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Review

Microbial Biomass Sulphur—An Important Yet Understudied Pool in Soil

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Abstract: Soil microorganisms require a range of essential elements for their optimal functioning and store several elements in the microbial biomass (MB), such as carbon (C), nitrogen (N), phosphorus (P) and sulphur (S), as well as other secondary and trace elements. The C, N and P content of the microbial biomass has been quantified in many studies for many years, whereas S has been the focus only in a few studies, despite the availability of methods and the relevance of MBS for the S turnover in soils. To illustrate the relevance of MBS, this review aims at summarizing the current state of knowledge on the quantities of MBS in different soils, influencing environmental and agricultural management factors, methodological shortcomings, and prospects for soil microbial biomass research. Median MBS contents were 6.0 μg g^{-1} soil in arable, 7.6 μg g^{-1} soil in grassland, and 5.7 μg g^{-1} soil in forest soils. All extractants used led to similar MBS contents in soils with similar soil organic (SO) C contents. MBC and soil pH positively explained MBS, using multiple linear regression analysis. Median MB-C/S ratios increased in the order arable (55), grassland (85), and forest (135) soils. As the overall quantity of MBS data is still small, future studies are required to verify these observations. Moreover, future research needs to more strongly consider stoichiometric relationships of elements in the soil and the soil microbial ionome. The role of S and its complex relationship with the availability of other elements in soils for the soil microbial biomass and its functions remains to be elucidated.

Keywords: microbial biomass; stoichiometry; fumigation extraction; soil organic S; microbial ionome

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1. Introduction

Sulphur (S) is quantitatively the fourth most important nutrient element for most plants and microorganisms and is thus critical for crop production and soil fertility. In most soils, total S is predominantly (more than 93%) present as soil organic S (SOS), which has a similar distribution to total nitrogen (N) in soil. Exceptions are saline soils, which may contain large amounts of Na_2SO_4 [1], gypsiferous soils, which contain large amounts of $CaSO_4 \times 2H_2O$ [2], and iron sulphide minerals (FeS_2) in hydric soils. In soil microorganisms, S fulfils a wide range of functions. For example, in combination with Fe, S is an important component of many redox systems in all cells [3] and, thus, to an unknown extent, contributes to the anti-oxidation potential of soils [4,5]. The presence of sufficient S in soil is inevitably necessary for maintaining or increasing soil organic matter (SOM) stocks [6,7].

A large variety of organic S components exists, which can all be metabolized by soil microorganisms, so that the microbial biomass (MB) plays a pivotal role as sink and

source in the S turnover of soils. Consequently, plant availability of S is facilitated by soil microorganisms in systems with no or limited mineral S input. In bacteria, C–S bonds are dominant, whereas in fungi a large part of S is bound in C–O–S esters [8], which might be reflected by the ratios of microbial biomass C/S and fungal/bacterial biomass [8–10] as well as by differences in S turnover [11]. Organic and inorganic S forms are both metabolized by soil microorganisms, so that MBS originates from both S sources [11]. Elemental S and metal sulphides can be oxidized to SO_4^{2-} mainly by Gram negative *Thiobacillus* spp., although many other microbial groups contribute to this process, *Arthrobacter*, *Pseudomonas* and *Actinobacteria*, but also fungi [12]. Conversely, SO_4^{2-} can be used by obligate anaerobic soil bacteria, e.g., *Desulfuromonas* spp., as an electron acceptor for respiration processes (desulphurization), so that soils may lose S not only by SO_4^{2-} leaching but also as H_2S.

Sulphur deficiencies in crops have been increasingly identified in Central Europe, due to the strong reduction in industrial emissions, the decrease in S concentration of fuels, and the increase in S demand with increasing yields, especially of oil-seed rape (*Brassica napus* L.) and legumes [13,14], but also winter wheat (*Triticum aestivum* L.) [15]. In contrast, other areas, such as India and East China, receive higher doses of atmospheric SO_2 from industry and traffic [16,17], so that the sink function of MBS may be important for buffering acidification [11]. Although MBS contributes only 0.9 to 2.6% to SOS, it plays a crucial role for S supply to plants by mineralization, due to the high microbial turnover intensity in arable, grassland and forest ecosystems [11,18,19].

In addition to MBS, recently formed and excreted organic S present in extra-polymeric substances (EPS) of microorganisms is important in this context. This fraction was mineralized more rapidly than the bulk SOS, which has been shown in laboratory studies by using the radioactive isotope ^{35}S [13,20,21]. Experiments for elucidating the relevance of microbial S turnover in the field, using the stable isotope ^{34}S, which contributes 4.21% to all S isotopes, are rare in soil biogeochemistry [22,23]. Not only the failure to use ^{34}S labelling or the $^{32}S/^{34}S$ ratio [24] but also the absence of any studies investigating the response of MB-C/S ratios to nutrient supply is astonishing, considering the huge interest in investigating microbial stoichiometry for more than a decade [25–27]. Interestingly, most of these studies were restricted to C/N/P relationships [28–30]. A rare exception was the incubation study of Khan and Joergensen [31], who investigated the interacting effects of N, P, and S limitation by applying four different organic components.

In the current study, we extended the review of Banerjee and Chapman [11] with the data on MBS additionally published over the last 25 years, separated according to the three land-use forms arable, grassland, and forest. In addition, soil microbial (MBC and MBN) and soil chemical properties (soil pH, SOC, total N, and SOS) were compiled to analyze the relationships between total storage of C, N, and S in SOM and in the active MB fraction (Table 1). The central objective of the current review was to renew the current state of knowledge on MBS in different soils, as affected by land-use management, methodological shortcomings, and future prospects for MBS research. The underlying hypotheses were the following: (1) MBS is closely related to the SOS content, which increases in the order arable < forest < grassland soils. (2) The MB-C/S ratio depends on S supply and is related to the SO-C/S ratio, which increases in the order arable < grassland < forest soils.

Table 1. Median, 25% and 75% as well as number of observations (*n*) for basic chemical and biological properties in arable, grassland, and forest soils.

Variable		Arable Soils	Grassland Soils	Forest Soils
Soil pH-H_2O	Median	6.7	5.9	3.9
	25%	6.0	5.4	3.8
	75%	7.5	6.6	4.7
	n	68	37	7
SOC (mg g^{-1} soil)	Median	13.0	35.4	36.1
	25%	8.6	23.8	20.3
	75%	22.8	45.4	53.2
	n	65	41	8
Total N (mg g^{-1} soil)	Median	1.24	2.41	1.55
	25%	0.81	1.60	1.22
	75%	1.82	3.44	1.83
	n	64	27	4
SOS (µg g^{-1} soil)	Median	224	385	189
	25%	172	257	152
	75%	362	654	360
	n	72	39	4
MBC (µg g^{-1} soil)	Median	274	755	776
	25%	151	442	462
	75%	402	1709	1122
	n	67	35	7
MBN (µg g^{-1} soil)	Median	35	95	107
	25%	20	57	87
	75%	49	138	259
	n	47	24	3

2. Data Acquisition, Handling, and Statistical Analysis

All papers citing Saggar et al. [18] and Khan et al. [32] were checked to determine whether they contain information on MBS contents, at least in one soil. Saggar et al. [18] published the first paper on describing the fumigation extraction for MBS, and Khan et al. [32] published a paper on the use of the fumigation extraction method for measuring the microbial ionome, inclusive MBS. Papers not citing any of these two references were additionally searched in Scopus with the key words "MBS", "Smic", "microbial S", and "biomass S". This resulted in a total number of 33 papers (Supplementary Materials), which give information on MBS for at least one soil. Information on basic soil chemical (soil pH, SOC, total N, and SOS) and additional soil microbial properties (MBC and MBN) were collected if present (Table 1). This means not all MBS data were accompanied by all of these additional data. SOS was calculated from total S, if not present, using the relationship:

$$\text{SOS} = \text{total S} \times 0.93,$$

obtained from Khan et al. [2], excluding all saline soils. Soil pH-H_2O was calculated from pH-$CaCl_2$, if not present, using the formula:

$$\text{pH-H}_2\text{O} = (\text{pH-CaCl}_2 + 0.373)/0.923,$$

given by Ahern et al. [33]. All $CHCl_3$ labile S data were recalculated to MBS, using a uniform conversion value (k_{ES}) of 0.35, which is the most widespread due to the strongest experimental foundation [11]. Outliers were rejected according to Doerffel [34]. This led to the removal of two papers with data of two grassland soils, due to excessively large MB-C/S ratios of 379 and 1271 [35]. Two arable soils were removed due to excessively high MB-N/S ratios of 38 and 81 [36,37]. Two soils listed by Banerjee and Chapman [11] were

not considered, as they were corrected for SO_4 adsorption in a non-comprehensible way, without using a k_{ES} value [38,39].

The results presented in the table (Supplementary Materials) and figures are expressed on an oven-dry basis. Normality was tested by the Shapiro-Wilk test and equal variance by the Levene test. As most data did not fulfil these two requirements, they were ln-transformed. The confounding effects of SOC on extractant comparison and of soil pH and land use were investigated by analysis of covariance. Multiple linear relationships were calculated between MBS, MB-C/S, and MB-N/S as a dependent variable and SOC, total N, SOS, and soil pH as independent variables, selected by stepwise forward regression analysis. All regression models were tested for normality (Shapiro–Wilk), constancy of variance, the absence of correlation between the residuals (Durbin–Watson statistics) and the absence of multi-collinearity, calculating the variance inflation factor (VIF). Variables were removed from the model if the VIF value exceeded 4.0. All statistical analyses were performed using SigmaPlot 13.0 (Systat, San José, CA, USA).

3. Land-Use Effects on MBS

Median MBS contents were 6.0 µg g^{-1} soil in arable, 7.6 µg g^{-1} soil in grassland, and 5.7 µg g^{-1} soil in forest soils (Figure 1a). The difference between arable and grassland soils was significant, due to the higher SOC and SOS contents in the grassland soils, reflecting the higher C input by plants. The differences of the soils from these two land-use forms to the forest soils were not significant, due to the low number of cases (n = 11) and the high variation between soils. Analysis of covariance showed that the difference between arable and grassland soils in MBS content was mainly caused by differences in soil pH. In line with this, MBC and soil pH positively explained MBS (r^2 = 0.77, n = 102), according to multiple linear regression analysis (Figure 1b).

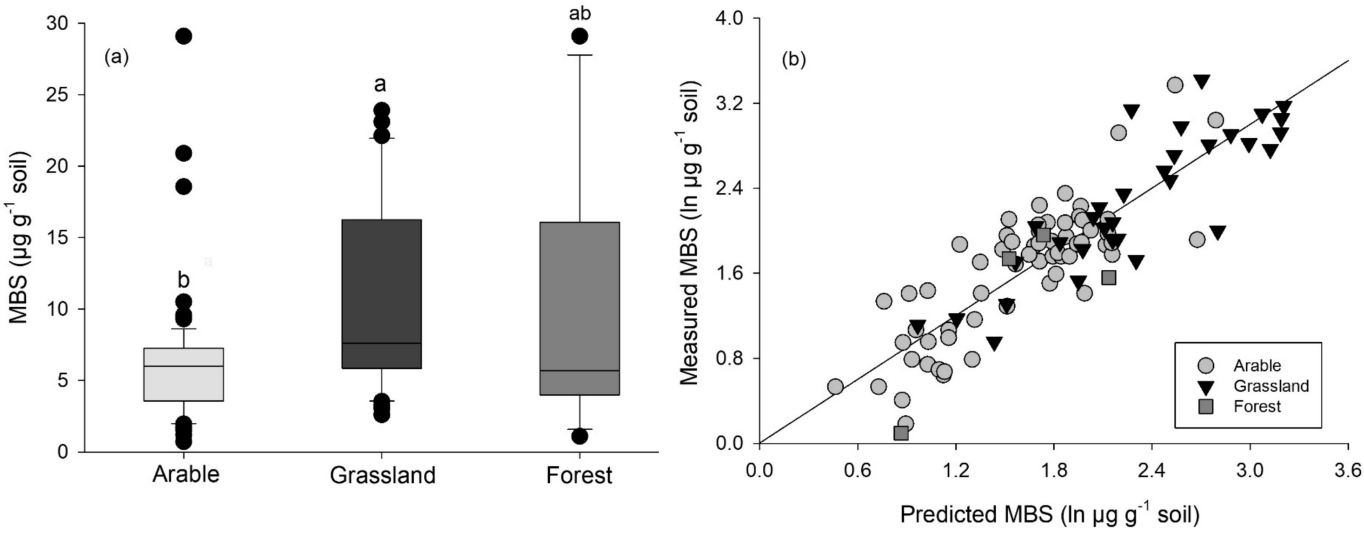

Figure 1. (**a**) MBS contents (µg g^{-1} soil) in arable (n = 77), grassland (n = 41), and forest soils (n = 11), different letters on top of outliers or whisker caps indicate a significant difference ($p < 0.05$, Holm-Sidak test); (**b**) multiple linear relationship between measured MBS as a dependent variable and predicted MBS by MBC and soil pH in water as an independent variable: ln MBS = −4.010 *** + 0.782 *** × ln MBC + 0.127 *** × pH-H_2O, n = 102, adjusted r^2 = 0.77 ***; *** $p < 0.001$.

Site-specific MBS contents of the three land-use systems ranged from 1.1 to 29.1 µg g^{-1} soil, i.e., a ten-fold smaller range than that reported by Banerjee and Chapman [11], because we excluded two organic soils, provided in their review. The median MBS/SOS ratio was 2.2%, without any effect of land-use system, i.e., MBS and SOS contents were similarly affected by SOM content and soil pH. Consequently, the SOS availability to soil microorganisms does not differ between the land-use systems (Figure 2a). In contrast,

the MBC/SOC ratios (Figure 2b) increased in the order forest (1.5%), arable (2.1%), and grassland soils (2.3%), indicating an increase in SOM availability [40,41].

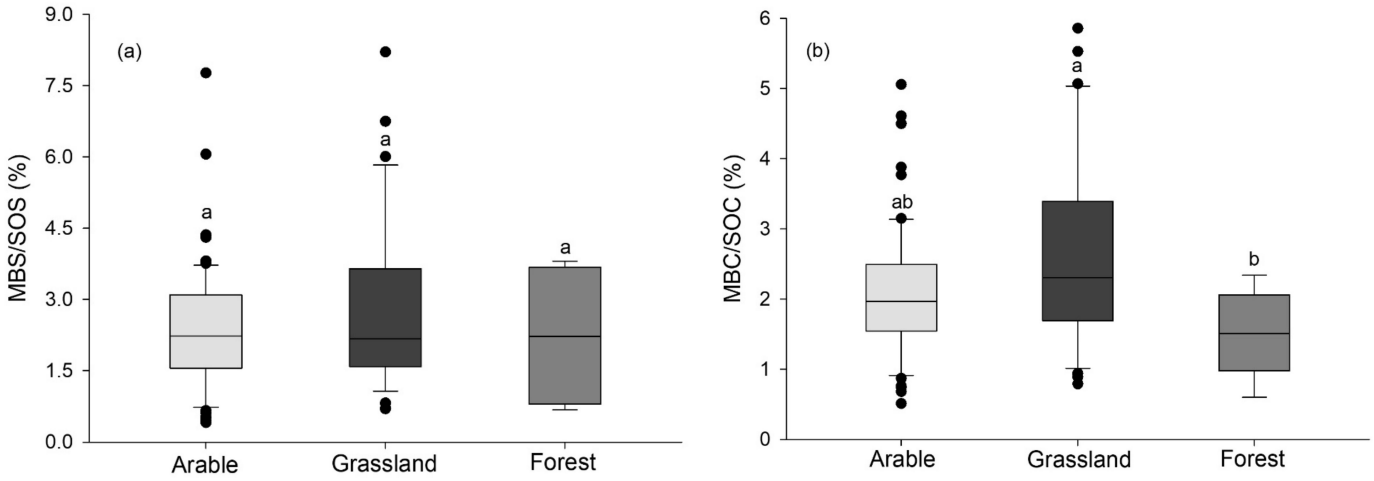

Figure 2. (**a**) MBS/SOS ratios in arable (n = 72), grassland (n = 39), and forest soils (n = 4) as well as (**b**) MBC/SOC ratios in arable (n = 65), grassland (n = 36), and forest soils (n = 5); different letters on top of outliers or whisker caps indicate a significant difference ($p < 0.05$, Holm–Sidak test).

MBS decreased more rapidly during fast growth of pearl millet (*Pennisetum glaucum* L.) than in the unplanted soil [42], indicating that soil microorganisms supplied S to deficient crops. On the other hand, the application of compost with large C/S ratios resulted in strong S immobilization in pot experiments [42], indicating that crops and soil microorganisms compete for available S. In an incubation experiment with low molecular weight, organic N, P, and S sources, P limitation decreased MB-C/S ratios, emphasizing the importance of microbial S metabolism in this case [31]. This is in line with the observation that sulpholipids can replace phospholipids to a certain extent under P limited conditions [43]. Sulpholipids were found in a wide range of bacteria and eukaryotes [44,45].

Banerjee and Chapman [11] stated 25 years ago that MBS measurements have been mainly restricted to temperate arable and grassland soils. Consequently, they requested more information on MBS from forest soils and tropical soils. However, only a minute amount of MBS data has been added from forest soils to the current dataset during the last 25 years. This is also true for organic soils, where Banerjee and Chapman [11] found maximum MBS values of 140 μg g^{-1} in a forest layer and 311 μg g^{-1} in a peatland sample. In such organic soils, the importance of the microbial biomass for nutrient storage is often more important than in mineral soils [46,47].

The general lack of MBS data for most ecosystems is astonishing, considering there have been numerous publications dealing with stochiometric relationships in the last decade, mainly focusing on C/N/P relationships in microorganisms and soil [29,48]. Kirkby et al. [6] already highlighted the importance of S for SOC storage. This suggestion has been taken up in several studies on the relevance of SOS [2], but without considering MBS as a central control of S turnover in soil.

4. Methodological Remarks on MBS Determination

The MBS contents extracted with 10 mM $CaCl_2$ significantly exceeded those extracted with 1 M NH_4NO_3 (Figure 3). However, analysis of covariance showed that this difference could be explained by differences in SOC content, as mainly grassland soils were extracted with 10 mM $CaCl_2$ and mainly arable soils with 1 M NH_4NO_3; i.e., all extractants led to similar MBS contents in soils with similar SOC contents.

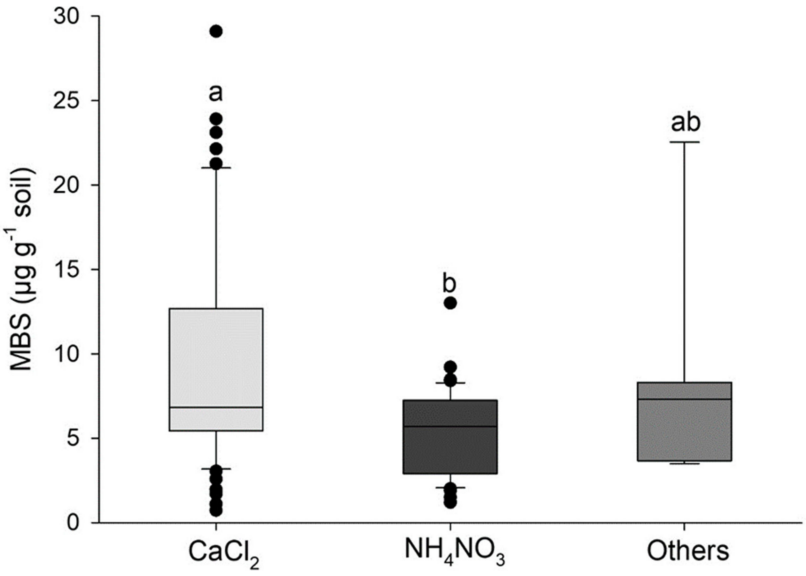

Figure 3. MBS contents extracted with 10 mM $CaCl_2$ (n = 75), 1 M NH_4NO_3 (n = 45), and other extractants (n = 9: 8 mM $Ca(H_2PO_4)_2$ = 1; 0.1 M $NaHCO_3$ = 2; 16 mM KH_2PO_4 = 2; 0.5 M KCl = 2; 16 mM NaH_2PO_4 = 2); different letters on top of outliers or whisker caps indicate a significant difference ($p < 0.05$, Holm–Sidak test).

The fumigation extraction (FE) method for MBS proposed by Saggar et al. [18] was the first FE method for the determination of specific nutrients within the microbial biomass. However, the number of citations of this method is relatively small in comparison with the methods for MBP [49], MBN [50], and MBC [51]. Although the approach of Saggar et al. [18] was highly innovative at the time of publication, the method gives a rather immature impression from a present-day perspective. Saggar et al. [18,52] added liquid $CHCl_3$ to the soil samples, did not evacuate the $CHCl_3$, used two different extractants, and proposed two different k_{ES} values to convert $CHCl_3$-labile S into MBS, i.e., 0.35 for $CaCl_2$ and 0.41 for $NaHCO_3$ as extractant. Saggar et al. [18,52] also did not exactly describe the method used for S determination in soil extracts. Although none of these uncertainties is a serious problem for the performance of the method, they might have restricted its use.

In contrast to MBC, MBN, and MBP measurements, a larger range of extractants have been used to determine soil MBS, such as 10 mM $CaCl_2$ [8,18,35,51–54], 100 mM $NaHCO_3$ [8,18,53,55,56], 16 M $Ca(H_2PO_4)_2$ [57,58], 16 mM KH_2PO_4 [59,60], 16 mM NaH_2PO_4 [53,61], 0.5 M KCl [62], and 1 M NH_4NO_3 [9,10,32,63–66]. The salt of an extractant should flocculate the soil colloids and prevent adsorption of $CHCl_3$-labile S-components during extraction. Amino acids can be absorbed by negatively charged surfaces of clay minerals, whereas SO_4^{2-} can be adsorbed by positively charged iron oxides. The use of 10 mM $CaCl_2$ and 16 mM NaH_2PO_4 as extractants allows total S measurements by ion chromatography in soil extracts after H_2O_2 oxidation [32,35,54,61,67,68]. The high salt concentration of 1 M NH_4NO_3 and 0.5 M KCl reduces the decomposition of $CHCl_3$-labile S-components during measurement. The different extractants mainly lead to different S levels in the non-fumigated soil extracts [32], whereas the extractant effects on the MBS contents are generally negligible, summarizing all data (Figure 3) and correcting for the SOC bias.

In contrast to ion-chromatography, X-ray fluorescence spectroscopy was rarely used for S detection in soil extracts [8,55]. The same was true for the sole determination of HI-reducible S [19,60]. The reduction method of Johnson and Nishita [69], followed by colorimetric detection, was repeatedly used in several studies [20,70–75]. However, most S data were measured by inductively coupled plasma optical emission spectrometry, i.e., ICP-OES [9,10,32,38,39,53,62–64,66,76,77]. ICP-OES is certainly the preferred method. However,

the lower detection limits of modern ICP-systems are combined with sensitivity against higher salt concentrations, so that 1 M NH_4NO_3 extracts require a 1/10 dilution.

The available literature on the determination of the k_{ES} value has been extensively reviewed by Banerjee and Chapman [11] and no further attempt has been made since the study of Wu et al. [35]. All fumigation extraction approaches need appropriate conversion (k_E) values to calculate the total amount of an element kept in the biomass from the extractable $CHCl_3$ labile fraction [78]. Although these conversion values were sometimes subject to debate [79,80], they have the advantages (1) of drawing attention to the uncertainties of a specific method, (2) of forcing the uniform use of a method without modifications, and (3) of drawing quantitative relationships to the total amounts stored in soil organic matter as independent quality checks [81].

The reported k_{ES} values ranged from 0.17 to 0.46 [11]. They were mainly based on the application of several cultured single bacterial and fungal species to one soil. One attempt has been made to add mixed cultures of unknown soil bacteria and fungi, which led to a k_{ES} value of 0.39 [58]. Randlett et al. [82] and Wu et al. [35] labelled soil MBS with ^{35}S directly in situ and both obtained a k_{ES} value of 0.35, using a soil/extractant ratio of 1/5. The application of cultured organisms requires that these cultures do not contain any necromass, which is usually not tested. Direct in situ labelling has the drawback that an unknown percentage of microbial metabolites has left the biomass [81]. The soil/extractant ratio should not decrease below 1/4, as this reduces the extractability of $CHCl_3$ labile material [83]. For this reason, the soil/extractant ratio should be increased to 1/20 [76] or even 1/40 [46] in soils containing large amounts of organic matter, such as litter layers or peat samples. A k_{ES} value of 0.35 for MBS is at the lower end of the corresponding conversion values k_{EP} = 0.40 for MBP [49], k_{EC} = 0.45 for MBC [84], and k_{EN} = 0.54 for MBN [50].

It was sometimes assumed that soil type may affect k_{ES} values [11]. However, it is known that the k_{EC} [78] and k_{EN} [85] values were not affected by soil type. In contrast, it is possible that the ratio of fungal to bacterial biomass affects k_{EC} and k_{EN} values, as fumigation rendered more $CHCl_3$-labile material extractable from fungi than from bacteria [86,87]. However, the effects of microbial community composition on conversion values are generally small, as the ratio of fungal to bacterial biomass varies in most soils in a rather small range [2,88]. Nonetheless, in contrast to MBC and MBN, cultured fungi released on average only 34.9% $CHCl_3$-labile S (±1.7 SEM, n = 15) after fumigation, i.e., significantly ($p < 0.05$, t-test) less than bacteria, at 40.7% (±1.7 SEM, n = 15) [11,18,19]. This means that the extractable fraction of the FE method is usually more related to the cytoplasm and the non-extractable fraction than to cell membrane and cell wall components [11,89,90]. Consequently, a higher percentage of MBS is related to the stable cell fraction in comparison with MBN, contrasting the view that S has high turnover rates [11,19,21]. This discrepancy cannot be solved by the currently available knowledge.

Based on current knowledge, we propose to use 10 mM $CaCl_2$ as extractant after 24 h fumigation and repeated evacuation at least at a soil/extractant ratio of 1/5 as well as a k_{ES} value of 0.35 proposed by Saggar et al. [18] and Wu et al. [35]. ICP-OES is certainly the most recommend analytical tool, although ion chromatography and the reduction method give reliable and useful MBS data with less technical effort.

5. MBS Stoichiometry

Median MB-C/S ratios significantly increased in the order arable (55), grassland (85), and forest (135) soils (Figure 4a). These differences reflect the respective median SO-C/S ratios, which were similar and significantly increased in the same order, i.e., 61, 88, and 150, respectively. The MB-C/S ratio could be positively explained by the SOS content and negatively by the soil pH (Figure 4b), although these two independent variables explained only a third (r^2 = 0.33) of the MB-C/S variance (Figure 4b). Analysis of covariance showed that land-use effects were mainly due to differences in soil pH, changing S supply and quality of the C input by plants [2,31].

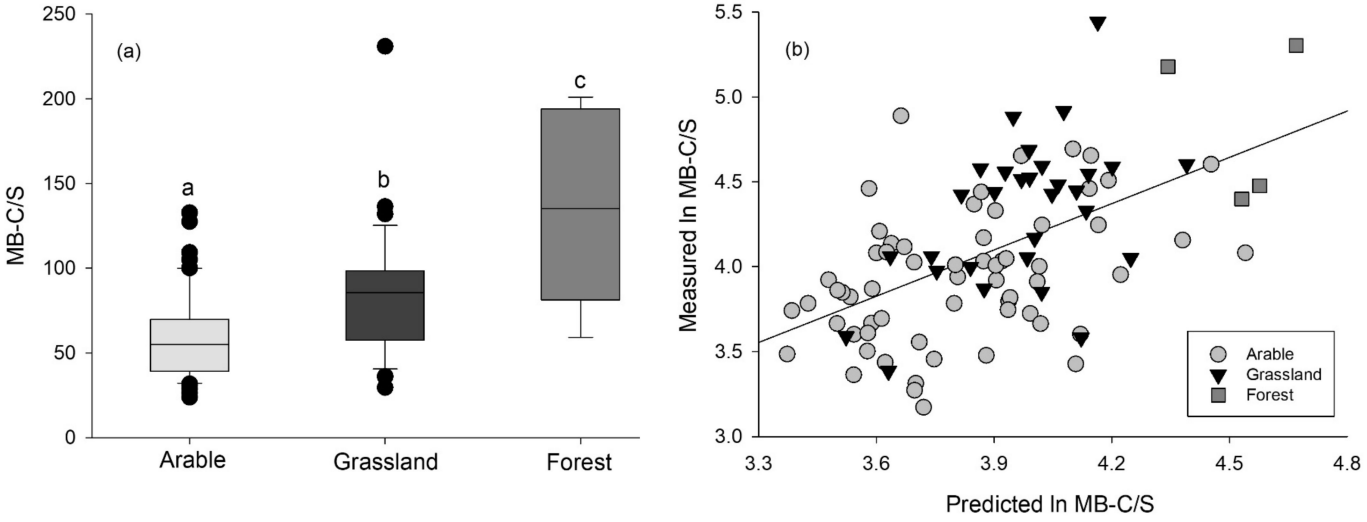

Figure 4. (**a**) MB-CS ratios in arable (n = 67), grassland (n = 33), and forest soils (n = 7), different letters on top of outliers or whisker caps indicate a significant difference ($p < 0.05$, Holm–Sidak test); (**b**) multiple linear relationship between the MB-C/S ratio as a dependent variable and the SOS content as well as soil pH in water as independent variables: ln MB-CS = 4.802 *** + 0.138 * × ln SOS − 0.232 *** × pH-H_2O, n = 97, adjusted r^2 = 0.33 ***; * $p < 0.5$, *** $p < 0.001$.

It is a striking feature of the current results that the MB-C/S ratio is similar to the SO-C/S ratio, i.e., S is not specifically enriched in the microbial biomass. However, the soil-specific correlation between these ratios is rather weak, as some studies of the current dataset showed lower MB-C/S ratios in comparison with the SO-C/S ratios [31,32,35,67]. Consequently, future studies may draw a different picture than the current review. In contrast, the MB-C/P ratio is always lower than the SO-C/P ratio (or SOC/total P ratio) and also the MB-C/N is in most cases lower than the SOC/total N ratio [2]. Exceptions are often tropical [91] and subtropical soils under saline conditions [2], where the MB-C/N and SOC/total N ratios are similar, most likely due to P or micro-nutrient limitation of soil microorganisms [31,92]. The absence of specific S accumulation in the microbial biomass is explicable by the following three reasons, considering the large variation of S availability in soil: (1) The MB-C/S ratio depends on the S supply of soils; (2) The MB-C/S ratio does not differ from that of microbial necromass but presumably also not from that of plant residues, which are the two main SOC sources; (3) S metabolism in soil microorganisms does not require homoeostasis, e.g., due to the ability to store an excess S supply.

The view has been stated that N and S turnover in soils are closely interconnected [93], which might be a reason for neglecting the microbial S turnover. However, this close relationship is surprising, as C-bonded S contributes a highly variable range of 33–85% to SOS, whereas 15–60% are contributed by non-protein C–O–S esters, i.e., hydrogen iodide-reducible S [59,60,62,70,94]. C-bonded S is dominated by S-containing amino acids, such as cysteine, cystine, and methionine. C-bonded S also occurs in minor percentages in co-enzymes and co-factors, e.g., CoA, CoM, α-lipoic acid, biotin, molybdopterins, S-adenosyl methionine, thiamine-pyrophosphate, etc. [95–98]. Antioxidants, such as glutathione, also contain C-bonded S [99,100].

Less is known about the chemical nature of C–O–S esters in soil microorganisms. An important SO_4^{2-} ester in soil may be chondroitin-sulphate, which is composed of glucuronic acid and N-acetyl-galactosamine esterified with SO_4^{2-} at various positions [101]. In addition to animals, bacteria and fungi also are known to produce chondroitin-sulphate [101]. Consequently, fungal and bacterial EPS may contain chondroitin sulphate and might be an important source of soil galactosamine, as proposed by Joergensen [102]. This would also explain the observation of Fitzgerald et al. [103] that soil microorganisms immobilized $^{35}SO_4$ as C–O–S ester into water-soluble polysaccharides, i.e., EPS.

Fungi contain a large percentage of SO_4-esters, metabolized to a large variety of C-O-S components [104]. In addition, some fungi contain vacuoles [105], which might be able to store excess SO_4 immediately after uptake, as observed by Saggar et al. [18,52]. They also observed that cultured fungi can increase their biomass S concentration by about 50–130%, whereas cultured bacteria can enhance their biomass S concentration by only 20%. Consequently, soil fungi may contain more S in their biomass and exhibit lower MB-C/S ratios than soil bacteria. The combined application of cellulose, which specifically promotes fungi [106,107], and SO_4^{2-} led to lower MB-C/S ratios than the application of glucose [108].

In line with this view, Heinze et al. [9,10] and Murugan et al. [63] reported a decrease in the MB-C/S ratio with increasing contribution of saprotrophic fungi to the microbial biomass in long-term fertilization trials, as assessed by ergosterol. However, such a relationship has not always been observed [62] and the current dataset is too limited to give further information on the relationship between MB-C/S and the ratio of fungal to bacterial biomass. Further research is needed to elucidate the level of stoichiometric heterostasis in fungi and bacteria, the two dominating functional taxa of the soil microbial biomass [79].

6. Conclusions

Fumigation extraction is still a useful tool for estimating the contribution of soil microorganisms to the S turnover in soils. The method is rather robust against methodological variations, so that MBS may be measured by all scientists interested in investigating this important and neglected nutrient quantitatively, despite distinct differences in laboratory equipment. MBS and the performance of microorganisms mineralizing SOS is an important source of plant available SO_4^{2-}. Future research needs to more strongly consider stoichiometric relationships of elements in the soil and the soil microbial ionome and how they are related to soil functions and soil type. Further, it is crucial to understand how chemical and physical soil properties influence the soil microbial ionome in the soil system and under which conditions S becomes limited for soil microbial functions.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11081606/s1. Overview of the datasets used for the review paper "Microbial biomass sulphur-an important, yet understudied pool in soil".

Author Contributions: The conceptualization was done by S.H., R.G.J. and F.W.; calculations with statistical software for the manuscript was conducted by R.G.J. Validation was done by S.H., F.W., K.S.K., M.H. and S.A.S. Resource acquiring was done by S.H. and F.W.; the main writing was done by R.G.J. and S.H., while the work improved due to comprehensive reviewing process by K.S.K., M.H., S.A.S. and F.W. Project administration and coordination were done by S.H. All authors have read and agreed to the published version of the manuscript.

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