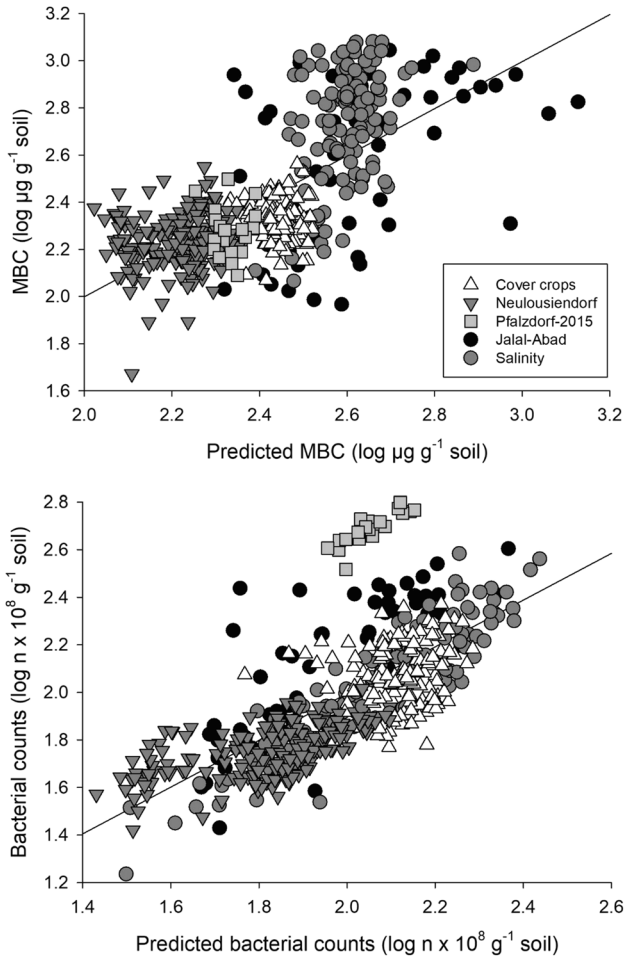

Fig. 1 Relationships between measured and predicted (a) MBC and (b) bacterial gene abundance according to multiple regression analysis of dsDNA, soil pH, and clay content (Table 4), separated according to 5 datasets

Table 5 Median ergosterol content and ratio of fungal gene copies/ergosterol for the total number of soil samples and separated according to 8 datasets

Dataset	Number	Ergosterol (µg g^{-1} soil)	Ergosterol/MBC (%)	Fungi/ergosterol ($n \times 10^8$ µg^{-1})
Pfalzdorf-2019	17	0.30	0.22 ab	7.0 ab
DOK	32	0.31	0.14 b	4.8 b
Askov	20	0.30	0.29 a	4.7 b
Issyk-Kul	20	2.98	0.44 a	1.1 c
Jalal-Abad	57	0.96	0.20 b	1.2 c
Salinity	98	0.47	0.10 b	13.2 a
Frass	21	1.16	0.51 a	3.4 b
Total number	265	265	260	263
Median		0.42	0.20	4.7
25% percentile		0.27	0.11	1.7
75% percentile		1.76	0.35	11.0

Different letters within a row indicate a significant difference (Dunn's pairwise multiple comparison procedure, $P < 0.11$)

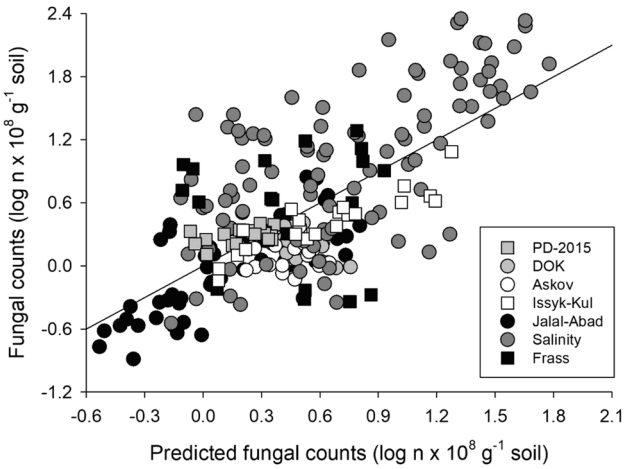

Fig. 2 Relationship between measured and predicted fungal gene copies according to multiple regression analysis of ergosterol, soil pH, and clay content (Table 4), separated according to 7 datasets

et al. 1984). Similarly, also a higher soil pH lowers the microbial demand for maintenance energy (Anderson and Domsch 1993, 2010). In addition, a high soil pH has positive effects on bacteria (Strickland and Rousk 2010) in agreement with the current results. These relationships largely explain clay and pH effects on the link between MBC and dsDNA, particularly as bacteria are the dominant and highly variable source of dsDNA (see discussion below).

The conversion of ATP (Jenkinson 1988) and substrate-induced respiration (SIR) (Anderson and Domsch 1978) to MBC are affected by the physiological activity of soil microorganisms. For this reason, ATP and SIR require a pre-incubation period after sieving (Jenkinson 1988). It is unknown, whether dsDNA measurements need a similar pretreatment if the data should be used as microbial biomass index.

Extracellular relic DNA (eDNA) from dead microorganisms is abundant in soil (Ascher et al. 2009; Levy-Booth et al. 2007) and contributes to the blurring of the relationship between MBC and dsDNA. Carini et al. (2016) examined eDNA in a wide range of soils, using the PCR viability based on the photoreactive DNA intercalating dye propidium monoazide. In their study, they found that on average 40% of both prokaryotic and fungal DNA was extracellular or from cells that were no longer intact. This value is in line with Gómez-Brandón et al. (2017), who observed that approximately 25% of dsDNA were not part of the microbial biomass. However, propidium monoazide may not work in every soil and provided sometimes only qualitative assessments of eDNA (Wang et al. 2021).

A largely unknown percentage of bias is introduced by the soil conditions at sampling time and the sample

Table 6 Mean conversion factors from dsDNA into MBC obtained from the previously published studies: MBC (µg g^{-1} soil) = F_{DNA} × dsDNA (µg g^{-1} soil)

dsDNA detection dye / extraction	MBC	F_{DNA}	Number	Reference
Hoechst 33258 / FastDNA	CFE	2.2	6	Agnelli et al. (2004)
PicoGreen / Marstorp and Witter (1999)	CFE [a]	3.2	8	Marstorp et al. (2000)
PicoGreen / own procedure	CFE [a]	3.9	3	Marstorp and Witter (1999)
PicoGreen / FastDNA	CFE	4.4	18	Semenov et al. (2018)
PicoGreen / Marstorp and Witter (1999)	CFE	4.6	8	Gong et al. (2001)
PicoGreen / Blagodatskaya et al. (2003)	SIR	5.0	44	Anderson and Martens (2013)
PicoGreen / FastDNA	CFE	5.2	5	Loeppmann et al. (2018)
Hoechst 33258 / FastDNA	SIR	5.6	4	Lloyd-Jones and Hunter (2001)
PicoGreen / FastDNA	SIR	5.9	6	Chernysheva et al. (2023)
QuantiFluor / FastDNA	CFE	6.0	1	Watson et al. (2021)
PicoGreen / own procedure	SIR	7.2	24	Blagodatskaya et al. (2003)
PicoGreen / own procedure	PLFA [b]	8.4	3	Widmer et al. (2001)
PicoGreen / Fornasier et al. (2014)	CFE	14.2	3	Bragato et al. (2016)
Ethidium bromide / FastDNA	CFE [a]	14.5	6	Leckie et al. (2004)
Hoechst 33258 / FastDNA	CFE [a]	18.6	12	Gangneux et al. (2011)
PicoGreen / FastDNA	SOC [c]	21	40	Bardelli et al. (2018)
PicoGreen / ISOIL + BB SP1	CFE [a]	24	35	Yokoyama et al. (2017)
Hoechst 33258 / FastDNA	CFE	25	7	Tomlinson et al. (2008)
PicoGreen / own procedure	CFE	28	8	Fornasier et al. (2014)
NanoDrop / PowerSoil	CFE	38	28	Gong et al. (2021)
Weighted mean (≈ median)		15	269	

FastDNA FastDNA spin kit for soil; [a] *CFE* Chloroform fumigation extraction method using a conversion value of 0.45 (Joergensen 1996); [b] using a conversion factor of 5.8 from total PLFA to MBC (Joergensen and Emmerling 2006); [d] assuming an MBC/SOC ratio of 1.6% (Bhople et al. 2019)

handling, e.g., dry matter determination, weighing, and pipetting. Also, different DNA extraction kits always showed significant differences (Fredricks et al. 2005; Zielińska et al. 2017). However, most studies (Table 6) used the FastDNA Spin Kit for Soil as extraction tool without systematic bias on the MBC/dsDNA ratios in comparison with other approaches. The datasets used in our study all implemented an additional washing step according to Hemkemeyer et al. (2014) to remove contaminants and considered DNA losses during the extraction as described by Hemkemeyer et al. (2024). Most studies used PicoGreen as dye (Table 6), again without apparent systematic differences to QuantiFluor, used in the current datasets, and Hoechst 33258.

MBC can be calculated from dsDNA, in sand and silt loams, using a conversion factor of 15, based on the current datasets and the weighted mean obtained by the literature survey (Table 6). Due to the large variation between the current datasets, we recommend checking this relationship for unknown soils by measuring both indices. In clayey and tropical soils, particularly with high contents of iron oxides (Huang et al. 2016), the calculated MBC data should be related to SOC as plausibility check.

Contribution of the main taxonomic groups to gene abundance and dsDNA

Bacteria contributed approximately 94% to the microbial marker gene abundance, archaea 4%, and fungi only 2%. These percentages agree with several other publications (e.g., Beule et al. 2019; Hartmann et al. 2015; Meyer et al. 2021; Tamez-Hidalgo et al. 2016). The close relationship between dsDNA and bacterial marker genes indicates that soil dsDNA is mainly of bacterial origin. Different reasons for the low contribution of fungi to the microbial gene abundance are known. The most important reason is the lower DNA concentration in the biomass of eukaryotic fungi compared to prokaryotic bacteria.

In the cultured marine species *Cycloclasticus oligotrophus*, Button and Robertson (2001) measured a bacterial biomass C-to-bacterial DNA ratio of 3.0 (µg $µg^{-1}$), assuming 48% C in bacterial biomass dry weight. This ratio was considerably lower in starving bacteria in their natural environment. Starvation has been reported to decrease the DNA content of marine bacteria from 30 to 1 fg per cell (Moyer and Morita 1989), which finally leads to cytoplasm-less ghost particles (Hessenberger et al. 1996). This generally

means the lower the gene copy numbers, the lower the microbial activity (Stoddard et al. 2015). In contrast to bacteria, Tellenbach et al. (2010) obtained a fungal biomass C-to-fungal DNA ratio (μg μg^{-1}) for endophytic symbionts colonizing Norway spruce roots, which varied at least between 4,400 and 8,200, assuming 48% C in fungal biomass dry weight. Similarly, Baldrian et al. (2013) measured a mean fungal biomass C-to-fungal DNA ratio (μg μg^{-1}) of 5,300, varying from 2,100 to 13,300 for different soil and litter colonizing fungi.

One reason for this strong variation is that fungi can contain up to several hundred copies of rRNA genes, which are interspaced by ITS sequences (Heidrich and Beule 2022) and vary by orders of magnitude across different fungal species (Lofgren et al. 2019). However, even among isolates of a single fungal species, copy numbers of 18S rRNA genes per genome can vary largely (Herrera et al. 2009; Lofgren et al. 2019; Zhao and Gibbons 2018). Nonetheless, the ratios of cell surface, cell volume, and genome are most likely more variable in laboratory cultures than in soil organisms, which has been shown for ATP (Contin et al. 2001; Dyckmans et al. 2003; Jenkinson 1988) and ergosterol (Djajakirana et al. 1996; Joergensen and Wichern 2008).

The thick and complex cell walls of fungi may result in poor release of DNA from the cells during extraction and after cell death (Fredricks et al. 2005; Tellenbach et al. 2010; Starke et al. 2019). In addition, the fungal DNA is densely packed in protein complexes in the nucleus (Galliano et al. 2021). However, according to the manufacturer, the FastDNA Spin Kit for Soil is also able to destroy even spores and, thus, presumably also fungal cell walls. Also, the friction by soil particles during bead beating may affect fungal mycelia stronger than prokaryotic cells.

Another reason for the low contribution of fungi to microbial gene abundance is that the primers NSI1 and 58A2R targeting ITS, used in the current datasets, are designed for Dikarya and are, thus, not representative for many fungal species from other phyla, especially Mucoromycota (Bonfante and Venice 2020). For example, arbuscular mycorrhizal fungi belonging to the Glomeromycota (Bodenhausen et al. 2021; Lekberg et al. 2018; Řezáčová et al. 2016; Victorino et al. 2020) can contribute 30% or more to the soil fungal biomass (Faust et al. 2017).

Based on amino sugar measurements, the contribution of fungal biomass to total soil microbial biomass was in most soils 75% and that of bacteria 25% (Joergensen and Wichern 2008), whereas archaea are not covered by this type of measurement. From the mean bacterial-to-archaeal gene abundance ratio, the biomass ratios of these prokaryotic microorganisms can be estimated. However, as the number of 16S rRNA gene copies per cell is 6.5 times lower for archaea (majority 1, ranging from 1–5) than for bacteria (majority 6–7, ranging from 1–21) according to the database rrnDB 5.8 (Stoddard et al. 2015), the bacterial-to-archaeal biomass ratio would decline from 23.5 to 3.6. In this case, fungi contribute on average approximately 70%, bacteria 23% and archaea 7% to the soil microbial biomass. This contribution of archaea would be markedly above the 2% proposed by Joergensen and Emmerling (2006), solely based on just one study by Gattinger et al. (2002), measuring phospholipid ether-lipids in soil.

Relationship between fungal indices

A median fungal gene abundance-to-ergosterol ratio of 4.7×10^8 μg^{-1} is close to the lower range Meyer et al. (2021) found in their soil (5.3×10^8 μg^{-1}), but much higher compared to findings from sources other than soil (Table 7). These different comparisons of genome markers with the fungal cell membrane component ergosterol reveal that fungal cells are much larger in energy-rich litter, liquid cultures, and faeces as compared to C- and energy-limited soil ecosystems (Meyer et al. 2021).

The high ratio of fungal gene abundance-to-ergosterol after rice straw addition (Salinity, Wichern et al. 2020), indicates that the fungal cells remain small, although the organic amendment promoted fungal activity. The combination of low ergosterol-to-MBC ratio (Sardinha et al. 2003; Wichern et al. 2006) and high fungal gene-abundance-to-ergosterol indicates unfavorable conditions for soil fungi. In contrast, N-rich black soldier fly larvae frass application caused a stronger increase in ergosterol than in fungal gene abundance (Frass, Rummel et al. 2021), indicating that this organic amendment led to larger fungal cells. However, a higher number of comparisons between fungal gene abundance and ergosterol would help to strengthen this view, which is based on the current dataset.

Table 7 Fungal gene abundance-to-ergosterol ratios from different sources

Source	Abundance/ergosterol $\times 10^8$ μg^{-1} (range)	Reference
Litter	0.10	Baldrian et al. (2013)
Liquid cultures of wood-decaying fungi	0.11	Song et al. (2014)
Cattle faeces	0.27 (0.01–1.11)	Meyer et al. (2021)
Fungal sporocarps	0.99	Baldrian et al. (2013)
Forest soil	1.34	Baldrian et al. (2013)
Different soil datasets	4.7 (1.2–13.2)	Current study
Silt loam soil	7.1 (5.3–9.3)	Meyer et al. (2021)

Conclusions

Microbial biomass carbon (MBC) and double-stranded desoxyribonucleic acid (dsDNA) are closely related, but their ratio is not 42 and declined with increasing soil pH and decreasing clay content. MBC can be calculated from dsDNA, in sand and silt loams, using a conversion factor of 15. However, in clayey and tropical soils, the calculated MBC data should be related to soil organic C as plausibility check. Bacteria contribute 94%, to the total microbial gene abundance and are, thus, the main but highly variable source of dsDNA. The fungal gene abundance is significantly related to the fungal cell membrane component ergosterol. A low fungal gene abundance/ergosterol ratio indicates large fungal cells, and a high ratio the reverse. Due to group specific difference in gene concentration within the biomass, bacteria contribute 23%, archaea 7% and fungi 70% to MBC. The reasons for the variation between the different microbial indices and their respective ratios require further and stronger experimental explanations, which would allow more precise insights on the physiological changes of soil microorganisms in response to their surrounding environment. We, thus, recommend combining fumigation-extraction with cell-membrane components and molecular genetic tools to deepen our understanding of soil microbial communities and their involvement in biogeochemical cycles.

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Authors contributions RGJ performed the statistical analysis and wrote the first draft. MH, ZO, SAS, and CW sampled the soil and conducted the laboratory work. FW contributed to the conception and design of the study. All authors contributed to the manuscript revision, read, and approved the submitted version of the manuscript.

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Data availability The datasets analyzed in the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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