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Abstract

1. As soils store more carbon (C) than the Earth's atmosphere and terrestrial biomass together, the balance between soil C uptake in the form of soil organic matter and release as CO_2 upon its decomposition is a critical determinant in the global C cycle regulating our planet's climate. Although plant litter is the predominant source of C fuelling both soil C build-up and losses, the issue of how litter chemistry influences this balance remains unresolved.
2. As a contribution to solving that issue, we traced the fate of C during near-complete decomposition of ^{13}C-labelled leaf and root litters from 12 plant species in a coarse-textured soil. We separated the soil organic carbon (SOC) into mineral-associated organic matter (MAOM) and particulate organic matter (POM) pools, and investigated how 14 litter chemical traits affected novel SOC formation and native SOC mineralization (i.e., the priming effect) in these soil fractions.
3. We observed an overall net increase in SOC due to the addition of litter, which was stronger for root than for leaf litters. The presumed stable MAOM-C pool underwent both substantial stabilisation and mineralization, whereas the presumably less stable POM-C pool showed substantial stabilisation and reduced mineralization. Overall, the initial increase in soil C mineralization was fully counterbalanced by a later decrease in native soil C mineralization. POM-C formation as well as MAOM-C formation and mineralization were positively related to the initial litter lignin concentration and negatively to that of the nitrogen leachates, whereas the opposite was observed for POM-C mineralization.
4. *Synthesis*. Our results highlight the importance of litter chemical traits for SOC formation, and stabilisation, destabilisation, and mineralization. In our coarse-textured soil, the amount of MAOM-C did not change despite large C fluxes through this pool.

The litter chemical traits that drove these processes differed from those frequently reported for fine-textured soils far from mineral-associated C saturation. To account for these discrepancies, we propose an integrative perspective in which litter quality and soil texture interactively control soil C fluxes by modulating several SOC stabilisation and destabilisation mechanisms. Irrespective, our results open new critical perspectives for managing soil C pools globally.

1. Introduction

Predominantly fuelled by plant litter, soils store more than twice the amount of carbon (C) contained in the Earth's atmosphere and terrestrial biomass together (Lehmann & Kleber 2015). By decomposing litters, soil organisms drive the formation of new soil organic matter (Cotrufo *et al.* 2013; Lehmann & Kleber 2015) (SOM) and the mineralization of litter C and subsequent CO_2 release into the atmosphere (Kuzyakov 2010; Perveen *et al.* 2019). Stimulated by fresh litter, decomposer communities may also accelerate native soil organic C (SOC) decomposition (priming effect; Kuzyakov 2010; Liang *et al.* 2018; Perveen *et al.* 2019). In combination, these processes contribute to regulate the Earth's climate as well as to the capacity of soils to support ecosystem functioning and provide ecosystem services (Lehmann & Kleber 2015; Lavallee *et al.* 2020).

It was supposed for some time that the inherent chemical properties of substrates added to soils determined their persistence. Over the last few decades, though, the view has emerged that the rate at which organic matter (OM) decomposes depends on its accessibility to decomposer organisms and the physicochemical environment in which they operate (Schmidt *et al.* 2011; Lehmann & Kleber, 2015). Substrates that can be rapidly decomposed (typically referred to as 'labile') may nevertheless persist for long a long time in soils when not accessible to decomposers, and inversely, substrates previously believed to resist decomposition (i.e.,

'recalcitrant') have been shown to decompose fairly well under appropriate conditions (Klotzbücher *et al.*, 2011, 2016; Marschner *et al.* 2008).

Long-term SOC sequestration, for centuries and more (Lavallee *et al.* 2020), occurs as plant litter and/or microbial decomposition products (hereafter 'biopolymers') associate with soil minerals forming mineral-associated organic matter (MAOM; von Lützow *et al.* 2006; Kleber *et al.* 2015). Organic matter in this fraction is protected from microbial degradation via several mechanisms, including its sorption onto mineral surfaces (von Lützow *et al.* 2006; Kleber *et al.* 2015), inclusion in nanometre- to micrometre-sized micro-aggregates (Chenu & Plante 2006; Hatton *et al.* 2012), and co-precipitation with metal (Fe and Al) oxides (von Lützow *et al.* 2006; Kleber *et al.* 2015). Carbon stabilisation in the MAOM fraction is limited, however, by soil texture, that is, the amount of fine particles (clay + fine silt; i.e., < 20 μm) that provide mineral surfaces for OM sorption, and function as nuclei for novel micro-aggregate formation (Lehmann *et al.* 2007; Totsche *et al.* 2018). Extensive OM occupation of reactive mineral sites brings this soil fraction close to its theoretical maximum C storage capacity, which Hassink (1997) refer to as the maximum capacity to preserve C. In view of inherent limitations when exclusively focusing on fine particles to quantify a soil's capacity to sequester C (see Stewart *et al.* 2009; Matus 2021), we hereafter refer to mineral-associated (rather than "soil") C saturation when considering the theoretical maximum C storage capacity related to a soil's fine particle fraction.

In contrast, the C contained in the particulate organic matter (POM), spanning a continuum of physically uncomplexed, partly decomposed litters to recalcitrant and mineral-free compounds, has shorter residence times, but still builds up over decades when physically protected from microbial degradation, for instance by occlusion in larger aggregate structures (Gregorich *et al.* 2006). For this fraction, no clear limits on C storage have yet been identified (Lavallee *et al.* 2020). Therefore, despite widespread focus on long-term MAOM-C

sequestration and its potential in view of climate change mitigation, a more integrative 'whole-soil' (MAOM + POM) approach to C storage is urgently needed (Barré *et al.* 2017).

The recently developed framework of SOM formation and stabilisation (Microbial Efficiency-Matrix stabilisation, MEMS; Cotrufo *et al.* 2013) holds that litters with high contents of labile (i.e., easily degradable) compounds enhance SOM formation more than those rich in recalcitrant compounds as the former are more efficiently converted to microbial biomass and metabolic products, which are believed to be the principal SOM precursors (Miltner *et al.* 2012; Ludwig *et al.* 2015; but see Angst *et al.* 2021). This superior efficiency is of particular relevance for SOM accumulation when the stabilisation capacity of the soil matrix is high. Yet, observations that roots contribute disproportionally to SOC stocks (Rasse *et al.* 2005; Kätterer *et al.* 2011; Clemmensen *et al.* 2013), despite their higher recalcitrance relative to leaf litters, raises some questions. Whether this high contribution of roots provides a challenge to the MEMS framework or, alternatively, points to other mechanisms enhancing root-derived SOC stocks, can be debated. For instance, predominantly labile root exudates (Lavallee *et al.* 2018; Berhongaray *et al.* 2019; Sokol *et al.* 2019), as well as root-associated fungi (Clemmensen *et al.* 2013), may favour the formation of root-derived SOC relative to leaf-derived SOC. The immediate proximity of root litters and root exudates to soil minerals has also been suggested to favour the rapid stabilisation of root decomposition products (Lavallee *et al.* 2018); but see Rasse *et al.* 2005). Laboratory studies incubating dead litters, thus excluding root exudation and litter-soil proximity as potential explanations, have found no effect of litter type (leaves *vs.* roots) on SOC (Steffens *et al.* 2015), nor enhanced incorporation of litter C in the SOC for root compared to leaf litters (Hu *et al.* 2016). These latter observations, then, raise questions about the microbial efficiency pillar of the MEMS framework.

Apart from playing a central role in SOC formation, the input of fresh OM can also stimulate native SOC decomposition (positive 'priming effect') by alleviating energy

constraints on microbial activity and biomass (Kuzyakov 2010; Fontaine *et al.* 2011). Native SOC mineralization is often reduced when nitrogen (N) is in sufficient supply as this may reduce the need for N mining (Fontaine *et al.* 2011; Chen *et al.* 2014). While priming has been studied extensively, its persistence up to complete decomposition of OM inputs—a recent meta-analysis reported that few studies only lasted longer than half a year (Luo *et al.* 2016), its control by litter quality, as well as its significance for different SOC fractions (e.g. MAOM-C *vs.* POM-C) are poorly understood.

Current theory holds that OM persists in soils as long as it is shielded from microbial attack. As a corollary, the protective capacity of organo-mineral complexes against microbial degradation of OM should reduce priming in the MAOM fraction relative to the POM fraction. Consistent with this expectation, in a grassland transect study, Chen *et al.* (2019) found SOM stabilisation through organo-mineral interactions to significantly reduce the priming effect. Specifically, priming was inversely related to the soil's capacity to provide C protection (high molar Fe/Al oxides and exchangeable Ca to SOC ratios) and occlusion in micro-aggregates (see Rasmussen *et al.*, 2007 for corresponding results). High clay + silt content also reduced C priming. In contrast, priming scaled positively with the proportion of C in macro-aggregates (see Tian *et al.*, 2016 for similar findings of priming in distinct aggregate size classes). The protective capacity of organo-mineral complexes appears limited, though. No protection provided by the soils' Fe and Al oxides nor by its clay content appeared in a large-scale study by Perveen *et al.* (2019) including 35 contrasting soils. In addition, root exudates are able to destabilize MAOM (Keiluweit *et al.*, 2015), both via the direct (acid-driven) destabilisation of organo-mineral complexes, as well as indirectly via enhanced (microbial) enzyme secretion (Jilling *et al.*, 2021). In combination, these results suggest that OM in organo-mineral associations may indeed provide SOC with some protection against microbial attack, but also indicate that not all OM in the MAOM fraction is irreversibly bound.

Here, we traced the fate of ^{13}C-enriched leaf and root litters from 12 herbaceous plant species, i.e. a total of 24 litters of contrasting chemistry (Fig. S1) until near-complete disappearance of litter residues with the aim to test the overall effect of litter input and chemistry on SOM (*i.e.*, POM and MAOM) formation, stabilisation and destabilisation by incubating them in a sandy-loam soil near mineral-associated C saturation. We expected (i) new SOC to mainly accumulate in the POM fraction (Stewart *et al.* 2009; Castellano *et al.* 2015), and this accumulation to be primarily driven by recalcitrant plant compounds, in particular lignin (Cotrufo *et al.* 2015). We expected novel MAOM formation, in contrast, to be limited and driven by labile plant compounds (Cotrufo *et al.*, 2013, 2015). As a corollary, we expected more novel SOC formation (through POM-C) for root than for leaf litters. We further expected (ii) to observe a positive priming effect on the whole soil level (Kuzyakov, 2010; Perveen *et al.* 2019), but less so for litters with high initial N and N leachate content, as this may reduce the need for N mining (Fontaine *et al.* 2011; Chen *et al.* 2014). In contrast, as priming occurs through the alleviation of microbial energy constraints, easily degradable C sources, in particular C leachates, were expected to enhance priming in the early decomposition phase. As organo-mineral complexes may protect OM from SOM decomposers to some extent, we expected to observe priming mainly in the POM fraction.

2. Material and Methods

2.1 Production of ^{13}C - ^{15}N labelled plant material

Twelve herbaceous species (*Bituminaria bituminosa, Brachypodium phoenicoides, Bromus erectus, Bromus madritensis, Clinopodium nepeta, Crepis foetida, Dactylis glomerata, Daucus carota, Medicago minima, Picris hieracioides, Tordylium maximum, and Trifolium angustifolium*), representative of southern France Mediterranean old-field succession, were selected on the basis of contrasting life histories (five annuals, two biennials and five

perennials), taxonomic groups (Poaceae, Fabaceae, Lamiaceae, Apiaceae and Asteraceae) and functional traits both above- and below-ground (Fig. S1; Birouste *et al.* 2012). Seeds from these species were collected in August 2014 and set to germinate in September for three weeks. Seedlings were then transplanted to pots filled with a nitrogen-poor soil containing <1 g kg^{-1} inorganic carbon (the same soil as used for litter incubation, as described below) at a density of 200 individuals per m^2. Pots were placed in an air-tight glasshouse under natural light conditions with automated air-cooling (temperature allowed to fluctuate between 15 and 28°C), irrigation, air humidity control, and CO_2 injection. Plants were grown under a $^{13}CO_2$-labelled atmosphere for 5 months by automated injection of 4 at% ^{13}C-CO_2 each time the chamber CO_2 concentration fell below 440 ppm. Soil released ^{12}C-CO_2 was responsible for temporal variation in the chamber ^{13}C-CO_2 concentration, with accumulation of ^{12}C-CO_2 inversely correlated with CO_2 fixation by plants and the intensity of photosynthesis. Measures made at mid-experiment indicated that the ^{13}C-CO_2 concentration in the chamber varied from 1.2 at% at dawn at the peak of overnight soil CO_2 accumulation to 1.4 at% in early afternoon at the peak of plant CO_2 fixation, providing a fairly constant concentration of ^{13}C-CO_2 of 1.27 at% (±0.05 SE) along the day. Pots underwent monthly addition of a ^{15}N solution (50/50 nitrate/ammonium in the form of 10 at% ^{15}N $Ca(^{15}NO_3)_2Na$ and $^{15}NH_4Cl$) in increasing amounts from 2 to 8 g N m^{-2} for a total of 18 g N m^{-2}. During the last month, irrigation was reduced step-by-step to induce plant senescence. None of the plant species had started to produce reproductive organs. At the end of the senescence period, plants were harvested and sorted into leaf, stem, tap roots and fine-roots (i.e., all roots except tap roots, corresponding here to roots of the three most distal orders). Root samples were carefully cleaned with water before sorting. All material was air-dried at 25-30°C. Leaf and fine-root material was subsequently considered as litter and used in trait analyses and decomposition experiments.

Leaf and fine-root samples were measured for ^{13}C and ^{15}N concentrations on a PDZ Europa

ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Three subsamples were used to assess the homogeneity of the labelling among leaves of the same species and fine roots of the same species. Although this varied among species, leaf litter showed an average enrichment of 2.56 at% ^{13}C (ranging from 2.19 to 2.82) and 6.49 at% ^{15}N (ranging from 5.00 to 8.32) and fine-root litter showed an average enrichment of 2.50 at% ^{13}C (ranging from 2.35 to 2.64) and 6.32 at% ^{15}N (ranging from 4.80 to 7.60).

2.2 Litter trait measurements

A set of 14 chemical traits was measured on the leaf and fine-root litter subsamples from our 12 species (Fig. S1). This included the concentrations of carbon (C) and five nutrients, nitrogen (N), phosphorus (P), Magnesium (Mg), Calcium (Ca) and Manganese (Mn), the concentrations of water-soluble compounds, hemicellulose, cellulose, lignin and condensed tannins, and the concentrations of C, N and P in litter leachates. Specifically, C and N were measured on a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). P, Mg, Ca and Mn were measured, after acid mineralization, by plasma emission spectrometry (ICP-MS, Thermo Scientific iCAP Q, Thermo Fisher Scientific GmbH, Germany). The concentrations of water-soluble compounds, hemicellulose, cellulose and lignin were obtained by the van Soest method (van Soest 1963) with a Fibersac 24 fibre analyser (Ankom, Macedon, NJ, USA). Condensed tannins were measured according to the acid butanol method (Waterman & Mole 1994; Coq *et al.* 2010). Litter leachates were obtained by extracting 0.5 g of litter (air-dried and cut in 1 cm long pieces) in 30 mL of distilled water for 30 min on an end-over-end shaker (20 rpm at 20 °C), then filtered at 0.45 µm. Total C, N concentrations in the extracts were analysed using an automated TOC-TN analyser (Shimadzu, TOC-Vcph, Japan). Total P concentration was determined

colorimetrically after digestion with sulfuric acid and hydrogen peroxide (35 min at 100°C and 2 h at 360°C) by the molybdenum blue method, with an autoanalyser (Evolution II, Alliance Instrument, Frépillon, France). Litter leachates correspond to easily-accessible compounds at early stages of the litter incubation and can influence the microbial decomposer community stoichiometry and nutrient limitation (Fanin *et al.* 2013).

2.3 Litter incubation experiment

The soil used for litter incubation, excavated in Villefort (France; 44°43'N, 3°92'E), was a brunisol developed on a schist parent material (https://www.geoportail.gouv.fr/donnees/carte-des-sols). The soil was sieved at 5 mm, spread in trays, and humidified to induce germination of the seed bank. After two weeks, the germinated seeds were manually removed and the soil was air-dried until constant mass, then sieved at 2 mm and homogenized. Sets of subsamples were used for soil physicochemical characterization and to estimate soil residual humidity (oven-dried at 105°C) and soil field capacity. The soil had a pH of 7 and a sandy-loam texture [9% clay (<2 µm), 17% fine silt (2-20 µm), 9% coarse silt (20-50 µm), 12% fine sand (50-200 µm) and 53% coarse sand (200-2000 µm)]. The soil had C and N concentrations of 15 g kg^{-1} and 1.1 g kg^{-1}, respectively, a P availability of 0.035 g kg^{-1} Olsen-P and a cation exchange capacity of 7.75 cmol kg^{-1}. Total soil C was considered equivalent to SOC since the soil did not contain significant amount of inorganic carbon.

Hassink (1997) proposed that the theoretical value of C saturation of a soil can be calculated as: 4.09 + 0.37 (% fine silt + clay). For the soil used in this study, the theoretical value for C saturation is therefore 13.7 g C kg^{-1}. This figure is, theoretically, the maximum amount of stable SOC (SSOC) that can be retained by organo-mineral interactions with fine silt and clay particles, referred hereafter as the (theoretical) mineral-associated C saturation. In our coarse-textured soil, the (initial) C in the MAOM fraction was 12.9 g C kg^{-1} (with minimal and maximal

values of 11.9 and 14.0 g C kg^{-1}, respectively) representing 86% ± 7% of total SOC, a value close to that of Angers *et al.* (2011), who estimated that the actual SSOC concentration of a soil represents ~85% of total SOC . Considering the theoretical value for C saturation of our soil calculated above, the soil used in this study has reached: [1 – (13.7 – 12.9)/13.7]*100 = 94.2% of its theoretical C saturation level (with minimal and maximal values of 86.5% and 101.8%, respectively). The C saturation level of our soil surpassed 90% whatever the calculation method used (see text S1).

Leaf litter and fine-root litter from each of the 12 species were cut in 1 cm^2 and 1 cm long pieces, respectively, thoroughly homogenized and 12 samples of 0.4 g were weighed for each of the 24 litter materials. Each of these samples was thoroughly mixed with 50 g of air-dried, 2 mm sieved soil and transferred to a 80 mL jar in two steps to allow the placement of a polyester mixed bed ionic resin capsule (PST1; Unibest, Bozeman, Montana, USA) in the centre of the jar. Fifteen jars of bare soil were prepared in the same way. This resulted in 303 jars, representing three replicates of four sequential harvests of 24 types of soil-litter mixtures and one bare soil. At the start of the experiment, soils were brought to 80% field capacity using distilled water and jars were closed with pierced lids allowing gas exchanges. Soil humidity decreased only moderately during the course of the incubation. The microcosms were kept in a dark chamber at 25°C for the whole duration of the experiment. Three replicates of control soil without litter input were harvested to measure soil C and ^{13}C at% concentration and conduct soil fractionation. Then, three replicates of each type of jar were harvested at four time steps, that is, after 10, 38, 157, and 367 days. Upon harvest the content of jars was spread on plates to dry at a temperature of 25°C. Subsamples of soil (including litter fragments) were analysed for C concentrations and ^{13}C at% on a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Litter decomposition dynamics were assessed on a 10 g subsample of incubated soil-litter mixture by

manually sorting litter pieces remaining in the soil. Litter samples were ground with a ball mill and measured for C and N concentrations using an elemental analyser (CHN model EA1108; Carlo Erba Instruments, Milan, Italy).

2.4 Soil fractionation

Both control soils and soils in which litter had decomposed for one year were fractionated. No litter fragments were taken from the latter soils prior to fractionation. As at that moment on average 97% of the initial litter C had decomposed, all remaining litter residues must have undergone strong decomposition, and should be considered as POM. Soil samples (6 g) were fractionated via a physical fractionation procedure as described in detail in Golchin *et al.* (1994). Briefly, soils were separated into a light particulate organic matter fraction (POM; <1.6 g cm^{-3}) and a dense mineral-associated organic matter fraction (MAOM; >1.6 g cm^{-3}) (Lavallee *et al.* 2020). The POM was isolated in two steps. First, soils were suspended in 25 mL of a sodium iodide (NaI) solution with a density of 1.6 g cm^{-3} within a 50 mL polypropylene centrifugation tube (Sarstedt AG & Co, Nümbrecht, Germany). The tubes were gently shaken manually 20 times with an end-over-end movement and centrifuged during 35 minutes at 8000 RPM. The free light fraction floating on top of the NaI solution was then aspirated with a filtration unit and rinsed with 200 mL of distilled water on a 0.45 µm filter. The remaining soil was resuspended with ~25 mL NaI and 12 glass beads (6 mm diametre) were added into the tubes. The latter were then capped, shaken for 1 h on a reciprocal shaker to destroy macro- and large micro-aggregates, and centrifuged 35 minutes at 8000 RPM and to liberate the occluded organic matter. The light fraction floating on top of the NaI solution was recovered and rinsed with 200 mL of distilled water on a 0.45 µm filter. Following the POM removal, the residual soil remaining at the bottom of the tube was resuspended with ~30 mL of distilled water to rinse the remaining dense fraction (i.e., MAOM). The tubes were capped again, vigorously shaken

manually for 1 minute to fully resuspend the residue, and centrifuged 15 minutes at 8000 RPM after which the water was removed with the aspiration unit. The rinsing procedure was repeated twice for a total of three rinsings. The cleaned fractions were oven-dried at 60°C. The dried weight of the POM and MAOM fractions were measured and their C concentrations and ^{13}C at% were analysed on a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

2.5 Community level physiological profiles

The community level physiological profile (CLPP) of the soil microbial community was assessed on approximately 22 g of air-dried soil from all harvested microcosms using the MicroResp™ system (Macaulay Scientific Consulting, Aberdeen, UK (Campbell *et al.* 2008). These soil sub-samples were homogenised and checked in order to ensure an even distribution of remaining litter fragments within MicroResp DeepWell Microplates. CLPP can be used to derive information on functional or metabolic diversity and to indicate shifts in microbial community structure and functioning (Bending *et al.* 2002). About 0.45 g of air-dried soil including remaining litter fragments was incubated in triplicate in 96-DeepWell Microplates (Fisher Scientific E39199) together with a solution containing 1.5 mg C of ^{15}C substrates (except for the low-soluble phenolic acids and cellulose, for which 0.75 mg C g^{-1} soil was added), so as to reach 80 % of field capacity. This was repeated for three C substrates, namely the caffeic, syringic and vanillic phenolic acids that correspond to lignin sub-units. Gel detection plates were prepared as recommended by the manufacturer with 1 % Oxoid Agar, 12.5 μg ml^{-1} Cresol red, 150 mM KCl and 2.5 mM NaHCO3. After an initial 2 h pre-incubation step at 25°C in the dark to account for the lag period, each microplate was covered with a detection plate using a silicone gasket (MicroResp™, Aberdeen, UK). The assembly was secured with a clamp and incubated for four additional hours. Optical density at 590 nm

(OD590) was measured in detection wells before and after incubation using a Victor 1420 Multilabel Counter (Perkin Elmer, Massachusetts, USA). Final OD590 were normalized using pre-incubation OD and converted to substrate induced respiration (SIR) rates expressed in μg $C\text{-}CO_2$ g^{-1} air-dried soil h^{-1}. The mean values for the triplicate wells were used for further data analyses.

2.6 Calculations

Decomposition rate constants (k) and limit values (A) were calculated from the equation:

$$LMR = A + (1 - A) \times e^{-kt} \quad (1)$$

where LMR is the litter mass remaining and t is the time (days).

The amount of C in the initial litter (hereafter 'initial litter C' (g)) was calculated as litter mass (0.4 g) multiplied by the initial litter C concentration, and varied between litter types and species (see Fig.S1).

The amount of C remaining in the decomposing litter (hereafter 'remaining litter C' (g)) at time t_n was calculated as the product of the litter mass remaining and its C concentration at harvest t_n.

Litter C loss at time t_n was expressed as initial litter C minus remaining litter C at harvest t_n.

The total litter-derived C remaining in the microcosm (hereafter 'litter-derived C' (g), i.e., remaining litter C + litter-derived SOC) at time t_n was assessed using the isotopic mixing model:

$$\text{Litter-derived C (g)} = (\delta_{treat} - \delta\delta_{cont})/(\delta_{litter} - \delta_{cont}) \times [\text{microcosm C}] \times MM \quad (2)$$

where $\delta\delta_{treat}$ and δ_{cont} represent the $\delta^{13}C$ of the microcosm (mix of soil and remaining litter) under litter treatment and in the control soil at harvest t_n, respectively. $\delta\delta_{litter}$ represents the $\delta^{13}C$ of the initial litter. [microcosm C] represents the C concentration in the microcosm (mix of soil and remaining litter) at harvest t_n (g g^{-1}) and MM is the initial dry mass (50 g) of soil in the

microcosm that was assumed to remain fairly constant during the incubation (<1% difference across microcosms and time if considering litter addition and C loss via respiration).

The amount of C in microcosm soil that originated from initial litter C (hereafter 'litter-derived SOC' (g)) at time t_n was calculated by subtracting remaining litter C (g) at harvest t_n from litter-derived C (g) at harvest t_n.

The amount of litter C respired from the microcosm (hereafter 'respired litter C' (g)) at time t_n was obtained by subtracting remaining litter C (g) at harvest t_n and litter-derived SOC (g) at harvest t_n from initial litter C (g).

The amount of litter C decomposed in the microcosm (hereafter 'decomposed litter C' (g)) at time t_n was obtained by subtracting the remaining litter C (g) at harvest t_n from the remaining litter C (g) at harvest t_{n-1}. The litter C loss and the litter C decomposed at any time t_n only differ in terms of time period assessed, which is relative to t_0 and t_{n-1}, respectively.

The rates of litter C loss, respired litter C and litter-derived SOC between two consecutive harvests were calculated by estimating the difference between harvest t_n and t_{n-1} and expressing it per day of the period $t_n - t_{n-1}$.

An estimate of C transfer efficiency (TE) from litter to soil across the entire incubation year was calculated as TE = 1 - (litter respired C / litter C loss), where the ratio represents the proportion of litter C respired as a fraction of the litter C loss (see also Fig. S6).

The amount of native C remaining in the microcosm soil (hereafter 'native SOC' (g)) at time t_n was calculated as:

Native SOC (g) = [microcosm C] × MM - remaining litter C – litter-derived SOC (3)

where [microcosm C] represents the C concentration in the microcosm (mix of soil and remaining litter) at harvest t_n (g g^{-1}), MM is the initial dry mass (50 g) of soil in the microcosm and remaining litter C and litter-derived SOC are measured at harvest t_n. Note

that at t_0, the term "remaining litter C - litter-derived SOC" equates the initial litter C.

Priming refers to changes in the mineralization of native soil C driven by fresh C supply (Kuzyakov 2010). The amount of primed C, that is, the amount of native SOC decomposed following fresh organic matter addition relative to the amount without such addition, at time t_n was calculated as the native SOC at harvest t_n in the control microcosms that had not received litter, minus the amount of native C remaining at harvest t_n in the microcosms where we added litter:

$$\text{Primed C (g)} = \text{native SOC}_{\text{cont}} - \text{native SOC}_{\text{treat}} \quad (4)$$

with positive values indicating higher soil C loss with litter addition. The primed POM-C and primed MAOM-C were calculated in the same way, by replacing whole-soil native SOC_{cont} and native SOC_{treat} by their POM and MAOM fractions, respectively.

The net C balance (mg C g^{-1} soil) was calculated as the litter-derived SOC minus the primed C. For the POM and MAOM fractions, the net C balance was calculated as the POM-C minus primed POM-C and MAOM-C minus primed MAOM-C, respectively. For these calculations, litter-derived SOC, POM-C, MAOM-C, primed C, primed POM-C, and primed MAOM-C were expressed as mg C g^{-1} soil.

In order to provide scaled numbers more appropriate to the comparison with other studies, all of the indices described above were expressed per initial C content of native soil or per initial litter C content.

2.7 Statistical analysis

All statistics were computed using R version 3.6.3 (www.r-project.org). We used model averaging (using the 'MuMin' package version 1.43.15) to assess the influence of the predictors (i.e., chemical traits) on the independent variables. For each independent variable, the set of predictors was reduced based on a priori knowledge of the role of chemical traits in soil

processes (section *Litter chemical trait predictors*). We performed model averaging on the set of models whose AICc values were similar to that of the best model (i.e., $\Delta AICc \leq 3$) to avoid unnecessarily restricting the number of potentially informative models yet keep their number lower than that of the number of predictors. We verified the robustness of our reported results by also running all analyses for $\Delta AICc \leq 2$, which hardly affected the results. Problems of collinearity were minimized by omitting predictors from the analysis when Pearson correlations were larger than 0.7 (Dormann *et al.* 2013) (Table S5). All predictors were centred and divided by their standard deviation prior to the linear modelling. Differences between leaf and root litters were assessed using pair-wise *t*-tests.

2.8 Litter chemical trait predictors

Litter chemical traits were used to predict observed patterns allowing generalisation beyond species identity effects. Litter type (*i.e.*, leaf versus root litters) was not included as predictor in the linear models we constructed as it co-varied with the majority of litter chemical traits (see Fig. S1). Total C concentration was omitted as predictor as its variation among species was approximately an order of magnitude lower than that of all other chemical traits (see Fig. S1). Predictors were chosen based on reported effects in the literature on all our dependent variables. In order to limit the number of model parameters, ratios and interactions between chemical traits were also omitted. Retained predictors were as follows:

Decomposition rate: cellulose (López-Mondéjar *et al.* 2016; Bhatnagar *et al.* 2018), hemicellulose (López-Mondéjar *et al.* 2016), lignin (Hobbie *et al.* 2006; Klotzbücher *et al.* 2011; Stewart *et al.* 2015), tannins (Coq *et al.* 2010), water-soluble compounds (WSC), containing (among others) compounds that are easily assimilated, and the nutrients nitrogen (N) (Zhang *et al.* 2008; Talbot & Treseder 2012; Ochoa-Hueso *et al.* 2020), phosphorus (P) (Cornelissen & Thompson 1997; Ochoa-Hueso *et al.* 2020) and their leachates (as well C

leachates), as well as calcium (Ca) (Hobbie *et al.* 2006; Zhang *et al.* 2008), magnesium (Mg), and manganese (Mn) (Ochoa-Hueso *et al.* 2020). The same predictors were used for the decomposed litter-C and the respired litter-C.

Priming: priming is sensitive to the quality of the fresh organic matter added (Di Lonardo *et al.* 2017; Shahbaz *et al.* 2017; Lyu *et al.* 2019), and diminishes with increasing soil nutrient availability, including N, P, Ca, and Mg (Liu *et al.* 2018). To test for the effect of C quality, we included hemicellulose and cellulose, lignin, tannins, and C leachates. To test for the effect of nutrient availability, N and P and their leachates, as well as Ca, Mg and Mn were further retained as predictors. Identical predictors were retained for whole-soil priming as well as that of the particulate organic matter (POM) and mineral-associated organic matter (MAOM) fraction.

Soil organic matter (SOM) formation: recent conceptual advances suggest that high quality litters enhance efficient soil organic matter (SOM) formation relative to low quality litters containing relatively high concentrations of recalcitrant compounds, particularly when the soil mineral matrix has a high organic matter stabilisation capacity (Cotrufo *et al.* 2013), but see (Castellano *et al.* 2015; Huang *et al.* 2019). C as well as the nutrients N, P, Ca, Mg, and Mn (as well as others not presently assessed) co-limit microbial decomposition and mediate the activity of certain exoenzymes (Ochoa-Hueso *et al.* 2020). Further, Ca^{2+}, Mg^{2+}, and Mn^{2+} cations may be involved in SOM stabilisation via polyvalent cation bridging (von Lützow *et al.* 2006; Wiesmeier *et al.* 2019). For the litter-derived SOC, the POM-C and the MAOM-C, we included the same predictors as for decomposition rate (above). The same predictors were also used to model net-C balance.

3. Results

3.1 Litter C decomposition and respiration

An average of 97% (±2.5 SE) of the initial litter C was decomposed after 367 days (Fig. 1a, Fig. S2), with small but highly significant (Table S2) differences between leaf and root litters, whose initial litter C was 98% (±1.7 SE) and 95% (±2.0 SE) decomposed, respectively. In the first half year (only), the graminoids decomposed more slowly than the legumes and forbs (Fig. S2), most likely due to their higher cellulose (and hemicellulose) but lower water-soluble compound concentrations (Fig. S3), which controlled the litter C decomposition in that period (Table S3). As the calculation of the remaining litter C is constrained by the difficulty of identifying remaining partially decomposed litter fragments, this number likely represents an upper bound. This limited precision notwithstanding, the estimated decomposition rates indicated that by decomposing the litters in highly favourable conditions, our one-year incubation effectively simulated multi-year decomposition in the field (Table S4). Leaf litters (k = 0.162 g g^{-1} d^{-1}) decomposed faster than root litters (k = 0.091 g g^{-1} d^{-1}; t_{11} = 3.840, P = 0.003; Table S2). Similarly, at each harvest but the first, significantly more leaf litter C than root litter C was respired (Fig.1b). Cumulatively, after one year, the amount of the initial respired litter C for leaf and root litters was 77% (±4.3 SE) and 66% (±6.6 SE), respectively. The respired litter C did not vary significantly at any moment among the three plant functional groups (all $P > 0.05$).

3.2 SOC formation

New SOC formation, representing the balance between litter-derived biopolymers entering the SOC pool and C leaving it as respired CO_2 (Fig. 1b), occurred rapidly initially but diminished subsequently (Fig. 1c). This new SOC represented a substantial part of the initial litter C, ranging from 18% to 45% after one year, and was significantly higher for root than for leaf litter (Fig. 1c, Table S2). At most harvests, less novel SOC was formed by the graminoids than by the legumes and forbs, even though this effect vanished for the leaf litters and weakened for the

root litter at the end of the year (Fig. S4). As for the decomposed litter C, variation across all litters were likely due to differences in cellulose, hemicellulose, and water-soluble compound concentrations (Fig. S3), as these controlled the formation of novel litter-derived SOC (Table S3).

Soil fractionation at near-complete litter decomposition (day 367) showed that litter-derived POM-C represented between 0.001 and 0.11 g C g^{-1} of initial litter C, and litter-derived MAOM-C between 0.16 and 0.38 g C g^{1} of initial litter C, depending on litter type, species identity, and chemical composition (Fig. 1d, Table S2). Differences among plant functional groups, though, were not significant(Fig. S5).

Litter-derived POM-C did not correlate with decomposition rate, and MAOM-C only weakly, but positively for leaf litter (Fig. 1e, f). Differences in litter-derived MAOM-C (and POM-C, albeit only across litter types; Fig. 1g) were strongly positively correlated to litter lignin concentration (Fig. 1h), but negatively to concentrations in hemicellulose and N leachates (Fig. 1i, j, Table S3).

3.3 Primed SOC

At the whole soil level, strong positive priming appeared limited to the early incubation phase before subsequently turning negative, and remaining so for root litters (Fig. 2a, Fig. S6). Cumulated over one year, the priming effect was not significant, but tended to be positive for leaf litters (0.003 g C g^{-1} initial soil C) and negative for root litters (-0.009 g C g^{-1} initial soil C, Fig. 2a, d, Table S2), with large differences between species (Fig. S6). The primed SOC did not scale (systematically) with any of the litter chemical traits measured (Table S3) nor vary significantly ($P > 0.05$) with plant functional groups (Fig. S6).

In terms of soil fractions, litter input generally induced positive priming from the MAOM fraction (0.059 g C g^{-1} initial soil C; Fig. 2c) yet negative priming from the POM fraction (-

0.062 g C g^{-1} initial soil C; Fig. 2b), with large species-specific differences in both fractions (Fig. 2bc). For comparison, over one year, the control soil lost 0.0537 g MAOM-C g^{-1} initial soil C and 0.1106 g POM-C g^{-1} initial soil C. Priming from both fractions were negatively correlated with each other (R^2 = -0.86, $P < 0.001$). The initial concentrations in litter lignin and N leachates controlled the priming of both POM-C and MAOM-C (the latter significant for N leachates only) (Fig. S7, Table S3) albeit in opposite ways; increasing concentrations of N-rich leachates decreased positive MAOM-C priming but lowered the negative POM-C priming.

3.4 Net SOC balance.

Our estimates of the SOC balance in POM and MAOM revealed strongly contrasting patterns among the 24 litters (Fig. 2), with the largest net SOC gain being about five times larger than the largest loss (Fig. 2h). For MAOM, the C gain from SOC formation was mostly cancelled out by positive priming in about half of the litters (Fig. 2c, g); consequently, the net MAOM-C balance was not significantly different from zero ($P > 0.05$). However, as the POM-C replenishment was typically larger than priming-induced C loss (Fig. 2b, f), and on average positive ($t_{23} = 5.075$, $P < 0.001$), the net effect of decomposing litter on total soil SOC remained positive ($t_{23} = 7.110$, $P < 0.001$), except for leaf litter from three species (Fig. 2h).

On average, the quantitatively realistic leaf and root litter input simulated here increased the total soil C stock by 4% and 8% (relative to its initial C content), respectively; both increases were significantly larger than zero (t_{11} = 3.593, P = 0.002 and t_{11} = 7.595, $P < 0.001$, respectively) and differed significantly from each other (Table S2). This differential effect of leaf and root litters was associated with the higher concentration in lignin and lower concentration in N leachates of root litters as compared to leaf litters (Fig. S1, Fig. S8). However, the total soil C stock did not vary significantly among functional groups for either leaf or root litters.

4. Discussion

As fresh litter deposition simultaneously drives novel SOC formation and the mineralization of native SOC, we studied both processes as well as their cumulative effect in terms of the net SOC balance as ^{13}C labelled leaf and root litters from 12 grassland species were incubated for one year. Specific to our study, our incubated soils were coarse textured and near their theoretical mineral-associated C saturation level. Out of 14 litter chemical traits, the initial litter lignin and N leachates concentrations consistently appeared as predictors for the formation of novel MAOM-C and POM-C as well as in the mineralization of native SOC in both soil fractions.

4.1 Recalcitrant litter compounds drive novel MAOM-C and POM-C formation

We expected novel SOC formation to primarily accumulate in the POM fraction and to mainly be driven by recalcitrant plant compounds, in particular lignin. Novel MAOM formation, in contrast, was expected to be limited and driven by labile plant compounds. Correspondingly, we expected root litters to enhance novel SOC formation as POM-C relative to leaf litters. These expectations were only partially confirmed. At near-complete litter decomposition (day 367), the amount of initial litter C recovered in the MAOM fraction was substantially larger than that in the POM fraction. The integration of novel C into the MAOM fraction despite it being near its theoretical mineral-associated C saturation (but see below) may indicate that the new C was adsorbed onto other organic molecules already in direct contact with mineral surfaces, forming an organo-mineral complex organized into multilayers or zonal structures (Kleber *et al.* 2007). Novel C may also have become incorporated in the MAOM fraction through entrapment in micro-aggregates (Totsche *et al.* 2018) or via co-precipitation with aluminium (Al) and iron (Fe) hydroxides (Kleber *et al.* 2015). In our low-clay soils, a considerable amount of

decomposition products may have become entrapped in newly formed small (< 53 um), but not larger, micro-aggregates (Schweizer *et al*., 2019). Co-precipitation with Al and Fe was likely moderate in our soils however, in view of the rather small molar Al/C and Fe/C ratios (Table S5) (Nierop *et al.* 2002) and the pH (= 7) of our soils (Rasmussen *et al*., 2018). In view of its low content, the contribution of exchangeable Ca (Rasmussen *et al*. 2018; Rowley *et al*. 2018) to our soil's SOC stabilisation potential was likely low, but that of short-order range minerals may have been more extensive. Our soil-texture derived theoretical C saturation may thus (moderately) underestimate our soil's true C storage potential. A substantial proportion of litter-derived C that was recovered in the almost C saturated MAOM fraction may indicate that the newly decomposed OM had competitively displaced native OM compounds in the organo-mineral complexes (Kaiser & Kalbitz 2012).

Initial litter lignin concentration drove the formation of both novel MAOM-C and POM-C. Although lignins are no longer believed to resist decomposition in the long-term (Marschner *et al.* 2008), their consistent effect indicates that they may nonetheless contribute substantially to POM-C and MAOM-C formation (Kallenbach *et al.* 2016; Huang *et al.* 2019). The 'recalcitrance' of lignins and other acid-unhydrolyzable residues select for decomposer communities with lignolytic activities (Fig. S9; Wickings *et al.* 2012). As the extracellular enzymatic repertoire of fungi surpasses that of bacteria (Datta *et al*., 2017), these communities were likely fungi dominated. This feedback induces different C recycling and anabolic processes, likely favouring fungal biomass, which is thought to contribute disproportionately to MAOM-C formation (Kleber *et al.* 2007; Miltner *et al.* 2012; Ludwig *et al.* 2015). In addition, or alternatively, lignin-derived phenols' strong sorptive affinity (Hernes *et al.* 2013) may favour their direct stabilisation (*i.e.*, without prior conversion into microbial biomass) in MAOM (Sokol *et al.* 2019). In comparison, degradation products of the more labile litter compounds may have been preferentially subject to microbial recycling, thereby augmenting

litter C respiration, and concurrently reducing the transfer to litter C to the soil, relative to lignin-rich litters (Geyer *et al.* 2016) (Fig. S10, Table S3).

In contrast to the positive influence of litter lignin concentration on MAOM-C and POM-C formation, high N leachates concentrations were associated with reduced MAOM-C and POM-C formation. Immediately, but only briefly available N-rich leachates that modify decomposer community stoichiometry and relieve nutrient limitation (Fanin *et al.* 2013) may favour lignin degradation at early stages of the litter decomposition process (Fig. S10) and thereby limit the direct stabilisation of their derived phenols.

Our results bring perspective to the hypothesized relation between litter quality and SOC formation efficiency in the MEMS framework (Cotrufo *et al.* 2013, 2015; Liang *et al.* 2018). One pillar of the MEMS framework concerns the importance of the decomposer substrate use efficiency (CUE when considering C as substrate), with efficiency being defined as the amount of microbial biomass production relative to the amount of substrate assimilated (which is partitioned into a microbial biomass production and a mineralization component). A high efficiency, as commonly observed for labile litters, implies relatively little C-CO_2 loss relative to microbial biomass production, with the latter deemed of primary importance following reports that a dominating part of stabilized SOC is of microbial origin (Mambelli *et al.* 2011; Miltner *et al.* 2012; but see Angst *et al.*, 2021). Although we did not assess the incorporation of litter C into microbial biomass, about 11% more litter C was respired from (labile) leaf litters than from (recalcitrant) root litters. Consequently, the denominator in the CUE calculation was larger for the leaf than root litters, perhaps due to enhanced microbial recycling (as suggested above). In a recent re-evaluation of existing data, Angst *et al.* (2021) found microbial necromass to constitute about 39% of the silt- and clay-sized MAOM. They also found lignin to be about twice as abundant in the silt-sized MAOM than in the clay-sized MAOM, which may help explain our results, since our soils contained about twice as much fine silt (and thrice as much

fine + coarse silt) as clay particles. At the least, our results call into question the generality of one pillar of the dually-motivated MEMS framework and are consistent with the view that the relation between litter quality and SOM formation efficiency may be inverted when the soil mineral matrix is near C saturation with reduced stabilisation capacity (Castellano *et al.* 2015). Under such conditions, often observed in surface soils, the effect of labile compounds on SOC formation may only be transient (novel SOC formation was stimulated by the nutrient-rich water-soluble compounds initially; Table S3), or largely overcome by that of recalcitrant litters at the later decomposition stages. As we neither manipulated the mineral-associated soil C saturation nor used different soils, however, it remains to be seen if and to which extent our findings are effectively related to our soil's C saturation and/or soil type, more generally.

4.2 Contrasting priming effects in the MAOM and POM fraction

We expected priming to be positive on the whole soil level, and this native SOC mineralization to be less for litters high in initial N and N leachates content (by reducing the need for N mining) and to be enhanced by easily degradable C sources in the early decomposition phase. Relative to the different soil fractions, we expected to observe priming mainly in the POM fraction but much less so in the MAOM fraction. Again, these expectations were only partially corroborated by the data. In our litter-soil incubations, on average, following the expected typical SOC over-mineralization early on (Kuzyakov 2010), the priming effect subsequently turned negative, and eventually became statistically indistinguishable from zero. These dynamics emphasize the importance of examining priming over longer periods than currently done, as it may qualitatively and quantitatively change our view on the effect of litter deposition on native SOM decomposition (see also Zhang *et al.* 2017). As our microcosm experiment simulated multi-year *in situ* decomposition outcomes, however, it remains to be investigated how these priming dynamics are altered as fresh litters are deposited to the soil before the (near complete)

decomposition of previously added litter material (Hamer & Marschner 2005; Wang *et al.* 2019). If such a novel deposition re-energizes decomposer communities, our results do not contrast the notion that priming contributes to native SOC losses in natural ecosystems (Perveen *et al.*, 2019). Alternatively, and speculatively, the observed initial positive priming may reflect the (over) mineralization and exhaustion of relatively easy degradable SOC, causing medium to long-term negative priming. In this case, repeated novel litter deposition may increasingly exhaust a soil's easily degradable SOC leading to a less positive, and maybe even vanishing priming effect. Hamer and Marschner's (2005; experiment 4) study, in that regard, suggests that both mechanisms may be operative, but in a soil-dependent manner.

In contrast to our expectation, across one year, the litter addition had instigated C over-mineralization in the MAOM fraction yet under-mineralization in the POM fraction. The loss of MAOM-C, although surprising since it is commonly considered more stable than POM-C (Lavallee *et al.* 2020), underlines its dynamic equilibrium with the soil solution (Kleber *et al.* 2007, 2015), and indicates that younger, more sorptive compounds may displace older, previously adsorbed OM (Kaiser & Kalbitz 2012). In our microcosms, this dynamic was likely enhanced by the experimentally-induced disturbances to the soils, and its significance under natural, relatively undisturbed conditions requires testing. Previous work has indicated that root systems, through their exudates, may destabilize MAOM (Keiluweit *et al.*, 2015), both through the exudates' acidity as well as by increasing microbial enzyme secretion (Jilling *et al.*, 2021). As in our microcosms root exudates were absent, it seems likely that increased enzyme secretion following litter deposition either liberated organic C from the organo-mineral complexes or led to the destabilisation of small aggregates in the MAOM fraction.

The negative correlation between the priming from the MAOM and POM fractions indicate an opposite behaviour of these fractions, as also observed with respect to their C stabilisation (Stewart *et al.* 2009), perhaps with transfer between these two fractions. Such a transfer may

occur if desorbed OM, rather than being entirely mineralized, is rapidly used by microorganisms and further protected from decomposition by occlusion into aggregates, whose formation is stimulated by increased litter-induced microbial activity (Six & Paustian 2014), particularly in surface soils where micro-sites are saturated with OM (Tisdall & Oades 1982; Angers 1998).

In contrast to our expectation, the measured initial litter traits did not explain the priming on the whole soil level. Also unexpected, the priming of POM-C decreased with initial litter lignin concentration, and enhanced by the initial litter N leachates concentration. The negative impact of the latter on the MAOM-C priming did follow our expectation. Decreasing positive MAOM-C priming with increasing N-rich leachates likely indicates a reduced microbial N-mining (Fontaine *et al.* 2011), whereas its lowering of the negative POM-C priming may imply a reduced transfer of native soil C from MAOM to POM. Importantly, the negative correlation between the primed POM-C and MOAM-C indicates that litter input may affect native SOC far more than currently highlighted by assessments of bulk SOC, at least in soils near C saturation. In such (mostly) coarse-textured soils, the dynamic nature of the equilibrium characterizing MAOM in the soil solution (Kleber *et al.* 2007, 2015) may be enhanced relative to fine-textured soils as relatively more OM is bound to the outer, weaker binding zones of the organo-mineral complexes (Kleber *et al.* 2007).

Our results suggest that failure to take into account soil type may render management strategies to enhance long-term C sequestration inadvertently counter-productive. They further indicate that potential for C storage in coarse-textured soils may lie in an efficient management of medium-term C storage in the POM pool (Barré *et al.* 2017), as its C storage capacity is less limited, if at all, than that of the long-term MAOM pool (Cotrufo *et al.* 2019; Lavallee *et al.* 2020).

4.3 Net SOC storage in soils close to C saturation is limited to the POM fraction

Despite the commonly acknowledged central role of litter in soil organic matter dynamics, surprisingly little data are available on its effects on the relative 'input-output' balance (Liang *et al.* 2018). Our expectation, in that regard, was that litter addition would enhance SOC accrual mainly through gains in the POM fraction. This expectation was largely confirmed (albeit through different pathways as anticipated; see above). In our soils close to their theoretical mineral-associated C saturation limit, the consistent C gain from SOC formation in the MAOM fraction was mostly cancelled out by positive priming, so that on average, the net balance for MAOM-C did not differ significantly from zero. In contrast, the mostly negative POM-C priming added to the POM-C formation, rendering the net POM-C, and consequently overall net SOC positive. These results confirm previous assumptions that any gain in soil C occurs as POM once the MAOM fraction is saturated with organic matter (Hassink 1997; Stewart *et al.* 2009; Castellano *et al.* 2015, Cotrufo *et al.* 2019).

The net SOC gain for root litters was twice as large as that for leaf litters, which is consistent with previous findings that most SOC is root derived (Rasse *et al.* 2005; Kätterer *et al.* 2011). Our present findings highlight that the larger contribution of belowground versus aboveground plant material to SOC holds in the absence of root exudation. In fact, as expected from the effects of litter chemistry on both SOC formation and priming, this differential effect of leaf and root litters was associated with the higher concentration in lignin and lower concentration in N leachates of root litters as compared to leaf litters (Fig. S1, Fig. S8). The absence of a plant functional-group effect on total SOC stocks after one year, despite initial differences in litter-derived SOC formation, may be due to smaller differences in these two litter traits than observed between leaf and root litters (Fig. S3). Overall, the mostly positive but variable effect of plant litters on the balance between carbon formation and destabilisation is key for our understanding of plant effects on the global C cycle and must be extended to effects of living plants, including root exudation processes.

4.4 Litter chemistry effects on soil carbon storage and priming: a potential way forward

Mineral-associated soil C saturation modifies the relation between litter quality and SOC stabilisation (Castellano *et al.* 2015), but it remains unclear how. We offer an integrative perspective to that effect linking litter quality, microbial decomposition, and a number of well-documented SOC stabilisation and destabilisation mechanisms consistent with our data (Fig. 3). Following numbers in the text, from **(1)** to **(13)** refer to processes illustrated in Figure 3. In soils far from mineral-associated C saturation (Fig. 3b), a substantial part of decomposition products associates with native organo-mineral complexes (grey dots) varying in sorption strength (the darker the grey the stronger bound) and mineral surfaces (brown lines) or, especially in acidic soils, co-precipitate with Al and/or Fe oxides (Rasmussen *et al.*, 2018) **(1)**. In neutral to alkaline soils, as in our soils, the released decomposition products more likely associate with clay and calcium (Rasmussen *et al.*, 2018; Rowley *et al.*, 2018). These decomposition products may replace some weakly bound native organic matter (light grey dots) through sorption-desorption (back-and and-forth arrows, scaled to rate (Guggenberger & Kaiser 2003; Kaiser & Kalbitz 2012; Kleber *et al.* 2015) **(2)**. As labile litter stimulates the "in vivo" pathway more effectively than recalcitrant litters (Cotrufo *et al.* 2013), most newly formed SOC is rapidly stabilized as MAOM as sufficient mineral binding sites are available. Primed C losses (CO_2 leaving the soil – red arrows) are presumably primarily of POM-C origin (unbound and aggregated grey dots) as most MAOM-C is bound **(3)**. With progressing decomposition (Fig. 3c), the proportion of recalcitrant litter compounds increases and the decomposer community becomes more fungi dominated, leading to overall more recalcitrant decomposition products (including microbial necromass; brown dots) that associate with native organo-mineral (and -metal) complexes and mineral surfaces **(4)**. These decomposition products may replace some previously bound decomposition products from the initial decomposition stage (back-and forth

arrows) that often reveal a weaker sorptive affinity (Sokol *et al.* 2019) **(5)**. At this decomposition stage, the proportion of litter-derived recalcitrant compounds may increase in the soil, favouring aggregation, and thus, increasing POM-C formation **(6)**. The rates of processes **(4)-(6)** should be greater for lignin-rich litters, which release a higher proportion of recalcitrant compounds with strong sorptive affinity (Kaiser & Zech 1997).

In soils close to mineral-associated C saturation (Fig. 3d), fewer mineral binding sites and organo-mineral (and -metal) complexes are available, leading to a rapid increase in the concentration of labile litter-derived decomposition and microbial products in the soil solution (a 'bottleneck effect'), which are ultimately mineralized **(7)**, or bind predominantly to the weaker binding outer zone of organo-mineral complexes (Kleber *et al.* 2007) **(8)**, destabilizing and replacing the weakly-bound fractions of the native MAOM **(9)**. The rates of processes **(7)** and **(8)** are likely higher for labile compared to recalcitrant litters due to their faster release and weaker sorptive affinity of the decomposition products (Sokol *et al.* 2019). Our observed enhanced respiration of litter-C of the more labile leaf litters relative to root litters (Fig. 1a) is consistent with this proposition (process **7**). In the absence of MAOM-C time series, the proposed enhanced rate of (**8**) for labile litters cannot be confirmed, but their initial faster SOC formation (Fig. 1c), driven by the easy degradable water soluble compounds (Table S3), are suggestive thereto. Process **(9)** is likely increasingly stimulated in soils approaching mineral-associated C saturation (Khandakar et al., 2021) as the ratio between weaker bound to stronger bound ('outer zone' light grey circles and 'inner zone' dark grey circles, respectively; Fig. 3d) organic matter may decrease. This leads to substantial MAOM destabilisation that can cause native SOC losses via priming **(10)**, as observed in our soils (Fig. 2c). Similar to processes occurring in soils far from mineral-associated C saturation, decomposition products of the more recalcitrant litter compounds become more abundant in the soil solution as decomposition progresses (Fig. 3e), which associate with native organo-mineral (and -metal) complexes and

mineral surfaces **(11)**, and may replace some previously bound decomposition products from labile litter compounds **(12)**, and may favour aggregation and increasing POM-C formation **(13)**. The rates of processes **(11)-(13)** are expected to be higher for lignin-rich litters (same as in soils far from mineral-associated C saturation); the positive effect of lignin (across one year) on novel POM-C and MOAM-C formation (Fig. 1g-h, Table S3) are consistent with, albeit not conclusive for, the propositions for process (**11**,**13**). However, overall, the rates of process (**12)** are likely lower than in soils far from mineral-associated C saturation, because less labile litter-derived compounds bound to organo-mineral complexes in the earlier phase of decomposition (Fig. 3d). However, POM-C formation may be enhanced **(13)**, resulting from limiting binding-sites, and thus, lower rates of process **(11)**. As most MAOM is entrapped in aggregates (Hatton *et al.* 2012), soil aggregation likely modulates the rates of **(1)-(13)** by modifying microbial decomposer access to organic matter and hence microbial community structure, abundance, and activity (Wilpiszeski *et al.* 2019), as well as through its effect on gas and liquid fluxes (Ebrahimi & Or 2016).

5. Conclusions

In the coarse-textured soil near mineral-associated C saturation we studied here, decomposing litters increased SOC concentration through two processes (Fig. 3 "synthesis", section 4.4). First, new litter-derived C was stabilized into POM and MAOM, and second, there was less native soil POM-C mineralization than in the absence of decomposing litters. Of all litter chemistry characteristics measured, lignin and N leachate concentrations were the best predictors of these two processes. Since root litter chemistry differed systematically from leaf litter chemistry, root litter contributed twice more to SOM than leaf litter on average, which corroborates earlier findings (Rasse *et al.* 2005; Kätterer *et al.* 2011). Our findings that litters have opposite effects on native soil MAOM-C and POM-C, and of common predictors of SOC

formation and priming, emphasize the potentially complex interplay between OM stabilisation and destabilisation processes and call for further integration in the study of these processes within and between C pools, and how they vary over time. Further, whereas our findings do not rule out that priming is an important cause of substantial SOC loss in natural ecosystems (Perveen *et al.* 2019), they point to a dynamic balance in the soil between co-occurring positive and negative priming effects (see section 4.2 above). Regardless, even in our soil that was likely close to its mineral-associated C saturation, the balance between SOC gains via formation and losses due to priming was predominantly positive, albeit limited to the POM fraction. Finally, the consistent key role played by lignin and soluble N-containing compounds in these processes brings perspective to the recently suggested conceptual framework of SOM formation (Cotrufo *et al.* 2013, 2015), according to which labile compounds favor SOM formation relative to recalcitrant compounds in soils with high matrix stabilisation capacity. A main reason for the diverging findings may be fundamental differences among soil types. Indeed, in our coarse-textured soils near their mineral-associated C saturation, recalcitrant lignin-rich litters rather than labile ones most efficiently drove novel SOC formation, indicating that the efficiency with which microbes decompose litter does not unequivocally translate into SOC accrual, as previously proposed for finer textured soils. As about 80% of the Earth's soils contain less than 30% clay (Shangguan *et al.* 2014), and are thus prone to mineral-associated C saturation, our findings imply that soil management strategies targeted at enhancing C storage to help mitigating climate change and promote sustained ecosystem functioning need to account explicitly for differences in soil types and should focus more specifically on medium-term C storage (Barré *et al.* 2017) and its interplay with long-term stabilisation and destabilisation processes.

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Figures and Tables

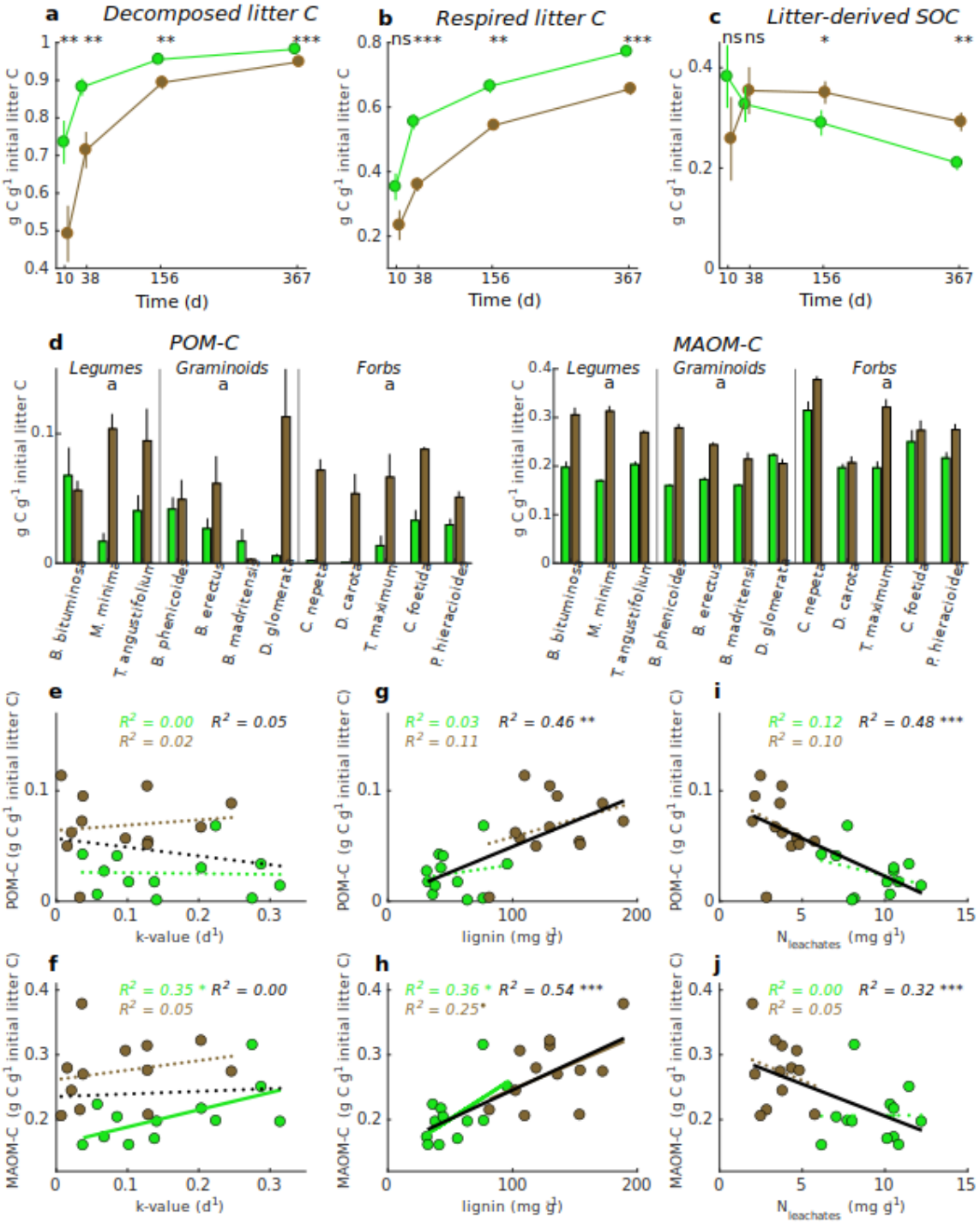

Figure 1. Litter carbon fate. The proportion of (**a**) decomposed litter C, (**b**) respired litter C, and (**c**) litter-derived soil organic carbon (SOC) for leaf and root litters (green and brown colour, respectively) across one year. The rate of litter-derived SOC formation turns negative for leaf and root litters from day 38 and 156, respectively, onward. (**d**) After one year, a

smaller proportion of the initial litter C was retrieved in the POM (left panel) than in the MAOM fraction (right panel) across all 12 plant species. Root litter contributed more strongly to both POM-C and MAOM-C than did leaf litter (Table S2), but novel C storage was independent of plant functional group for each soil fraction ($F_{2,21} = 1.090$, $P = 0.354$, $F_{2,21} = 2.256$, $P = 0.130$ for POM-C and MAOM-C, respectively, ANOVA type III). (**e**) Whereas the POM-C was independent of the litter decomposition rate k, (**f**) leaf litter-derived but not root litter-derived MAOM-C correlated significantly with decomposition rate k. (**g**) Whereas initial litter lignin concentrations drove POM-C only across litter types, (**h**) it did so both within as well as across litter types for the MAOM-C. Both (**i**) POM-C and (**j**) MAOM-C varied significantly with initial litter N leachate concentrations across but not within litter types. Error bars (in **a-d**) indicate standard errors. ".", "*", "**", and "***" represent significant leaf – root differences (**a-d**) and Pearson correlations (**e-j**) with $P < 0.10$, 0.05, 0.01, and 0.001, respectively. Solid but not dotted lines represent significant correlations (**e-j**).

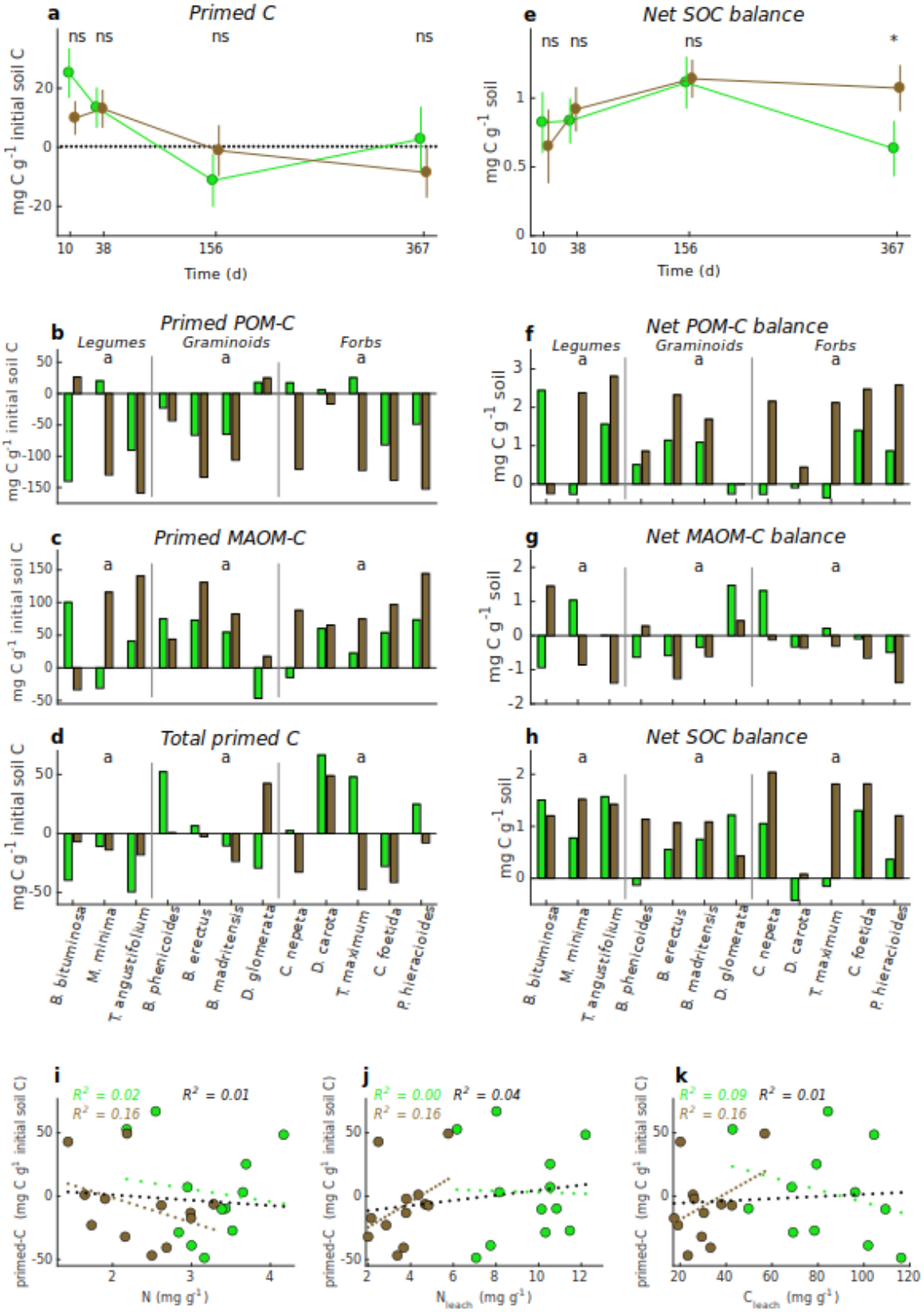

Figure 2. The priming effect and the net C balance. (**a**) Whole soil primed C for leaf and root litters (green and brown colour, respectively) over one year. Leaf – root differences never

reached significance. Error bars indicate standard errors. (**b,c,d**) Primed POM-C, primed MAOM-C, and whole soil primed C (= primed POM-C + primed MAOM-C) over one year for all 12 plant species and litter types. Primed POM-C was more negative for root litters (-89 mg C g^{-1} initial soil C) than for leaf litters (-36 mg C g^{-1} initial soil C; Table S2). No other effect of litter type or plant functional type was found (all $P > 0.05$; ANOVA type III). (**e**) Net C balance (= litter-derived SOC gains – primed SOC losses) over one year. Across SOC gains and losses, root litter decomposition increased SOC concentration more than leaf litter after one year. (**f**,**g**,**h**) The net C balance of the near-complete litter decomposition after one year reveals large differences among species, yet is overall positive for the POM fraction (**f**) and the whole soil (**h**), but neutral for the MAOM fraction (**g**). The difference between leaf and root litters was significant for net POM-C (0.65 and 1.63 mg C g^{-1} soil, respectively) and net whole-soil SOC (0.70 and 1.23 mg C g^{-1} soil, respectively), but not for net MAOM-C (Table S2). No effect of plant functional type was found (all $P > 0.05$; ANOVA type III). Neither N nor the N and C leachates predicted the primed-C (**i**,**j**,**k**). "*" and "ns" represent significant ($P < 0.05$) and non-significant ($P > 0.05$) leaf – root differences (**a**,**e**). None of the Pearson correlations (**i**-**k**) were significant, as indicated by the dotted lines.

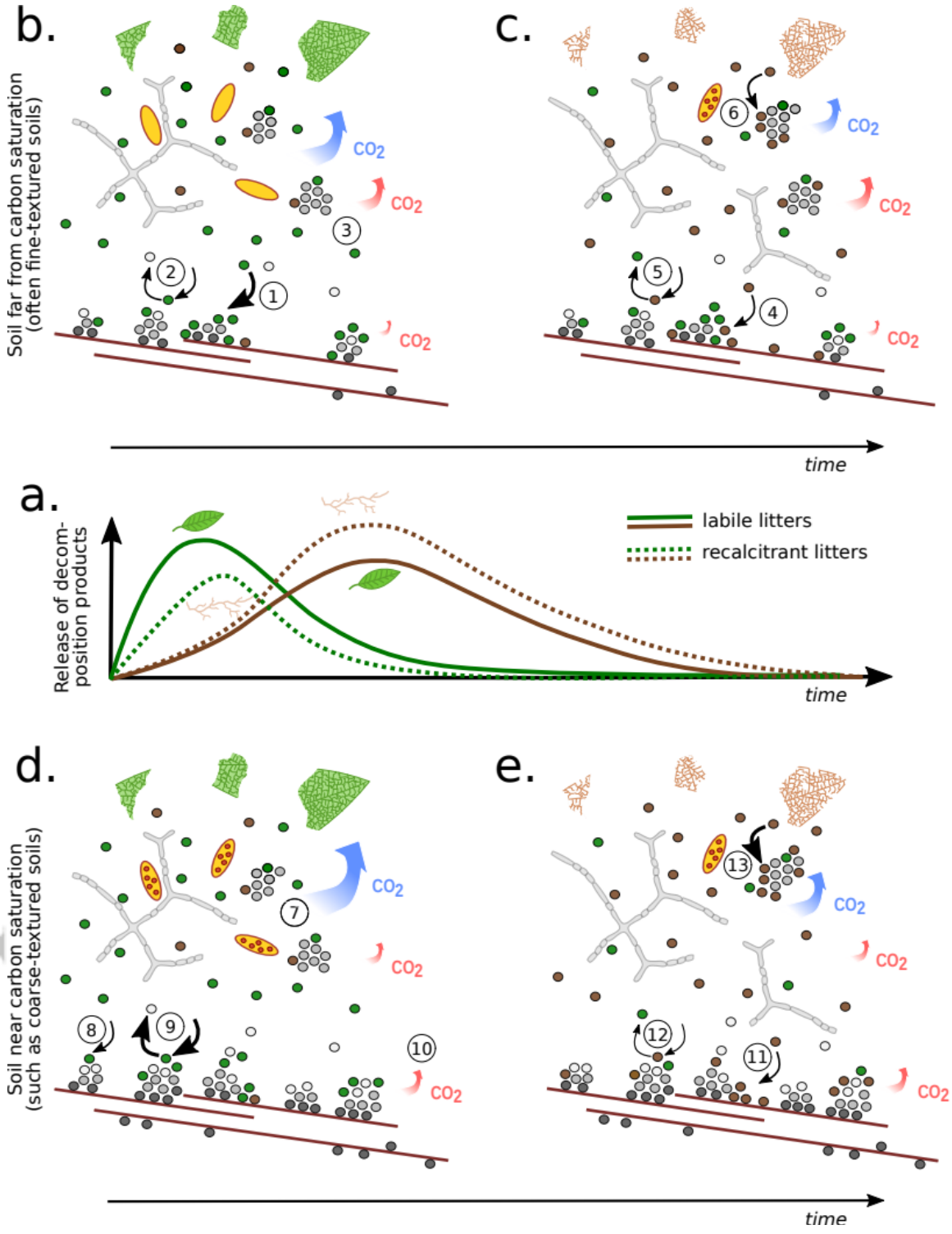

Figure 3. A renewed perspective on the influence of litter chemistry on soil carbon storage and priming. Initial litter chemistry is likely to have persistent effects during litter decomposition, although differently between soils of different soil mineral-associated carbon saturation (b, c unsaturated and d, e saturated). See main text, for more details on the proposed

mechanisms, numbered here from (1) to (13) on the Figure. a. After litter input to the soil, plant-derived degradation products are progressively released into the soil solution. Labile compounds (green lines; early peak) are released faster than recalcitrant compounds (brown lines; later peak). The relative abundance of labile *versus* recalcitrant compounds and the rapidity of their release varies with the initial litter quality (as exemplified by leaf *versus* root litter: solid *versus* dotted lines, respectively). Thus, the amount of rapidly released labile *versus* slowly released recalcitrant compounds (colour coded) scales with the initial litter quality (line-style coded); more labile compounds are released initially but less recalcitrant compounds are released later on for labile litters than for recalcitrant ones. b-e. Independent of soil C saturation, bacterial (yellow ellipses) and fungal (light grey lineal structures) decomposers rapidly mineralize part of the labile compounds (blue arrows, scaled to rate) of fresh litter material (panels b and d, corresponding to the green lines in a) and leaving behind mostly labile litter-derived decomposition (and bacterial-dominated microbial) products (green circles). In a later decomposition stage (panels c and e, corresponding to the brown lines in a) mineralization rates diminish and more recalcitrant litter-derived decomposition (and fungi-dominated microbial) products (brown circles) are released.