

## Review Paper

## Plant- or microbial-derived? A review on the molecular composition of stabilized soil organic matter

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## ABSTRACT

Soil organic matter (SOM) represents a major reservoir of stored carbon (C). However, uncertainties regarding the composition and origin of stabilized SOM hinder the implementation of sustainable management strategies.

Here, we synthesize data on the contribution of plant- and microbial-derived compounds to stabilized SOM, i. e., aggregates and mineral-associated organic matter (MAOM), and review the role of environmental factors influencing this contribution. Extrapolating amino sugar concentrations in soil based on molecular stoichiometry, we find that microbial necromass accounts for ~50% (agroecosystems) or less (forest ecosystems) of the C stabilized within aggregates and MAOM across studies. This implies that plant biomolecules, including lipids, lignin, and sugars, might account for a substantial portion ( $\geq 50\%$ ) of the organic matter protected by minerals and aggregates. Indeed, plant-specific sugars and lipids can each account for as much as 10% of organic C within mineral soil fractions, and most reported quantities of plant-specific lipids and lignin in mineral soil fractions are likely underestimates due to irreversible sorption to minerals. A relatively balanced contribution of plant and microbial biomolecules to stabilized SOM in aggregates and MAOM is inconsistent with recent suggestions that stable SOM is comprised mostly of microbial compounds.

Land use and soil type appear to profoundly affect the contribution of plant and microbial compounds to stabilized SOM. Consistent with studies of bulk soils, favorable conditions for microbial proliferation in grasslands or fertile Chernozems or Luvisols appear to increase the contribution of microbial compounds, while less favorable conditions for microbial proliferation in forest soils or Podzols/Alisols appear to favor the abundance of plant compounds in stabilized SOM. Combined with a tight link between substrate quality and the abundance of microbial compounds in stabilized SOM, and a potentially inverse relationship between substrate quality and the abundance of plant compounds, these results provide evidence that plant biomolecules might be preferentially stabilized by organo-mineral interactions in some ecosystems.

Various areas warrant further research. For example, difficulties in distinguishing direct and indirect effects of temperature and precipitation on the composition of stabilized SOM may be overcome by long-term observational studies that include climate manipulations. Knowledge gaps in the contribution of plant and microbial compounds to stabilized SOM in soil layers below 30 cm depth may simply be closed by extending the sampling depth. Moreover, a refined focus on soil fauna, with potentially strong effects on microbial and plant compounds in stabilized SOM, will provide new insights into SOM dynamics. Future studies should quantify both microbial and plant biomolecules in mineral soil fractions to allow direct comparisons and overcome limitations in existing data. For example, because biomarker-based estimates of microbial-derived C can only indirectly estimate the maximum amount of plant-derived C, exhaustive studies of plant biomarker concentrations could be conducted, including estimates of plant-specific lipids, sugars, and lignin (and biomarkers released following mineral dissolution). Generally, more integrative studies, e.g., combining molecular and isotopic tracers of organic matter inputs with targeted sampling of mineral fractions, are required to improve knowledge of the formation and persistence of stabilized SOM.

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## 1. Introduction

Soil organic matter (SOM) represents the largest terrestrial carbon (C) reservoir, stores more C than the atmosphere and vegetation combined, and its maintenance is vital to soil fertility, climate change mitigation, and in turn, human well-being. Despite this importance, current practices are only partly effective in sustainably managing SOM reservoirs and protecting them from natural and anthropogenic disturbances. For example, less stable SOM pools, such as particulate organic matter (POM), can relatively easily be manipulated via (organic) amendments or vegetation management, while it remains less certain how such manipulations affect the more stable SOM pools. This uncertainty arises from persistent knowledge gaps about the formation and composition of stabilized SOM (Blankinship et al., 2018).

Recently developing paradigms increasingly question the concept of SOM being composed of humic substances, i.e., fulvic and humic acids and humin. These SOM fractions are operationally defined and rather unsuitable to develop an understanding of the nature of SOM (Lehmann and Kleber, 2015). Soil OM represents a continuum of organic compounds (polymers and monomers) of different size and degree of decomposition that interact with their environment (Lehmann and Kleber, 2015), and it is these physicochemical interactions that protect some organic biomolecules from decomposition and enables their persistence in the soil (Schmidt et al., 2011). Thus, the mechanisms of SOM persistence appear to be best studied on the molecular level, in the context of environmental factors influencing SOM composition and stability.

Soil OM is primarily stabilized by two mechanisms: by the formation of *mineral-associated organic matter* (MAOM) and by the formation of *aggregates* (from here on, both of these mechanisms are subsumed using the term *stabilized SOM*). Mineral-AOM develops via interactions between molecules of SOM and mineral soil components that form stronger (e.g., via ligand exchange) or weaker bonds (e.g., via Van der Waals forces), increasing the protection of that SOM against decomposition (von Lützow et al., 2006). Clay- and, to a lesser extent, silt-sized mineral particles are mainly involved in this process owing to the abundance of reactive oxide and phyllosilicate surfaces in these particle sizes (Blume et al., 2015). Aggregates are intimate mixtures of minerals and organic compounds that hamper the access of microorganisms to the SOM within, increasing its stability (Six et al., 2004). These structures have commonly been divided into macro- (>250–2000  $\mu\text{m}$ ) and micro-aggregates (53–250  $\mu\text{m}$ ), which differ in stability, composition, and formation mechanism (Lehmann et al., 2007; Tisdall and Oades, 1982; Totsche et al., 2018). The separation of aggregates and MAOM from a bulk soil is usually achieved via a combination of consecutive sieving and density-separation steps, i.e., physical fractionation, as practiced and proven effective in many studies (Angst et al., 2018a; Christensen, 2001; Lavallee et al., 2020; Mueller et al., 2009; Sollins et al., 2009). A third and controversially discussed mechanism of SOM stabilization is *biochemical recalcitrance*, which refers to the effects of chemical properties on the rate and extent of microbial utilization of organic substrates (e.g., complex molecules, such as lignin, will persist longer in the soil than simple molecules, such as glucose; von Lützow et al., 2007). Yet, biochemical recalcitrance is potentially most relevant during the initial stages of decomposition or in organic layers, such as the forest floor or peat deposits, and might only play a minor role in the long-term protection of SOM in mineral soil (Marschner et al., 2008). In other words, an inherently “recalcitrant” biomolecule will not necessarily persist in the soil, and conversely, “labile” biomolecules are not necessarily decomposed and mineralized. Biochemical recalcitrance is, thus, not within the focus of this review. However, some biomolecules can be ‘selectively preserved’ if their chemical properties enhance their ability to be stabilized by physicochemical interactions within the soil matrix. For example, aromatic and aliphatic acids, such as lignin monomers, appear to be selectively retained on mineral surfaces in studies of mineral sorption of dissolved organic matter (e.g., Feng et al., 2005).

Likewise, polysaccharides and protein appear to be selectively preserved in organo-metallic complexes in Andosols (Nierop et al., 2005b; Tonneijck et al., 2010). Other types of studies often show that nitrogen-rich, microbial-derived biomolecules like amino sugars/acids appear to be selectively bound to mineral surfaces (e.g., Kleber et al., 2007; Kopitke et al., 2018; Sollins et al., 2006). These physicochemical forms of selective preservation are clearly relevant to the stabilization mechanisms and molecular composition of MAOM.

While a plethora of studies has investigated MAOM and aggregates in various settings (e.g., under different land use, in different climates or soil depths), a common ground for their formation or composition has not been established (Blankinship et al., 2018). Recent conceptualizations, based on evidence that microbial residues accumulate in MAOM and aggregates, suggest that an environment optimal to the effective microbial utilization of organic matter could lead to a higher amount of stabilized SOM (Cotrufo et al., 2013; Miltner et al., 2012). Central to this concept is the ‘quality’ of organic substrates entering the soil, with higher ‘quality’ indicated by lower carbon to nutrient ratios and lower concentrations of recalcitrant compounds. Accordingly, the higher the quality, the higher the microbial substrate use efficiency, and the higher the build-up of microbial biomass, whose residues eventually interact with the soil mineral phase to form aggregates and MAOM (Cotrufo et al., 2013). While evidence for the validity of this microbial-centric conceptualization of SOM formation and stabilization is increasing (e.g., Kallenbach et al., 2016; Liang et al., 2019; Cyle et al., 2016), such microbial-centric perspectives have not been fully reconciled with observations that plant biomolecules can also be preferentially retained in stable SOM pools (Castellano et al., 2015). Indeed, some recent studies report a substantial contribution of plant-derived organic matter in stabilized SOM pools, like MAOM (e.g., Angst et al., 2017a; Van der Voort et al., 2017), even when the conditions are favorable for microbial growth (Córdova et al., 2018; Hernes et al., 2013; Mikutta et al., 2019). Further, other conceptual and empirical studies suggest the relative abundance of microbial and plant-derived organic matter in soil depends on the context (Castellano et al., 2015; Liang et al., 2017), including land use and land cover (e.g., Khan et al., 2016; Liang et al., 2019; Pisani et al., 2015), and environmental conditions that determine the potential decomposition and stabilization of plant-derived substrates (e.g., Pisani et al., 2014; Rumpel et al., 2015; Sokol et al., 2019). Moreover, many studies either focus on plant or microbial components of SOM, but there is currently a lack of studies combining these more holistically across multiple scales, particularly with respect to stabilized SOM. Thus, despite the growing number of assertions that stabilized SOM is, or could be, dominated by microbial biomolecules (e.g., Castellano et al., 2015; Cotrufo et al., 2013; Grandy and Neff, 2008; Liang et al., 2017; Miltner et al., 2012), there is much uncertainty about whether and under which circumstances most stabilized SOM is comprised of microbial compounds.

To address some of these uncertainties and establish a more fundamental basis on which SOM dynamics may be interpreted, we here review the recent knowledge about the molecular composition of stabilized SOM and the environmental factors influencing this composition. We focus on studies that directly isolated MAOM (and/or aggregates) and quantified molecular ‘biomarkers’, i.e., studies that involved some kind of physical soil fractionation and measured the abundance of distinct biomolecules that are mainly or exclusively derived from plants (e.g., lignin, lipid biopolymers like cutin and suberin, neutral sugars) or from microbes (e.g., amino and neutral sugars). Based on the selected literature, we first review the contribution of plant-vs. microbial-derived compounds to MAOM and aggregates; then, we discuss factors influencing the relative abundance of plant and microbial-derived biomolecules, aided by a compilation of data from 23 studies. For the data compilation, we focused on lignin and amino sugar biomarkers, given their frequent application in studies on SOM dynamics. When available, we also (qualitatively) discuss other biomarkers (e.g., neutral sugars or lipids) which have been less frequently

quantified in aggregates and MAOM. Finally, we consider the implications of the revealed patterns and discuss directions for future research.

## 2. Stabilized SOM

### 2.1. Microbial-derived SOM

Permanent and variable charges of soil minerals (mostly clay minerals or iron/aluminum oxides and (oxy)hydroxides) give rise to interactions of such minerals with functional groups of organic matter (Blume et al., 2015). Proteins, in particular, have the tendency to strongly bind to mineral surfaces due to their amphiphilicity, i.e., containing both hydrophilic and hydrophobic components that make them intrinsically surface active (Horbett and Brash, 1995; Kleber et al., 2007). Proteins are a major part of the microbial cell wall, and this may be the reason why many studies, independent of land use or soil type, report an enrichment of microbial-derived amino and neutral sugars with decreasing mineral particle size (especially in clay-sized MAOM), i.e., with an increase in the abundance of reactive surfaces (Amelung et al., 1999a; Angst et al., 2019a; Dao et al., 2018; Griepentrog et al., 2014; Guggenberger et al., 1995; Jolivet et al., 2006; Kiem and Kögel-Knabner, 2003; Ludwig et al., 2015; Mastrolonardo et al., 2015; Ni et al., 2020; Puget et al., 1998; Rumpel et al., 2010a; Solomon et al., 2000; Spielvogel et al., 2007). A strong correlation between  $^{14}\text{C}$  ages and the amount of microbial-derived neutral sugars reported in dense soil fractions (i.e., MAOM) of forest soils indicates a potentially long residence time and, perhaps, a high stability of microbial residues (Rumpel et al., 2010b).

Another factor potentially contributing to the enrichment of microbial residues in MAOM is that microbial communities may favorably dwell in micropores between clay surfaces, reducing the distances between microbes and their substrates and between microbial necromass and sorption sites (Wei et al., 2014). A recent study using advanced nuclear magnetic resonance methods found that bacterial cells can attach directly to clay surfaces non-specifically (Olivelli et al., 2020). As some studies report a dominance of bacterial instead of fungal amino sugars in MAOM across land uses (Griepentrog et al., 2014; Zhang et al., 1999, Fig. 1), combined, these results indicate that the bacterial biomass near reactive mineral surfaces and the factors influencing the accrual of this biomass may be important variables that determine the amount of microbial residues stabilized in MAOM. A re-evaluation of published amino sugar data in stabilized SOM (Fig. 1a), though, shows that the amino sugar content substantially varies between each fraction, of which that in clay-sized and silt-sized MAOM ( $59.2$  and  $65.8$   $\text{mg g}^{-1}\text{C}$ )

is effectively lower than that in macro- and microaggregates ( $\sim 123.8$  and  $102.1$   $\text{mg g}^{-1}\text{C}$ ). Apart from being attached to mineral surfaces, microbial biomass and microbial residues may be entrapped in small pores in such aggregates, which enzymes or organisms are unable to reach (Totsche et al., 2018). This entrapment may increase the stability of microbial residues and the contribution of these residues to the SOM in aggregates. However, compared with studies inferring a dominance of microbial residues in stabilized SOM, and specifically MAOM, these values are rather low (e.g., Simpson et al., 2007). Perhaps this indicates that microbial-derived proteins and amino acids, rather than amino sugars, are primarily responsible for the frequently observed enrichment of ‘microbial necromass’ or ‘microbial-derived’ C and N in MAOM and aggregates.

General statements about whether macro- or microaggregates are dominated by bacterial or fungal residues are difficult to draw based on the available literature and seems to depend on ecosystem properties. The ratio of glucosamine (fungal) to muramic acid (bacterial) compiled from 14 observations (from agricultural soils only; Fig. 1b) provides no insight about whether macroaggregates or microaggregates are enriched in fungal or microbial residues, while both aggregate classes seem to be dominated by fungal residues as compared to clay-sized MAOM. This observation agrees with fungal hyphae being involved in aggregate formation, particularly in that of macroaggregates (Six et al., 2004).

Estimates on microbial compounds in stabilized SOM may be biased by an incomplete extraction of such compounds; however, we are not aware of any study that systematically quantifies the content of irreversibly bound amino or neutral sugars to mineral surfaces, which may, thus, warrant further research.

### 2.2. Plant-derived SOM

In contrast to the increase in microbial residues with decreasing particle size, the contribution of lignin, a major cell wall component of vascular plants (Kögel-Knabner, 2002), has commonly been reported to decrease from POM to mineral soil fractions, and to decline with decreasing particle size (Clemente et al., 2011; Grünewald et al., 2006; Kiem and Kögel-Knabner, 2003; Ma et al., 2014; Mastrolonardo et al., 2015; Schmidt and Kögel-Knabner, 2002; Solomon et al., 2000; Thevenot et al., 2010). Based on our synthesis of data from 20 studies, covering grassland, forest, and agricultural sites, the average content of lignin-derived phenols in POM is  $30.1 \pm 3.6$   $\text{mg/g C}$  (data not shown), that in silt-sized MAOM is  $25.6 \pm 2.4$   $\text{mg/g C}$ , and that in clay-sized MAOM is  $12.0 \pm 1.0$   $\text{mg/g C}$  (Fig. 2). The fact that lignin needs to be depolymerized before its subunits may react with mineral surfaces

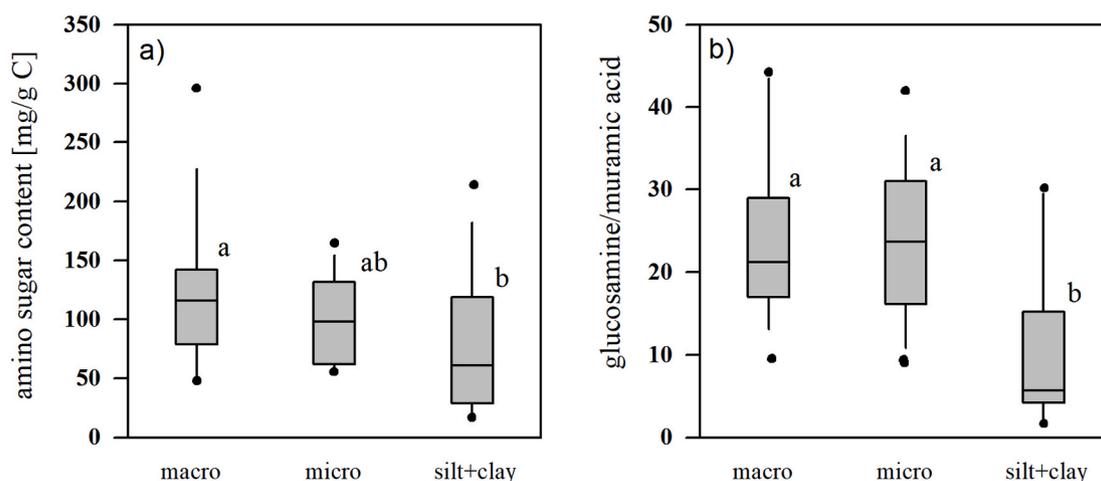


Fig. 1. a) amino sugar contents based on 14 observations from four studies for macro- and microaggregates, and 23 observations from eight studies for clay- + silt-sized MAOM; b) ratio of glucosamine to muramic acid based on the same data sources. Horizontal lines in the boxes are the median, whiskers contain 95% of the data, black points are outliers, i.e., above or below the 95% data range.

explains why the lignin in MAOM is typically highly oxidized (Guggenberger et al., 1995; Kiem and Kögel-Knabner, 2003; Thevenot et al., 2010). Yet, exceptions to these often reported patterns (Wang et al., 2014; Clemente et al., 2011; Mastrolonardo et al., 2015) indicate that the depletion of lignin in MAOM can be offset by certain factors. For example, vanillyl monomers are more persistent than syringyl monomers (Hedges et al., 1988). Soils under plants with a higher content of vanillyl units, which persist longer during initial decomposition processes and have a higher chance to associate with mineral surfaces, may thus feature higher contents of stabilized lignin (cf. Clemente et al., 2011). Surprisingly, the number of studies having extracted lignin from intact aggregate structures is low. The few available reports indicate a greater lignin content and a lower degree of lignin oxidation in macroaggregates than in microaggregates (Monreal et al., 1997; Panettieri et al., 2017; Xiao et al., 2007).

Neutral, plant-derived sugars (mainly derived from hemicelluloses) show a similar pattern, i.e., their contents generally decline with decreasing particle size (Amelung et al., 1999a; Guggenberger et al., 1995; Kiem and Kögel-Knabner, 2003; Solomon et al., 2000). These sugars are preferred microbial substrates over other forms of SOM (Gunina and Kuzyakov, 2015), such as lignin, and likely contribute to the build-up of microbial necromass. The contribution of plant- vs. microbial neutral sugars has often been evaluated relatively, via calculation of the ratio of galactose and mannose (microbial) to arabinose and xylose (plant), which typically increases with decreasing particle size (Guggenberger et al., 1995; Kiem and Kögel-Knabner, 2003; Spielvogel et al., 2007). Notably, the contents of mainly plant-derived neutral sugars (i.e., xylose and arabinose) in MAOM (52–128 mg g<sup>-1</sup> C) may exceed those of lignin by several factors (Córdova et al., 2018; Guggenberger et al., 1995; Kiem and Kögel-Knabner, 2003) and may substantially contribute to the C in stabilized SOM (values of about 130 mg/g C have been reported; Córdova et al., 2018).

Similar to lignin and plant-derived neutral sugars, the content of plant-derived lipids, or their abundance relative to microbial-derived lipids, has been reported to decline with decreasing particle size, and from particulate to mineral soil fractions (e.g., Angst et al., 2018b; Ludwig et al., 2015). Yet, some plant-derived lipids have been found to

accumulate in stabilized relative to non-stabilized SOM pools, including long-chain *n*-alkanoic acids and certain monomers of cutin and suberin (with hydroxyl and/or carboxylic acid groups; Angst et al., 2017a; Jandl et al., 2004; Quenea et al., 2004). Consistent with studies of bulk soils (e.g., Mueller et al., 2013), this indicates that stabilization of plant-derived lipids via organo-mineral interactions could jointly depend on the monomeric composition of lipid biopolymers and the chemical properties of those monomers. The contribution of plant-derived lipids to stabilized C ranges between ~2% and ~10% for MAOM (i.e., ~20–100 mg lipid per g C; Angst et al., 2020, 2019a, 2018b; Angst et al., 2017a; Carrington et al., 2012; Wiesenberger et al., 2010). Studies extracting these compounds from intact aggregates do, to the best of our knowledge, not exist. Although the number of studies on plant-derived lipids employing soil physical fractionations is scarce, the numbers presented above indicate that the contribution of these compounds to MAOM may be higher than that of lignin (cf. Fig. 2).

The above values for plant-derived SOM are likely underestimates because the most common extraction methods almost certainly fail to completely extract monomers of lignin, cutin, and suberin from bulk soils and mineral soil fractions. For example, sorption experiments of lignin onto various iron oxides and clay minerals indicated that up to 56% of lignin was irreversibly bound to these minerals (Hernes et al., 2013). Incomplete extraction of plant-derived lipids from mineral soils by commonly employed base hydrolysis techniques was also reported by Lin and Simpson (2016) and Naafs and Van Bergen (2002). These authors and others have found that, for several locations and land uses, up to 80% of total quantifiable lipids in soil were only extractable after mineral dissolution, which has rarely been utilized prior to lipid hydrolysis and extraction (Cai et al., 2017; Li et al., 2018; Li and Liang, 2020; Lin and Simpson, 2016). These methodological and analytical limitations could blur our perception of the composition of MAOM and aggregates, which may contain more plant-derived compounds than acknowledged in some recent studies. For example, when the contributions of lignin to MAOM are traced via an isotopic label (instead of extraction and quantification of lignin monomers), lignin-derived C can be strongly ‘selectively preserved’ relative to other forms of C in plant litter. In one incubation study of tropical soils with iron-oxides, a larger fraction of C derived from lignin (48%) was retained in soil compared to C derived from bulk litter (31%), and only 6% of lignin was mineralized compared to 51% of bulk litter (Huang et al., 2019; see also Hall et al., 2017). In a similar incubation study that utilized isotopic tracers, C in acid-unhydrolyzable residues, which includes lignin, cutin and suberin, was retained in the mineral soil fraction (<53 μm) to a greater extent than C added in more ‘labile’ forms (like biomolecules present in hot water extracts of plant litter; Almeida et al., 2018). Although these incubation studies cannot identify whether the plant-derived C was ultimately preserved in their original forms (e.g., lignin or cutin) or as microbial biomolecules following microbial utilization of plant-derived substrates, some preservation of plant biomolecules is likely given the affinities of aliphatic acids and phenolic acids for mineral surfaces (e.g., Feng et al., 2005; Polubesova et al., 2010).

### 2.3. Plant- vs microbial-derived SOM

An inherent shortcoming of the biomarkers presented in the previous sections is that they only account for a portion of SOM (Otto and Simpson, 2007). As such, these biomarkers offer valuable, but narrow insights into the composition of SOM. One way to overcome, or at least partly offset, this shortcoming is the calculation of the proportion of microbial necromass C in SOM. This can be achieved by quantifying amino sugar biomarkers and multiplying their abundance by conversion factors based on molecular stoichiometry, following Appuhn and Joergensen (2006), Engelking et al. (2007), Joergensen and Wichern (2008), and Liang et al. (2019). This approach has been shown to be reliable for the estimation of bacterial and fungal necromass C across various soils within narrow confidence limits (Appuhn and Joergensen, 2006).

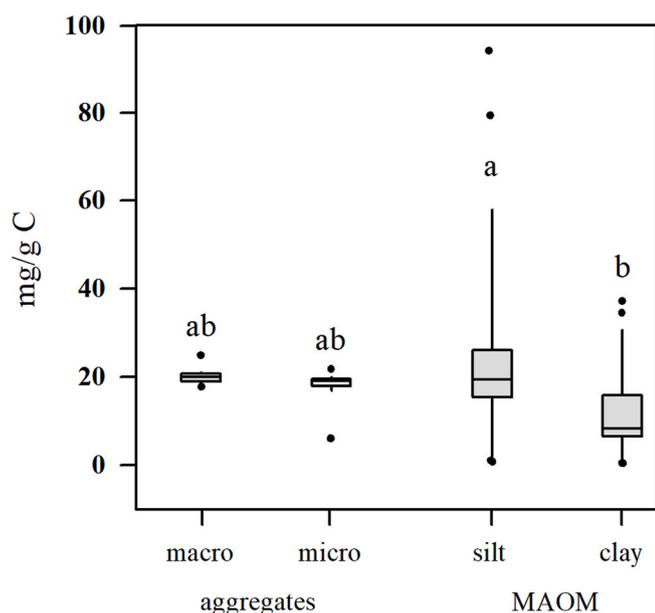


Fig. 2. Lignin contents (mg/g C) based on six observations from three studies for macro- and microaggregates, on 41 observations from ten studies for silt-sized MAOM, and on 60 observations from 13 studies for clay-sized MAOM. For comparison, the average lignin content of POM based on 44 observations from seven studies is  $30.1 \pm 3.6$  mg/g C.

Assuming this approach produces a reasonable estimate of microbial necromass C, it is plausible that the remaining C in SOM is mainly derived from plants; in other words, a rough approximation of plant-derived C in SOM can be estimated by quantifying the fraction of C that is not accounted for by microbial necromass. We estimated bacterial, fungal, and microbial necromass C in macro- and microaggregates, and silt- and clay-sized MAOM from 60 published observations (Fig. 3), including data from forest and agricultural soils; the results indicate that microbial necromass C in stabilized SOM does not exceed 50%, on average (47.2% for macroaggregates, 49.7% for microaggregates, and 38.6% for MAOM in the silt and clay fraction). Microbial necromass accounted for more of stabilized SOM pools in agricultural soils (56.7, 53.7, and 44.1% for macro- and microaggregates, and silt- and clay-sized MAOM, respectively) and much less in forest soils (11.6, 11.6, and 15.2% for macro- and microaggregates, and silt- and clay-sized MAOM, respectively), likely due to the less favorable conditions in forests for bacterial growth (e.g., low pH, variable quality litters). While the values from agricultural soils are in the range of those compiled by Liang et al. (2019) and Khan et al. (2016) for bulk soils under the same land use, they are by ~12–21% lower than the values those authors reported for forest bulk soils (Khan et al., 2016; Liang et al., 2019). Because forest soils can be dominated by POM (Cotrufo et al., 2019), microbial colonization of such POM and microbial death *in situ* may result in higher amounts of microbial necromass in fractions other than aggregates or MAOM in forests as compared to other ecosystems. These results, partly based on the same studies (three studies included in our synthesis were also included in the synthesis of bulk soil data by Liang et al., 2019), suggest that the contribution of microbial necromass to bulk soils might not mirror its contribution to stabilized SOM.

Notably, macro- and microaggregates feature a higher fungal microbial necromass C than silt- and clay-sized MAOM (marginally significant at  $p < 0.1$ ); this reinforces the role of fungi in aggregate formation (Six et al., 2004) and highlights the relevance of aggregates in the stabilization of microbial necromass C. By contrast, OM associated with silt and clay particles tends to contain more bacterial necromass C, while being dominated by other, likely plant-derived C (Fig. 3). These values indicate that microbial residues contribute more to stabilized SOM than assumed in some earlier studies (cf. Simpson et al., 2007), but also suggest that this contribution is ecosystem dependent (see below) and that a substantial amount of stabilized SOM is likely derived from plants. The results of some pyrolysis GC-MS studies are in line with this interpretation, finding a relatively balanced contribution of microbial and plant compounds largely independent of fraction (Ludwig et al., 2015; Rovira and Grasset, 2019). The strong emphasis on microbial necromass in stabilized SOM pools put forward by many recent studies, thus, appears to understate the direct sorption pathway of plant compounds to mineral surfaces (Sokol et al., 2019) and the fact that plant-derived debris is considered the nucleus for aggregate formation (Tisdall and Oades, 1982), i.e., an integral part of aggregates.

The above interpretations should be made with caution, given the inherent uncertainties of stoichiometric conversion and wet chemical

extraction methods. For example, a shift from Gram (+) to Gram (-) bacteria in soil, whose abundance can vary across ecosystems (Fanin et al., 2019), leads to an underestimation of bacterial residues (Appuhn and Joergensen, 2006; Joergensen, 2018). Moreover, the C not accounted for by microbial necromass C does not need to be entirely composed of plant-derived C. In addition, we found only a limited number of observations from forest (and grassland) sites. We encourage further studies of amino sugars and microbial necromass (and plant biomolecules) in forests and grasslands, and we suggest that interpretations of microbial necromass might be improved if it is measured along with the relative abundance of Gram (+) and Gram (-) negative bacteria, given this abundance correlates with that in microbial necromass. We also think it would be informative to quantify the distribution of total microbial necromass among different parts of the soil matrix (e.g., POM vs. MAOM).

### 3. Influencing factors

To effectively manage C pools in soils requires better knowledge of the factors determining the amount of microbial and plant C in stabilized SOM (Liang et al., 2020). Below, we review how the molecular composition of stabilized SOM is impacted by key factors related to climate, land use, and other aspects of environmental change. These environmental factors control the relative abundance of plant- and microbial-derived C in stabilized SOM by influencing microbial C use efficiency, the relative abundance and chemical composition of plant and microbial inputs to SOM, or the potential for preferential stabilization of biomolecules with different spatial distribution in soil or different chemical properties.

Because we rely on a comparably large dataset of mean annual temperature (MAT) and mean annual precipitation (MAP; Table 1), values reported along the study design in many papers, we directly tested these climate variables in relation to the concentrations of amino sugars and lignin in stabilized SOM via simple regressions (except for lignin contents in macro- and microaggregates;  $n = 2$ ). Regarding the other factors reviewed here (substrate quality, soil type and depth, mineralogy, soil fauna, and land use), we only performed statistics on the compiled data when the number of replicates for each category was at least four.

#### 3.1. Temperature and precipitation

Changes in climate may affect plant community composition, the allocation of above- and belowground inputs to the soil, microbial community composition, and the kinetics of biogeochemical processes, such as mineral weathering. As such, differences in climate factors should also influence the composition of stabilized SOM.

We performed simple regressions with MAT and MAP on amino sugar contents in macro-/microaggregates and clay-sized MAOM, based on our dataset compiled from the literature (four and nine studies and 14 and 23 observations for macro-/microaggregates and silt- and clay-sized

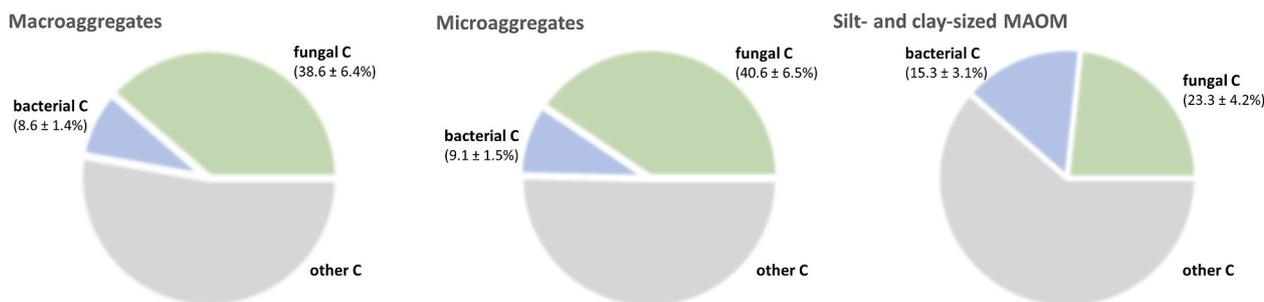


Fig. 3. Microbial necromass C calculated following Appuhn and Joergensen (2006), Engelking et al. (2007), Joergensen and Wichern (2008), and Liang et al. (2019), separated into bacterial, fungal, and other (likely plant-derived) C.

**Table 1**

Basic characteristics of the compiled dataset used to aid our review of the influence of environmental factors on amino sugars and lignin in stabilized SOM.

Dependent variables	min	max	mean	CV	Climate variables								# of studies	# of observations			
					MAP				MAT					Total	Land use (n)	Soil type (n)	
					min	max	mean	CV	min	max	mean	CV					
<i>Amino sugars</i>																	
macroaggregates	55.8	164.9	102.2	38	380	1459	837.8	47.4	5	20	11.1	64.3	4	14	agriculture (13)	Podzol (1), Chernozem (7), Luvisol (4), Alisol (2)	
microaggregates	48.3	296.4	123.8	54.7	380	1459	837.8	47.4	5	20	11.1	64.3	4	14	agriculture (13)	Podzol (1), Chernozem (7), Luvisol (4), Alisol (2)	
clay- + silt-sized MAOM	17.2	150	75.6	55.4	380	1350	808.2	36.4	6	26	13	54.7	9	23	agriculture (16), forest (5), grassland (2)	Gleysol (5), Alisol (2), Luvisol (8), Chernozem (7)	
<i>Lignin</i>																	
macroaggregates	19.8	25.0	22.4	11.6	-	-	1650	-	-	-	20	-	1	2	grassland (2)	Luvisol (2), Cambisol (2)	
microaggregates	6.0	16.8	11.4	47.3	-	-	1650	-	-	-	20	-	1	2	grassland (2)	Luvisol (2), Cambisol (2)	
silt-sized MAOM	0.8	94.1	25.1	74.5	300	2200	828.4	68.4	0.9	26	12.1	67.4	10	41	forest (1), grassland (22), agriculture (12)	Chernozem (28), Luvisol (7), Ferralsol (6), Alisol (1)	
clay-sized MAOM	0.4	35.5	11.6	68.1	115	2200	704.2	81.1	0.9	26	13.3	54.2	13	60	forest (18), grassland (23), agriculture (13)	Chernozem (25), Luvisol (12), Ferralsol (6), Alisol (13), Cambisol (2)	

Data from: Amelung et al. (1999b); Angst et al., 2017a; Angst et al. (2019a); Carrington et al. (2012); Clemente et al. (2011); Ding and Han (2014); Gillespie et al. (2014); Griepentrog et al. (2014); Guggenberger et al. (1995), 1999; Heim and Schmidt (2007); Hong et al. (2011); Kiem and Kögel-Knabner (2003); Ma et al. (2014); Murugan et al. (2019); Neufeldt et al. (2002); Peinemann et al. (2005); Schmidt and Kögel-Knabner (2002); Simpson et al. (2004); Six et al. (2001); Solomon et al. (2000); Spielvogel et al. (2007); Zhang et al. (2003). Soil classification according to IUSS Working Group WRB (2014); if other classifications were used in the referenced studies, we translated the soil order from these classifications into the WRB classification for consistency. More details are given in Supplementary Table S1.

MAOM, covering arable land, grassland, and forest, respectively). These regressions indicate positive correlations of the amino sugar content (in mg g<sup>-1</sup> of soil organic C) in aggregates and MAOM with MAT (marginally significant -  $p < 0.1$  - to highly significant -  $p < 0.001$ ), but none with MAP (Table 2). The consistent positive correlation of MAT and amino sugars may be explained by an increased investment of microorganisms in anabolism over catabolism at higher temperatures, perhaps, leading to a higher production of microbial biomass and, consequently higher contents of microbial necromass (cf. Ding et al., 2019).

Some manipulative experimental (Liang et al., 2015; Shao et al., 2018) and observational studies (Amelung et al., 1999a) have documented contrasting effects, with lower amounts of amino sugars in bulk soils and particle size fractions in response to warming; these results have mainly been attributed to water stress that occurs due to experimental warming and enhanced decomposition of non-mineral protected (microbial) SOM (Amelung et al., 1999b; Shao et al., 2018). In another observational study by Amelung et al. (1999b), the content of

**Table 2**

Regression results for amino sugar and lignin contents against MAT and MAP in different fractions.

biomarker	fraction	MAT		MAP	
		p	r	p	r
amino sugars	microaggregates (n = 14)	0.03	0.59	0.69	-
	macroaggregates (n = 14)	0.056	0.52	0.9	-
	silt + clay-sized MAOM (n = 21)	<0.0001	0.73	0.6	-
lignin	silt-sized MAOM (n = 41)	0.4	-	0.6	-
	clay-sized MAOM (n = 60)	0.9	-	<0.001	-0.4

plant-derived neutral sugars decreased in clay-sized MAOM with an increasing MAT in favor of microbial-derived sugars. Experimental and observational studies on bulk soils also found shifts in microbial necromass contribution from fungal to bacterial sources or vice versa with changing climate parameters (Liang et al., 2015; Shao et al., 2018; Xia et al., 2019). These inconsistent results from long-term observational and manipulative warming studies might result from the fact that the former record effects of plant and microbial communities adapted to cold/warm temperatures, while the latter show the rather short-term impacts of plant and microbial communities and soil suddenly exposed to warmer temperatures. Thus, in long-term observational studies, the content of amino sugars in soil might rather be related to altered vegetation patterns in response to a different climate, e.g., changing plant communities with a different tissue composition (e.g., Bowman et al., 2017), which may have stronger controls on microbial communities than climate directly (Classen et al., 2015). Conversely, for manipulative warming studies, the short- to medium-term physiological sensitivity of a soil system to altered climate parameters may be decisive for the response of amino sugars. As such, it is difficult to outline general responses of microbial necromass C (and plant C) in stabilized SOM, as it will likely be determined by both direct (e.g., temperature) and indirect climate effects (such as an altered vegetation).

The same statistical analyses for lignin in clay- and silt-sized MAOM indicated a weak negative correlation of MAP with the lignin content in clay-sized MAOM ( $p < 0.001$ ,  $R^2 = 0.16$ ) and no correlation between MAT and lignin content (based on a dataset compiled from ten and 13 studies, 41 and 60 observations for silt- and clay-sized MAOM, respectively, covering forest, grassland, and arable land; Table 2). This negative correlation with MAP may be due to the fact that other SOM compounds in this fraction may strongly increase in response to an

increasing MAP (Amelung et al., 1999b), decreasing the relative contribution of lignin to the total C content. By contrast, other studies did not find correlations of the lignin content in bulk soil or stabilized SOM with MAP, and instead, found evidence of temperature effects (Amelung et al., 1999b). For example, a weak positive correlation between temperature and lignin contents was reported by Feng et al. (2008) for warmed bulk soils. They attributed the decrease in lignin to an increased abundance of fungi, who are the main decomposers of lignin. Amelung et al. (1999b) investigated lignin in particle size fractions from a grassland climosequence and, while not reporting any changes in the lignin content, found a decreased degree of lignin oxidation (i.e., decomposition) with increasing MAT in MAOM. The authors attributed these patterns to a lower input of readily available substrates to the soil under higher temperatures (such as carbohydrates) for a co-metabolic decomposition of lignin. These minor changes in lignin with increased temperature are in line with the results of Zhou et al. (2012), reporting an insensitivity of microbial populations involved in the decomposition of lignin to warming.

Artificial warming and observational studies on bulk soil and stabilized SOM indicate decreased contents of plant-derived neutral sugars upon warming (Amelung et al., 1999a; Feng et al., 2008), likely due to a shortened microbial dormancy phase under increased temperatures, so that the typically labile sugars are more likely to be decomposed or metabolized before they can interact with reactive surfaces and be stabilized. In contrast, plant-derived (specifically leaf-derived) lipids accumulated in bulk soil relative to lignin and neutral sugars in an artificial warming experiment (Feng et al., 2008). An observational study of six sites in North and South America showed that warmer sites tended to have higher concentrations of plant-derived lipids in soil, including components of leaf waxes and cutin (Pisani et al., 2014). It remains unknown, though, what caused these experimental and observational effects of temperature on the relative abundance of plant-derived lipids in SOM. As with amino sugars, the content of plant-derived compounds in stabilized SOM is likely directly influenced by the effects of climate variables on microbial biomass, C use efficiency, and community composition and indirectly via controls on plant growth and altered organic matter inputs to the soil.

### 3.2. Substrate quality

In recent years, there has been a steady rise in evidence that microbial derived compounds accumulate in stabilized SOM and may considerably contribute to the total soil organic C. Yet, although it seems reasonable that the C use efficiency of microorganisms and C-substrate quality interact to determine the amount of microbial necromass C that can be stabilized in MAOM (Kallenbach et al., 2016; Cotrufo et al., 2013; Bradford et al., 2013), direct evidence for effects of substrate quality on MAOM C pools and composition is limited. Recent studies on the application of organic substrates and nutrients to soil indicate that the content of amino sugars in aggregates and MAOM generally responds positively to substrate additions (Ding et al., 2015; Ding and Han, 2014; Griepentrog et al., 2014; Xu et al., 2003). For example, N additions were shown to increase the amount of amino sugars in silt- and clay-sized MAOM (Gillespie et al., 2014), which, however, only appeared to be true when microorganisms were not C-limited. In a set of fertilizer application studies, N fertilizer alone did not have any effect on the content of amino sugars in stabilized SOM (Ding et al., 2015; Ding and Han, 2014); only when combined with an easily degradable organic C source did the content of amino sugars increase with N fertilization. Likewise, bulk soils under tree species with higher amounts of N and less acid insoluble compounds in their leaves and roots (i.e., high quality substrates) also had SOM that was more stable and more likely to be derived from microbes (based on  $\Delta^{14}\text{C}$ ,  $\delta^{15}\text{N}$ , and other indices; Angst et al., 2019b).

Specifically fungal amino sugars appear to increase more in bulk soil and stabilized SOM than those from bacteria upon the addition of N and

various high-quality substrates (Bipfubusa et al., 2008; Ding and Han, 2014; Griepentrog et al., 2014). It is unclear whether this greater apparent sensitivity of fungal residues to substrate quality is due to differences in the stoichiometry or C use efficiency of bacteria and fungi (Manzoni et al., 2012; Silva-Sánchez et al., 2019; Six et al., 2006; Soares and Rousk, 2019), differences in the stabilization potential of fungal and bacterial residues, or a potentially increased turnover rate of some fungi particularly sensitive to substrate quality (De Vries et al., 2009; Rousk and Bååth, 2007).

Apart from these uncertainties, substrates with a high quality generally seem to stimulate the accumulation of microbial residues in stabilized SOM, which reinforces the assumptions of the latest conceptualizations (Cotrufo et al., 2013). Yet, this accumulation does not necessarily lead to a higher C sequestration in soil. For example, Córdova et al. (2018) found higher contributions of microbial residues to MAOM upon the addition of high-quality plant litters, but, when related to the bulk soil organic C, the concurrently increasing respiration rates in their study offset the C gain by the accumulation of microbial residues. Common garden experiments in forests show similar evidence that litter quality has dual, and partly counteracting, effects on C stocks, where positive effects of litter quality on formation and retention of microbial-derived C are at least partly offset by positive effects of litter quality on mineralization of plant-derived C (Angst et al., 2019b; Mueller et al., 2015; Vesterdal et al., 2008, 2013).

Although plant inputs are utilized by the soil microbiome, the distinct biomolecular structure of plant inputs seems to be, to some extent, maintained in stabilized SOM and bulk soils, independent of substrate quality. For example, Tamura et al. (2017) found more cutin and plant-derived SOM in micro- and macroaggregates under litter with a higher content of these compounds. Ma et al. (2014) found more lignin in clay- and silt-sized MAOM following the input of woody material (higher lignin) as opposed to the input of leaf material (lower lignin). Another study reported high contributions of plant-derived neutral sugars to MAOM under high quality inputs (Córdova et al., 2018). Many studies of bulk soils show that plant species and communities with distinct compositions of plant biopolymers have correspondingly distinct compositions of lignin and lipid monomers in soil (Bull et al., 2000; Kuhn et al., 2010; Mueller et al., 2012, 2013; Otto et al., 2005; Otto and Simpson, 2006). Collectively, these results are not consistent with the dominance of the 'in-vivo', microbial pathway of SOM formation and stabilization (Liang et al., 2017).

A central factor for determining the preservation of plant biomolecules in soil may be the point of entry of plant-derived C. Since microbial communities are inherently sessile and located at distinct spots in the soil, they need to 'wait' for substrate entering their habitat to gain access to it (Sokol et al., 2019). If plant material enters areas with low microbial density, exoenzymatic modifications may dominate the transformation of these plant compounds, breaking down complex materials but not necessarily resulting in mineralization or assimilation by microbes (Liang et al., 2017). In contrast, if plant material enters a microbial hotspot, such as the rhizosphere, it may readily be metabolized (resulting in mineralization and C loss, or assimilation and potential C retention). Future studies may employ spatially resolved sampling designs, such as rhizosphere vs. bulk soil, to provide further insights into the location-dependent relevance of different formation pathways of stabilized SOM.

In summary, while substrate quality seems to have a major influence on the content of microbial residues in stabilized SOM, the amount of stabilized plant compounds in soil may be inversely related to, or partly uncoupled from, the quality of the original plant substrate (see also Castellano et al., 2015). However, comprehensive studies investigating the effects of substrate quality on microbial- and plant-derived compounds in stabilized SOM in one experimental design (e.g., Córdova et al., 2018) are very rare. Such studies would allow for more integrated inferences about the role of substrate quality in influencing microbial and plant signatures in stabilized SOM.

### 3.3. Soil type, soil depth, and mineralogy

Different soil types are related to different processes, such as illuviation/eluviation or redox reactions, that determine the amount, reactivity, and vertical distribution of available surfaces for the sorption of SOM (Blume et al., 2015). The mineralogy of a soil mainly depends on its parent material and the extent of weathering, and determines the content of (oxy)(hydr-)oxides and type of dominant clay minerals (e.g., Kaolinite, Illite, or Montmorillonite). Consequently, mineralogy impacts the availability of reactive surfaces for the sorption of SOM, and this subsequently alters reactive surfaces for additional SOM-SOM interactions (Mitchell et al., 2018). It can, thus, be expected that these factors may also influence the composition of stabilized SOM. The abundance of plant- and microbial-derived C is likely to also vary with depth, as substrate inputs and physicochemical soil properties vary with depth (Rumpel and Kögel-Knabner, 2011), but the potential for synthesis regarding these factors is limited due to the dearth of data and associated studies. We, thus, only performed statistics on our dataset when enough data were available (at least four observations for each category, which was the case for amino sugars in aggregates and MAOM in Chernozems and Luvisols and for lignin in clay- and silt-sized MAOM in Chernozems, Luvisols, Ferralsols, and Podzols), and else, qualitatively evaluated the data for soil type and reviewed the available literature for the effects of mineralogy and soil depth.

Based on the compiled data, amino sugar contents in stabilized SOM tend to be highest in Chernozems and Luvisols, while Gleysols and Podzols have lower contents (Fig. 4). The content of amino sugars in Alisols seems to strongly depend on whether considering macro- or microaggregates, or silt- and clay-sized MAOM. Yet, the data for Alisols and Podzols are little informative, being based on two and one data point(s) only. Nevertheless, combined with the typical characteristics of each soil type, we can make some general assumptions on the reported patterns. For example, the conditions in Chernozems and Luvisols, which are either rich in easily decomposable SOM (Chernozems – mostly found under tall-grass prairie) or basic and well aerated (Luvisols; IUSS Working Group WRB, 2014), may be favorable for microbial growth, while a part of Gleysols is permanently flooded, and Podzols and Alisols generally feature low pH values; conditions less conducive to the growth of (aerobic) microorganisms. Moreover, frequent redox processes in Gleysols and the dissolution of minerals upon low pH values in Podzols (Blume et al., 2015) likely further reduce the capacity of such soil types to stabilize C in MAOM (be it microbial- or plant-derived).

While preferential sorption of amino acids, which can have both plant and microbial sources, to certain minerals has been reported (Hedges and Hare, 1987; Lambert, 2008), it is less clear whether other microbial-derived compounds are also preferentially retained by sorptive interactions. Some studies reported an insensitivity of amino sugars and microbial-derived SOM to differing clay mineralogy (Kallenbach et al., 2016; Pronk et al., 2015). By contrast, in a study by Creamer et al. (2019), mineralogy determined whether dead microbial cells were

directly sorbed to reactive surfaces or had to be further processed prior to sorption, while there were no differences in the content of microbial residues adsorbed to different mineralogies. It, thus, appears that qualitative mineralogical effects might be less important to the sorption of microbial than to the sorption of plant-derived compounds (see below). This is in line with the reactive nature of proteins in microbial cell walls, having a strong affinity for any soil mineral surface.

Many studies focus on topsoil, neglecting deeper soil layers. Studies on bulk soil, mostly conducted in forests, generally report an increased proportion of amino sugars in SOM at depth as compared to that in the topsoil (Angst et al., 2018a; Ding et al., 2017; Liang and Balsler, 2008; Ni et al., 2020). This was also confirmed by Spielvogel et al. (2008), who investigated microbial-derived neutral sugars in MAOM. These patterns were attributed to a decreasing input of plant compounds with increasing depth. Yet, exceptions to these patterns in MAOM and bulk soil, as reported by Mikutta et al. (2019) and Moritz et al. (2009), indicate some dependence on soil type and climate, i.e., both of the cited studies were conducted in regions with high precipitation (>2500 mm/y). Under such conditions, dissolved organic matter from plants and decomposing litter is more likely to reach deeper soil layers, diluting the microbial signature in the subsoil. Moreover, the permeability of a soil and spatially distinct inputs of organic matter (e.g., Chabbi et al., 2009; Heinze et al., 2018; Preusser et al., 2019) may further influence the amount and distribution of microbial (and plant) compounds in the subsoil. Based on the compiled data and, partly, the lack thereof, there clearly is a need for more research on the microbial contribution to stabilized SOM as affected by soil type, clay mineralogy, and depth, particularly in agricultural and grassland ecosystems.

For lignin, soil type only had a significant influence on clay-sized MAOM in our statistical analysis ( $p < 0.0001$ ; based on 58 observations from 13 studies), where Alisols had higher lignin contents than Chernozems, Ferralsols, Luvisols, and Cambisols (Fig. 4). This could indicate that the stability of lignin is favored at low pH values and in the presence of Al oxides and hydroxides (Mikutta et al., 2007), which are features of Alisols. Interestingly, independent of soil type, lignin tends to be more than two times as abundant in silt-sized relative to clay-sized MAOM (Fig. 4), as also reported by Heim and Schmidt (2007) and Panettieri et al. (2017), and similar to the patterns observed for different land uses (see below). While the reasons for this enrichment remain unknown, the fact that silt-sized MAOM generally contains less oxides and hydroxides (Blume et al., 2015), to which lignin preferentially and strongly adsorbs, may result in a better extractability of lignin biomarkers from silt-sized MAOM.

As with amino sugars, the maximum depth in many studies to which lignin contents in stabilized SOM have been reported is ~30 cm. Some studies on forest subsoils indicated a decreased proportion of lignin in clay- and silt-sized MAOM with increasing depth in favor of microbial-derived compounds (Rumpel et al., 2004, 2012; Vancampenhout et al., 2012). Lignin-derived phenols in dissolved organic matter moving down the soil profile are preferentially preserved on oxide and hydroxide

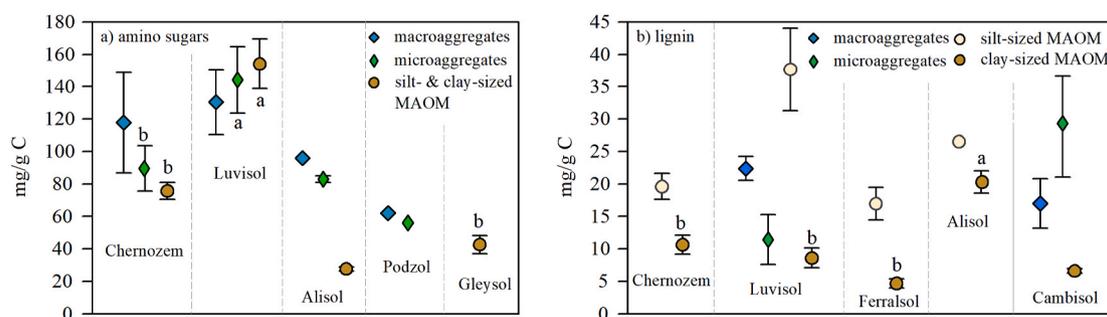


Fig. 4. Contents of (a) amino sugars and (b) lignin in stabilized SOM separated by soil type (according to IUSS Working Group WRB, 2014). One-way ANOVA was performed when at least four datapoints were available. This was the case for amino sugars in aggregates and silt- and clay-sized MAOM in Chernozems, Luvisols, and Gleysols and for lignin in clay-sized MAOM in Chernozems, Luvisols, Ferralsols, and Alisols.

surfaces in shallow soil layers (Kaiser et al., 2004; Kaiser and Zech, 2000), possibly explaining the decreasing trend with depth reported in these studies. By contrast, the proportion of root-relative to leaf-derived lipids in SOM has often been reported to increase with depth (Angst et al., 2016; Feng and Simpson, 2007; Nierop, 1998; Nierop et al., 2006; Spielvogel et al., 2014). Some studies also assume a major proportion of SOM to be derived from roots, and increasingly so with depth (Rasse et al., 2005). The potential preservation of plant-derived lipids in MAOM is supported by observations of sorption of aliphatic carboxylic acids to some clay minerals, including montmorillonite and kaolinite (Feng et al., 2005; Kang and Xing, 2007; Olshansky et al., 2014). Some minerals even appear to preferentially sorb aliphatic carboxylic acids (e.g., kaolinite; Feng et al., 2005), and some minerals appear to be more likely to retain such acids, even if they selectively sorb aromatic C forms (Adhikari and Yang, 2015). Yet, direct comparisons between root-, leaf-, and microbial-derived compounds in stabilized SOM in subsoil and in bulk subsoil do, to the best of our knowledge, not exist.

### 3.4. Soil fauna

Soil fauna mix litter and organic matter into and in the soil, and mechanically break up and ingest and egest litter material, resulting in a redistribution of SOM and chemical and structural changes to SOM (Frouz, 2018). While soil fauna is often neglected in SOM models, it can in fact, substantially alter soil C sequestration patterns (Filser et al., 2016). For example, the invasion of North American ecosystems by European earthworm species impressively clarifies how profound earthworms may affect their (soil) environment, such as by altering soil chemistry, microbial dynamics, or thinning litter or organic layers (Eisenhauer et al., 2007; Ferlian et al., 2020; Filley et al., 2008). Moreover, depending on the context (e.g., lab vs. field scale or the species (assemblages) investigated), the activity of earthworms may either stabilize SOM against decomposition in aggregates and MAOM (Angst et al., 2017b; Barthod et al., 2020) or increase soil CO<sub>2</sub> emissions via the stimulation of the microbial community (Lubbers et al., 2013, 2017). Such processes also appear to alter the contribution of plant- and microbial-derived compounds to stabilized SOM. In laboratory incubations, Angst et al. (2019a) found that the presence of earthworms increased the content of amino sugars in the more stable soil fractions (i. e., microaggregates and MAOM) and decreased plant compounds in the less stable fractions (i.e., free POM). Similar results were reported by Vidal et al. (2019). Other studies found that specifically the content of lignin (Hong et al., 2011; Ma et al., 2014) and root-derived lipids (Angst et al., 2020; Nguyen Tu et al., 2020) increased in the presence of earthworms in bulk soils, microaggregates, and silt- and clay-sized MAOM. It has been reported that earthworms disrupt fungal hyphae and decrease fungal abundance during passage through the earthworm's gut (Bernard et al., 2012; Ferlian et al., 2018), which may result in the observed increase in lignin in stabilized SOM in the presence of earthworms. Likewise, earthworms may actively forage for more easily degradable substrates in soil (i.e., leaf-derived organic matter; Curry and Schmidt, 2007), relatively increasing the amount of recalcitrant compounds (i.e., root-derived organic matter) in stabilized SOM and SOM in general (Angst et al., 2020). By moving and mixing minerals and organic matter, earthworms could also influence sorption and desorption of biomolecules to mineral surfaces (Guhra et al., 2020; Lyttle et al., 2014; Shan et al., 2011). General conclusions about the effects of earthworms are hard to draw, though, due to the fact that these effects may vary with earthworm species, which have diverse functional traits (Brown et al., 2000; Curry and Schmidt, 2007), and between laboratory and field scale experiments, and may further be influenced by ecosystem type or climate change (Lubbers et al., 2013; Singh et al., 2019). Yet, the available literature indicates that earthworms may substantially impact the composition of stabilized SOM.

Studies on other types of soil fauna in connection with stabilized SOM are scant, but the few reports available indicate that also other

invertebrates may strongly affect the contribution of microbial and plant compounds to stabilized SOM. For example, Amelung et al. (2002) found strong increases in lignin in MAOM upon the reworking of SOM by termites. Jiang et al. (2018) reported significant influences of bacterivorous nematodes on the ratio between fungi and bacteria in micro- and macroaggregates, which could also alter the amount of microbial residues in these aggregates. Moreover, the fact that a variety of SOM-degrading enzymes have been ascertained in the guts of isopods, Collembola, mites, and mollusks (Wolters, 1998) points to potential direct and indirect effects of the soil fauna on the composition of stabilized SOM.

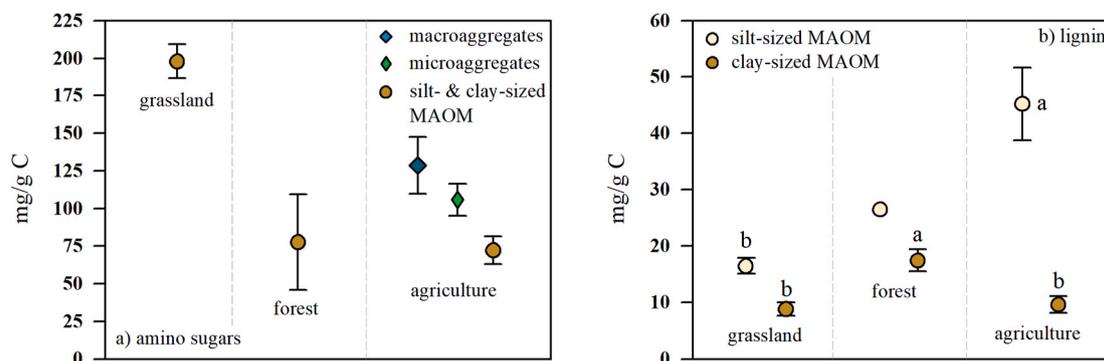
### 3.5. Land use

Differences in land use and land cover are coupled with differences in the amount and quality of organic matter entering the soil, in physico-chemical soil properties, and in climate. As such, land use and land cover should strongly affect the content of microbial necromass C.

Based on the available literature, grasslands seem to favor the accumulation of microbial necromass in stabilized SOM over other land uses. For example, Turrión et al. (2002) reported an increase in amino sugars in clay-sized MAOM with the conversion of forests to pastures; Ding et al. (2017) found an increase in the content of amino sugars in clay-sized MAOM after the conversion of arable soil to grasslands. Though there were only two data points for grassland soils, the concentrations of amino sugars in silt- and clay-sized MAOM in these soils were also higher than those in forest and agricultural soils in our dataset (198.0 mg/g C vs 78.6 and 72.3 mg/g C, respectively; Fig. 5).

In grasslands, a continuous input of plant material (Smith et al., 2005; Thoms and Gleixner, 2013), low C:N ratios and ratios of lignin to N in plants (Schimel et al., 1994), and a higher soil pH (Kaiser et al., 2016) likely result in high microbial biomass (relative to croplands; McGonigle and Turner, 2017) and high microbial C use efficiency (Qiao et al., 2019) and consequently, the accumulation of microbial necromass. These favorable environmental conditions probably make the retention of microbial necromass C in stabilized SOM a central feature of grassland soils (cf. Ma et al., 2018) as compared to other ecosystems, such as forests, with commonly lower pH and substrate quality (Berthrong et al., 2009), or arable land, with plant inputs to the soil mostly confined to a certain period (Smith et al., 2005). This inference is, in part, also supported by the fact that cultivation of grasslands (but also forests) led to a decrease in the content of amino and microbial neutral sugars in clay- and silt-sized MAOM and in bulk soil (Ding et al., 2020; Jolivet et al., 2006; Zhang et al., 1999). The importance of the microbial pathway to MAOM stabilization in grasslands is also highlighted by a recent isotopic tracer study, which showed that while the 'labile' fraction of grass litter was incorporated into MAOM, the 'structural' fraction of grass litter was not (Haddix et al., 2016); this contrasts with studies of forest litter and soils, which show substantial retention of C derived from 'structural' biomolecules (like lignin) in MAOM (Almeida et al., 2018; Huang et al., 2019). A reduced importance of microbial-derived C in forests (relative to grasslands) is also supported by evidence that MAOM accounts for a smaller fraction of SOM in forests (Cotrufo et al., 2019), and that fungi, which tend to be dominant in forests (Fierer et al., 2009), could have lower carbon use efficiency than bacteria (Silva-Sánchez et al., 2019; Soares and Rousk, 2019). However, due to the low availability of published results with respect to clay- and silt-sized MAOM and the lack of data for microbial necromass in intact macro- and microaggregates, as affected by land use, the hypothesis of a microbially dominated formation of stabilized SOM in grasslands put forward here clearly needs more empirical support.

The land use-related patterns for amino sugars in stabilized SOM seem to be partly mirrored by lignin, where some studies reported higher lignin contents in stabilized SOM and bulk soil in grasslands than in forests or arable land (Guggenberger et al., 1995; Panettieri et al., 2017; Solomon et al., 2000; Thevenot et al., 2010). Forest soils have



**Fig. 5.** Content of amino sugars (a) and lignin (b) in stabilized SOM separated by land use. One-way ANOVA was performed when at least four datapoints were available. This was the case for lignin in silt-sized MAOM for grassland and agriculture, and for clay-sized MAOM in grassland, forest, and agriculture.

generally lower pH values than grassland and agricultural sites; conditions conducive to the activity of fungi, which are the main decomposers of lignin. This could explain why the concentration of lignin in bulk soil has been reported to be typically lower in forests (given the higher abundance of fungi) as compared to grasslands and agroecosystems, and why the lignin in bulk soils is also typically more oxidized in forests (Thevenot et al., 2010). Yet, an insensitivity of lignin contents towards land use change (Leifeld and Kögel-Knabner, 2005; Clemente et al., 2011) or decreased contents of lignin after conversion from forests to grassland (Glaser et al., 2000) in silt- and clay-sized MAOM have also been reported. These inconsistent responses of lignin to land use changes are further mirrored by our assessment of published data, where forests featured the highest lignin contents in clay-sized MAOM (17.0 mg/g C (forest) vs. 8.8 and 9.7 mg/g C in grassland and agricultural soils, respectively; Fig. 5). These inconsistencies may arise from different site conditions (e.g., soil pH, substrate quality, soil type, and soil mineralogy) independent of land use or the different yields and composition of lignin for different plants, so that contents of lignin in stabilized SOM may be more related to a species-specific lignin composition than to land use in general. It is also plausible that enhanced decomposition of lignin in forests could simultaneously lead to a comparably lower retention of lignin in bulk soils (e.g., as POM), and a comparably higher retention of lignin in MAOM (e.g., as lignin monomers sorbed to mineral surfaces) because MAOM tends to account for a lower proportion of bulk SOM in forests as compared to grasslands (Cotrufo et al., 2019).

Studies on plant-derived lipid biomarkers in stabilized SOM as affected by land use are virtually absent. The only study we found reports a higher contribution of plant compounds to clay- and silt-sized MAOM in forest as compared to cultivated soils (Quéneá et al., 2006). Somehow supporting these results, Pisani et al. (2013) found higher amounts of plant-derived biomarkers in the bulk soil under pine as compared to soil under grassland, while the amount of plant lipids under cedar was not significantly different from that under grassland. The low pH of some forest soils, like Podzols, appears to favor selective preservation of plant-derived lipids, including monomers of cutin and suberin (Bull et al., 2000; Nierop et al., 2005a; van Bergen et al., 1998); these observations are supported by experiments showing stronger binding of carboxylic groups to minerals at lower pH, including muscovite mica and goethite (Newcomb et al., 2017).

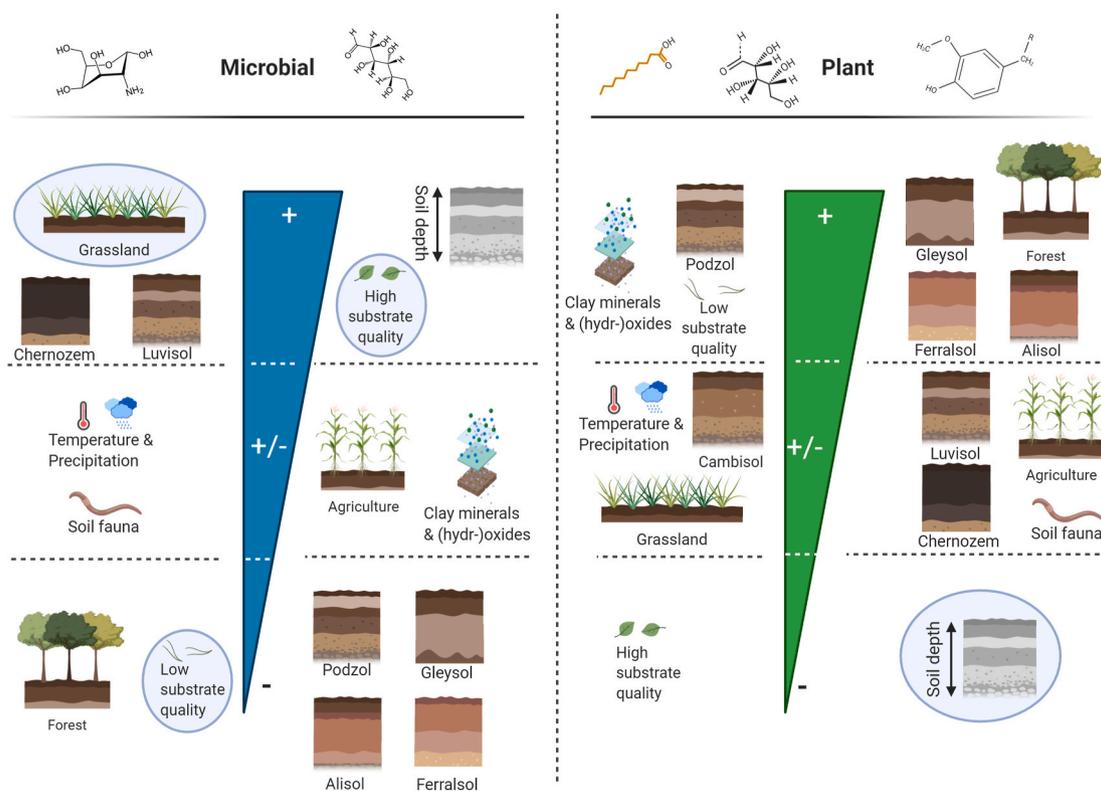
Plant-derived neutral sugars in silt- and clay-sized MAOM were reported to decrease or increase after the conversion from forest to arable land or grassland, respectively (Glaser et al., 2000; Jolivet et al., 2006). Moreover, Spaccini et al. (2001) reported a site-specific accumulation of carbohydrates in stabilized SOM in different forest and agricultural soils. Combined, these results indicate that, similar to our inferences on lignin, the content of plant-derived lipids and neutral sugars in stabilized SOM appears to be more related to site-specific factors and the content of these compounds in the specific plant material, which highly vary in different plant species (Mueller et al., 2012; Zech et al., 2012), than to

land use in general. Interactive effects of environmental factors are also likely; for example, the effects of land use on soil organic C and microbial physiology appear to depend on soil pH, with pH and land use potentially interacting to favor stabilization of microbial-derived C when the pH is greater than ~6.2 (Malik et al., 2018). Similarly, a meta-analysis suggests that conservation tillage increases microbial biomass, and thus, the potential for stabilization of microbial-derived C, but not in sandy soils (Chen et al., 2020). Thus, while some general effects of land use on microbial-derived compounds in stabilized SOM are evident (higher microbial-derived C in grasslands), evidence on the effects of land use or land cover on plant-derived SOM is less clear, though some preferential accumulation of lignin and plant lipids in acid forest soils is likely.

#### 4. Conclusions and future directions

Our literature review and compiled data on microbial- and plant-derived compounds in stabilized SOM indicate that microbial compounds substantially contribute to stabilized SOM. However, and very important to note, our compilation also shows that plant-derived compounds could account for ~50% of the SOM in aggregates and MAOM, and even more in forest soils. While the microbial pathway for the formation of stabilized SOM is certainly important, we call for a more balanced approach in conceptual and quantitative studies, which requires a renewed consideration of the direct contribution of plant compounds to stabilized SOM, which is still relevant in many contexts (Fig. 6). For example, soil types less conducive to the proliferation of microbes, such as Podzols or Alisols, may contain relatively more plant compounds than soil types with favorable conditions for microbial growth, such as Chernozems and Luvisols. Likewise, land uses and land covers with different plant communities and soil conditions may influence the relative proportion of microbial-vs. plant-derived compounds. Grasslands, in particular, with a continuous input of plant material, low C:N ratios and ratios of lignin to N in plants, and a higher soil pH, seem to favor the accumulation of microbial residues in stabilized SOM as compared to forest or agricultural soils. Building on recent conceptualizations of the variable roles that microbes can play in mediating the stabilization and composition of SOM (e.g., Khan et al., 2016; Liang et al., 2017), and on observations of differences in soil properties between ecosystems (e.g., Cotrufo et al., 2019; Hall et al., 2020), we hypothesize that plant-derived biomolecules contribute more substantially to SOM in forests, both as POM and MAOM.

These results and the available literature also support the notion that the content of microbial-derived compounds in stabilized SOM is related to the quality of the substrate the microorganisms feed on, while the content of plant-derived compounds is inversely related or uncoupled from substrate quality (see also the less quantitative review by Castellano et al., 2015). Notably, an increase in microbial-derived SOM upon the addition of high-quality substrates does not necessarily result



**Fig. 6.** Diagram summarizing the effects of environmental factors on the contribution of microbial- and plant-derived compounds to stabilized SOM. “+” indicates an increase in microbial or plant compounds in response to the respective factors located above the dashed lines, “+/-” either indicates inconsistent or unclear effects of the factors located in between the dashed lines, and “-” indicates a decrease in microbial or plant compounds in response to the respective factors located below the dashed lines. The effect of factors encircled in blue is strongly supported by evidence from the literature. Because the data and evidence this scheme is based on were taken from studies separately investigating plant or microbial compounds in different sites, the microbial side does not consistently display the inverse pattern of the plant side (and vice versa). “clay minerals & (hydr)oxides” indicate a qualitative effect, i.e., plant compounds may preferentially sorb to these minerals, while their effect on microbial compounds, specifically amino sugars, is less clear. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in higher total soil C contents. For example, trees with higher quality litter might increase stabilization of microbial C in mineral soils but decrease stabilization of plant-derived C organic and/or mineral horizons (Mueller et al., 2015; Angst et al., 2019b). Thus, we recommend considering the total C budget of a soil when investigating substrate effects on C in stabilized SOM.

We also identified many gaps in research and knowledge. For example, we found that climate variables, such as temperature and precipitation, have a substantial influence on microbial- and plant-derived compounds in stabilized SOM. However, robust conclusions are difficult to draw, given the difficulty to distinguish direct (e.g., the short-to mid-term response of a soil to changed temperature or precipitation) and indirect effects of climate variables (e.g., the long-term adaption of a soil to changed organic matter inputs as a consequence of a shifted vegetation). Long-term observational studies combined with climate manipulations may overcome this shortcoming. Moreover, most of the studies we found were limited to the upper 30 cm of the soil profile. The contribution of microbial- and plant-derived compounds to stabilized SOM in deeper soil layers remains widely unknown, even though deep soils can play a decisive role in C sequestration. Another field requiring further study refers to the role of soil fauna in directly or indirectly altering the composition of stabilized SOM. The available literature indicates potentially strong effects of soil fauna on microbial- and plant-derived compounds in stabilized SOM, but the number of relevant studies is stunningly low. Finally, more studies concurrently investigating microbial- and plant-derived compounds in stabilized SOM, along with potential influencing factors (e.g., climate, plant community composition, soil properties, soil pH and texture, etc.)

are crucially needed to further our knowledge on the formation and persistence of soil C in a changing environment. We suggest that crucial knowledge gaps would be addressed most directly by studies that: *i*) quantify amino sugars in aggregates and mineral fractions of non-cultivated soils, with further calculation of ‘microbial necromass C’ and its inverse, ‘plant-derived C’ (as hypothesized herein), *ii*) simultaneously extract and quantify plant and microbial ‘biomarkers’, including monomers of lignin, cutin, and suberin, after mineral dissolution, allowing for quantification of previously ‘hidden’ plant-derived C. Such biomarker approaches should ideally be integrated into a more holistic strategy which uses multiple, complementary methods to avoid myopic interpretations of SOM composition. The most informative studies might integrate quantification of biomarkers in soil with measures of microbial community composition, the biochemical composition of plant litter, isotopic approaches to trace the fate of C inputs, and/or spatially-explicit spectroscopic analyses (e.g., NanoSIMS or NEXAFS spectroscopy). Such integrated approaches may also partly alleviate the uncertainties associated with indirect estimates of microbial necromass and plant-derived C. Moreover, a more collaborative and cohesive approach using long-term research sites and monitoring of sites (with variable ecosystem properties) is needed, along with an emphasis on understudied ecosystems. Given the importance of SOM and the evolving understanding of its role in regulating food security, responses to global environmental change, and regulation of pollutants in the environment, a concerted effort and commonly accepted approaches should be developed and used consistently in SOM studies. As we have identified, more and better data are required to comprehensively test drivers of (stabilized) SOM composition and turnover. As such, it is

imperative that the scientific community develop accepted and standardized approaches that will collectively improve the fundamental understanding of SOM composition and the mechanisms that govern its stabilization and destabilization in various ecosystems.

#### Author contributions

G.A. conceived the study and wrote the first draft, K.E.M. helped with statistical analyses and with revision of all manuscript drafts, K.G.J. N and M.J.S. contributed to the final draft of the manuscript.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2021.108189>.

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