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Abstract

Soil organic matter is the dominant carbon pool in terrestrial ecosystems and its management is of increasing policy relevance. Soil microbes are the main drivers of soil organic carbon sequestration, especially through accumulation of their necromass. However, since the direct characterization of microbial necromass in soil is challenging, its composition and formation remain unresolved. Here, we provide evidence that *microbial death pathways* (the distinct processes of microbial dying) in soil affect necromass composition and its subsequent fate. Importantly, the composition of derived microbial necromass does not equal that of microbial biomass. From biomass to necromass, distinct chemical transformations lead to increases in cell wall : cytoplasm ratios, while nutrient contents and easily degradable compounds are depleted. The exact changes depend on environmental conditions and the relative significance of different microbial death pathways, e.g. predation, starvation or anthropogenic stresses. This has far-reaching consequences for mechanisms underpinning biogeochemical processes: (i) the quantity and persistence of microbial necromass is governed by microbial death pathways, not only the initial biomass composition; (ii) efficient recycling of nutrients within microbial biomass presents a possible pathway of organic carbon sequestration that minimizes nitrogen losses; (iii) human-induced disturbances affect the causes of microbial death and consequently necromass composition. Thus, new research focusing on microbial death pathways holds great potential to improve management strategies for soil organic carbon storage. Not only microbial growth, but also death drives the soil *microbial carbon pump*.

Introduction

Soil organic matter (SOM) represents the largest terrestrial organic carbon (C) stock, and thus managing this pool to balance rising atmospheric CO_2 levels is of increasing policy relevance, especially in agroecosystems[1]. To achieve a lasting increase of C stored in soil, it is essential to understand the nature of SOM and the processes that contribute to its formation. During the last

century soil science made tremendous progress in understanding basic principles: The traditional view of SOM as *humic substances* derived from *recalcitrant* plant litter was replaced by an emerging understanding that large portions of C compounds in soil are channeled through microorganisms. Major parts of SOM in fact consist of microbial residues, especially necromass[2]. This phenomenon is referred to as the *soil microbial carbon pump*[3]. Indeed, recent global assessments of biomarkers in soil indicate that, while living microbial biomass makes up less than 5% of soil organic carbon (SOC), microbial necromass accounts for more than half[4,5]. This several-fold increase indicates that residues of dead microorganisms accumulate in soil, persisting over time. Mineralization of these residues, but also interactions with mineral soil surfaces and the formation of bioorganic complexes are regarded as main factors driving necromass stabilization in soil, mechanisms which are dependent on soil type but also on the chemical composition of necromass[6,7]. Thus, further insights into the formation and chemical nature of necromass are urgently needed to conceptually integrate it into the existing framework of biogeochemical cycles and C sequestration dynamics. Developing this fundamental line of research may also reveal how soil management strategies can be adapted to exploit microbial pathways for optimizing soil C storage[8].

The direct characterization of necromass is analytically impeded by the complex nature of soil[9]. However, microbiological research offers insights into necromass characteristics and relevant knowledge about the processes of microbial dying. By taking a microbial perspective it becomes apparent that microbial biomass is not exposed to “sudden death”, but experiences a series of controlled events during its transformation to necromass, here referred to as *microbial death pathways (MDP)*. MDP comprise distinct chemical transformations dependent on the cause of death and the organisms involved, but inevitably result in microbial necromass that differs from microbial biomass (Table 1); hence we posit that microbial necromass does not equal microbial biomass in soil. In this Perspective we explore how soil microorganisms die and, based on microbial physiology, elaborate the chemical transformations during MDP that influence the molecular and nutrient composition of necromass. We thereby suggest that MDP themselves represent important

66 determinants of soil organic C sequestration, and their implementation in concepts of soil C and
67 nutrient cycling has far-reaching consequences for understanding the mechanisms underpinning
68 biogeochemical processes (summarized in Box 1).

69

70 Microbial death pathways in soil microorganisms

71 It can easily become a philosophical question whether a microbial cell is alive, dead or even
72 dormant[10]. We here focus on the unavoidable transition of microbial biomass to necromass, with
73 awareness that the duration and also complexity of this transition is highly variable. Current
74 biogeochemical models use microbial biomass and different compartments of SOM as individual
75 pools[11]. Accordingly, we treat living biomass as one pool, describe possible MDP that vary in extent
76 and speed of chemical transformation, and subsequently define necromass as an end-product of the
77 completed MDP. Microorganisms in soil (biomass pool) are exposed to a range of mortality risks,
78 including not only natural developmental aging (senescence), but also starvation, desiccation,
79 predation, viral attack, competition or anthropogenic disturbances. The relative contribution of each
80 is so far unknown, but likely depends on environmental conditions, soil types and organism groups
81 involved (Table 1, Fig. 1). To understand the underlying mechanisms, we will first focus on
82 senescence and other forms of programmed cell death (PCD), and then elaborate the link to other
83 relevant pathways affecting necromass characteristics.

84

85 Senescence and programmed cell death in microorganisms

86 The old paradigm of microorganisms being immortal under optimal conditions was dismantled some
87 time ago, first by the observation of asymmetric cell division in bacterial cells and yeasts, leading to
88 subsequent aging of mother cells[12,13]. Likewise, filamentous fungi show a “progressive loss of growth
89 potential” over time[14]. Such natural aging of individuals over longer time periods probably has
90 limited relevance in unstable soil environments, especially in unicellular organisms. However, in

filamentous fungi, senescence occurs as a by-product of growth and likely represents a prominent mechanism of necromass formation due to the modular fungal growth form. Fungi *move* through soil by the formation of new hyphae, consequently leaving behind large amounts of hyphal fragments[15]. Most importantly, new hyphal growth as well as fungal sporulation and fruiting are subsidized by internal recycling from older hyphal parts, resulting in efficient reuse of cytoplasmic components, lipids and even cell wall fragments like chitin[16,17]. This mechanism is of particular significance during nutrient limitations and starvation (Table 1)[18,19]. We describe this whole process as compartmental senescence (Fig. 1f), an analogue to leaf senescence in which valuable resources are withdrawn as a natural developmental process up to leaf abscission[20]. Similar to leaf litter, senescent hyphal parts diverge in their chemical composition from actively growing hyphae: Older hyphae (distant from growing tips) are characterized by vacuolization and cytoplasmic reductions following the activity of autocatalytic enzymes[21,22], and strong reductions in DNA content[23] and nutrient concentrations[24]. Senescence-derived fungal necromass will therefore be depleted in cytoplasmic components, storage compounds and growth-limiting elements relative to biomass[25] (Fig. 1f). The relative proportion of the cell wall fraction will increase, with consequences for C-to-nutrient ratios and chemical complexity: Fungal cell walls are rich in complex interlinked polysaccharides, with varying concentrations of chitin and melanin depending on species identity[26].

In bacteria, surprisingly similar recycling processes are induced under nutrient-limited or stressful conditions (common in heterogeneous soil environments) despite their unicellular growth form[27], especially within colonies and biofilms. This is represented in the literature by the generic concept of programmed cell death (PCD)[28,29], and will be categorized here as starvation- or stress-induced PCD (Table 1). Bacterial PCD is governed by a highly controlled genetic programme, involving cellular lytic degradation[30,31]. As a most simple mechanism, endospore formation induced under stressful conditions is supported by autocatalysis of the mother cell and even surrounding daughter cells[28,32]. Similarly, under starvation, PCD is a strategy in *Myxococcus* to provide nutrients for fruiting body formation, while individual cell death in filamentous *Streptomyces* colonies supports neighbouring

117 cells[28]. Such unicellular *suicide* may seem paradoxical, but several authors describe this widespread
118 phenomenon as a form of altruistic behavior with the main goal of nutrient replenishment for
119 genetically related survivors (kin selection)[28,29,33]. Especially in the context of recent suggestions that
120 the majority of soil bacteria and archaea occur in colonies embedded in EPS and biofilms, these
121 mechanisms may be highly relevant[33,34]. Within bacterial colonies, close proximity allows cellular
122 components released by cell lysis to be recycled efficiently by survivors, especially under resource-
123 limited conditions[27]. Again, the resulting necromass will be depleted in easily degradable cytoplasmic
124 compounds and growth-limiting elements. In addition, starvation induces further modifications to
125 bacterial cells, such as degradation of storage compounds and a reduction in cell size. Based on
126 geometric considerations, smaller cell sizes further increase cell wall : cytosol ratios.

127 Together, these lines of evidence suggest that death in microorganisms leads to significant
128 modifications in the molecular composition of necromass compared to biomass, mainly driven by
129 decreases in cytoplasmic components with related rising C-to-nutrient ratios, in parallel to a
130 reduction of limiting and easily degradable compounds (Fig. 1).

131

132 <u>Further types of MDP relevant in soil</u>

133 Though the relative contribution of different causes and mechanisms for MDP in soil are currently
134 unresolved (Table 1), it is clear that in natural soil environments additional (external) causes of
135 microbial death are common. Still, parts of the mechanisms described for senescence and PCD,
136 especially resource recycling, are also relevant for other MDP. Soil microorganisms form the base of
137 the soil food web, and are consumed by a diverse and abundant soil fauna community[35]. Bacteria can
138 also be killed by other predatory bacteria[36] and viruses that are highly abundant in soils[37,38] (Table 1,
139 Fig. 1). Evidently, predated microbial biomass is transformed to varying degrees to necromass
140 residues[39]. Here, the physiology and resource demands of the attacking agent are important in
141 determining the resulting chemical transformations. Predation by fauna and protozoa is known to

142 release excess N in mineral forms, a concept referred to as the *microbial loop*[40] (Fig. 1c). Fauna needs
143 to maintain homeostatic C:N:P ratios and invest C into both catabolism and anabolism, which
144 necessitates the release of excess mineralized N, especially following ingestion of bacteria with low
145 C:N ratios. In parallel, non-digested microbial necromass residues will be emitted in fecal pellets or
146 excreted (in the case of protozoa), with the chemical composition of these residues depending on the
147 digestive capacities of the predator species[41,42] and their specificity of prey intake[35]. The exact
148 chemical composition depends on many factors, but relative proportions of less easily degradable
149 compounds will generally increase (Fig. 1c). Predatory bacteria, by contrast, ingest mainly the
150 cytoplasm of bacterial prey, leaving behind cell wall fragments as necromass residues[36] (Fig. 1e). Even
151 viral attack produces modified bacterial residues that are depleted in N and especially P, and thus C
152 enriched[37] (Fig. 1d). Another relevant mechanism to consider during viral or pathogenic infection are
153 self-defence mechanisms present in bacterial colonies: Via quorum sensing infected bacterial cells
154 partly initiate cell lysis (PCD), which some authors also describe as a mechanism to make resources
155 available to neighboring daughter cells[28,43], again allowing resource recycling within colonies (Fig. 1d).

156 Other forms of MDP exist in soil, with the list discussed here likely being incomplete (Fig. 1b).
157 Antagonistic interactions during microbial competition can have lethal outcomes. Microbial species
158 strongly compete for resources in soil environments. On the one hand, more efficient resource
159 exploitation by one species may lead to starvation and subsequent (compartmental) death of
160 another, inducing PCD mechanisms as described above[44]. On the other hand, interference
161 competition involves the excretion of antimicrobial toxins with potentially lethal effects[45,46].
162 Regarding chemical transformations during MDP induced by antagonism, certain degrees of internal
163 resource recycling in response to this stressor are likely, depending on the lethality and
164 concentrations of toxins, especially during stress-induced spore formations in bacteria or within
165 fungal mycelia[47].

166 Another relevant mortality agent in light of global change is MDP induced by anthropogenic
167 disturbances. Examples are pesticide applications and other forms of soil pollution (Fig. 1g), soil

compaction, more intense drought or heat events as a consequence of climate change, or osmotic stress induced by salinity (as a consequence of inappropriate management). Water stress also occurs regularly under natural conditions and microorganisms have adapted to such soil conditions over evolutionary time[48] - abiotic stresses are likely relevant MDP in soil (including desiccation, starvation, freezing, anoxia, oxidative stress). However, sudden and severe stress events, including xenobiotic chemicals, may lead to more rapid death and subsequent release of complete cell components, characterized by lower resource retention and less efficient reuse than observed in natural MDP[49] (Table 1, Fig. 1g).

Relevance of MDP in environmental contexts

Different types of MDP differ not only in the relative amount of necromass produced, but also in the chemical transformations induced, with further contrasts created among different microbial groups. For example, while compartmental senescence in fungal mycelia produces necromass residues dominated by complex, C-rich cell wall fragments, lysis of bacterial cells likely releases more cytoplasmic compounds and cell wall fragments containing more N, since bacterial cell walls contain higher proportions of peptides[26,50]. Predation, by contrast, will retain more C within the soil food web but also release complex necromass residues together with mineral nutrients available to plants. Thus, it can be expected that fungal : bacterial ratios, predator load, viral abundances and competitive dynamics in soil communities will affect the relative importance of different MDP types, with consequences for necromass quantity and quality (Fig. 2a). Since the community composition of soil microbes, fauna and viruses varies among soils and further responds to environmental change, MDP and the strength of chemical transformations will vary accordingly (Fig. 2)[38,51,52]. In parallel, the efficiency of C versus nutrient recycling within microbial biomass will respond to soil nutrient limitations[53], affecting the C:N and N:P ratios of microbial necromass (Fig. 2a). Anthropogenic (lethal) stressors, especially extreme events[54], may have even more substantial impacts on necromass

193 formation and composition. Rapid, uncontrolled death of microorganisms in soil not only increases
194 the quantity of necromass produced, but also reduces its chemical transformation as opposed to
195 natural MDP (Fig. 1g, Table 1).

196 Not only microbial growth rates are affected by environmental parameters, but also MDP, which
197 subsequently determine the amount of C (i) remaining within microbial biomass or the soil food web,
198 (ii) being retained as SOC by mineral and organic interactions or (iii) available for mineralization by
199 necromass-degrading microbes. Predictions of the long-term persistence of necromass-derived SOC
200 are important research priorities, though challenging to conduct due to the different mechanisms
201 and high context-dependency involved (Fig. 2)[6]. Based on metabolic theory, necromass that is richer
202 in easily available C and nutrients (alongside C-rich molecules) will be more exposed to microbial
203 mineralization than more transformed complex polymeric necromass[55]. Indeed, meshbag
204 experiments indicate that necromass mineralization rates are positively correlated with N content,
205 and negatively with the abundance of highly complex components like melanin[56]. On the other hand,
206 mineral sorption is discussed as a more prevalent factor determining necromass persistence, which
207 acts most strongly on proteinaceous and phosphorylated molecules[57]. Thus, MDP and resulting
208 necromass chemistry are relevant to all stages of the *necromass continuum*[58], namely *production*,
209 *recycling* and *stabilization*, though the relative significance of each stage will likely depend on
210 microbial necromass composition and diversity, together with soil properties (Fig. 2a).

211 Another relevant factor for C persistence represents the heterogeneity introduced by spatial and
212 temporal diversity in soil[59], with this complexity further enhanced by diverse and spatially
213 differentiated MDP. The latter affect molecular composition and diversity of microbial necromass as
214 well as nutrient release from biomass and its spatial distribution. The way cells disintegrate, e.g.,
215 leakage of cytosolic compounds, altered particle size distributions and the spatial arrangement of
216 cellular remains within soil particles further increases spatial heterogeneity (Fig. 1). Additionally,
217 temporal effects on MDP will be present at various scales, from short term to seasonal fluctuations[60].
218 In conclusion, diverse MDP, chemical transformations and their responses to spatial and temporal

fluctuations will increase *molecular diversity*, *spatial heterogeneity* and *temporal variability* – all relevant factors determining SOC sequestration[59].

Conclusions and future research directions

We have detailed how microbial dying represents a distinct process with consequences for the quantity and molecular composition of microbial necromass. Despite a lack of direct measurements in soil, knowledge derived from (i) microbial physiology, (ii) trophic interactions and (iii) soil community ecology provides strong evidence for the mechanisms of MDP described. At the current stage the relation between microbial parameters and soil organic C storage is not fully resolved, often related to missing information on microbial traits, interactions of necromass with soil minerals and responses to environmental change[61,62]. Here, we argue that an understanding of the processes and rates of microbial death and resulting necromass characteristics will improve the implementation of microbial contributions to SOC sequestration in biogeochemical models. Microbial death, interactively with microbial growth, drives the soil *microbial carbon pump*, where the correlations among biomass and necromass abundance and composition are highly context-dependent (Fig. 2). Current soil organic matter research takes microbial growth and carbon-use efficiency as a proxy for microbial C inputs[63]. However, as presented here, mortality rates and chemical transformations will also determine the quantity and composition of microbial necromass in soil (Box 1).

The insights provided on biomass-to-necromass transformations add important novel perspectives on soil biogeochemical processes (Box 1). Still, it is clear that MDP represent only one component of a complex multifactorial soil system, which affects SOC sequestration interactively with microbial biomass characteristics and soil chemical properties (Fig. 2). An important opportunity provided by these new insights is to rethink the energy-efficient channeling of C within microbial communities as well as high nutrient-use efficiencies due to resource recycling mechanisms. It was proposed that C

sequestration in microbial residues may be limited by N demands[64]. However, if N is reused or released in mineral forms prior to necromass formation, N (or other nutrients) may under certain conditions not limit C sequestration. In line with this, increases in C sequestration also do not necessarily decrease nutrients available to plants, an aspect especially relevant for the management of agricultural soils with untapped C storage potential[4,5].

Future research must test these concepts experimentally in soil and microbial systems, but also establish whether the incorporation of MDP improves predictive strength of biogeochemical models (Box 1). We hope this Perspective will stimulate deeper exploration of the topic exploiting novel techniques and interdisciplinary approaches, and motivate a new research focus in the field of soil organic carbon cycling and microbial ecology. As the direct analysis of microbial necromass in soil is challenging, this also applies to the examination of MDP. However, analytical tools in soil science and microbiology are evolving rapidly: Detailed applications of stable isotope probing allow analyses of microbial growth and death within highly resolved food web dynamics[60,65], while higher resolution in imaging and spectroscopic techniques may provide insights on microbial interactions and processes at the level of individual organisms and populations[66,67]. Furthermore, by integrating MDP, we hypothesize that experiments with *real* necromass - not autoclaved biomass - will provide more detailed insights into its fate and stabilization (Box 1). Most likely, the often observed biphasic mineralization (*two-pool model*) is an artefact derived from this experimental bias driven by easily mineralizable cytosolic compounds[68,69]. Future experiments should also address responses of MDP to global change scenarios. Such knowledge and conceptual insight holds great potential to improve mechanistic understanding of organic C storage via microbial necromass, increase the precision of biogeochemical models and develop agricultural management strategies to exploit the C storage potential of soils.

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Author contributions

T.C. and J.L. generated the initial conceptual ideas. T.C. led the writing and literature search. K.M.-J., I.M., M.C.R., J.L. and T.C. developed the final concepts. All authors contributed to writing and editing.

Competing interests

The authors declare no competing interests.

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457 **Table 1**: Summary of described microbial death pathways (MDP) and their characteristics.

Microbial death pathway (MDP)	Affected organismal groups	Attacking/antagonistic agent	Internal resource recycling	Necromass characteristics[1]			Estimates of death rates and relevance
				C-to-nutrient ratio[2]	cell wall : cytoplasm ratio	easily degradable compounds	
(Compartmental) senescence	mostly fungi	-	high	↑	↑	↓	average hyphal length estimates for soil: 102 m cm^{-3}; turnover rate estimates 0.3 – 6.3 $month^{-1}$ [15]
Starvation/stress-induced PCD	bacteria; in fungi similar to senescence	-	high	↑	↑	↓	no data
Predation (soil fauna)	bacteria and fungi	fauna, protozoa	reuse by attacking agent	↓	?	↓	soil fauna abundance 60 - 11 × 10^7 individuals m^{-2} soil[70] (for individual groups see [71]); 16% reduction in mic. biomass by protozoa and nematodes[72]
Predation (bacterial)	bacteria	predatory bacteria	reuse by attacking agent	?	↑	↓	7.4% of bacteria belong to (facultative and obligate) predator groups[36]; death rates unknown
Viral attack	bacteria	bacteriophages	medium	↑	↑-	↓-	10^3 - 10^{10} virus particles (g^{-1} soil)[38]; 30-80% of bacteria in soil carry prophages[37]; death rates unknown
Interspecific competition	bacteria and fungi	bacteria and fungi	medium	↑	↑	↓	no data
Anthropogenic stress	bacteria and fungi	-	low	-	↑-	↓-	mostly unknown; drought events 25% death within 3 hours[60]; fire reduces mic. biomass by 33%[73]; pesticide effects on microbial biomass depend on chemicals and systems involved[74,75]

458

459 [1]necromass characteristics are described in direct comparison to microbial biomass. Arrows indicate expected transformations by MDP

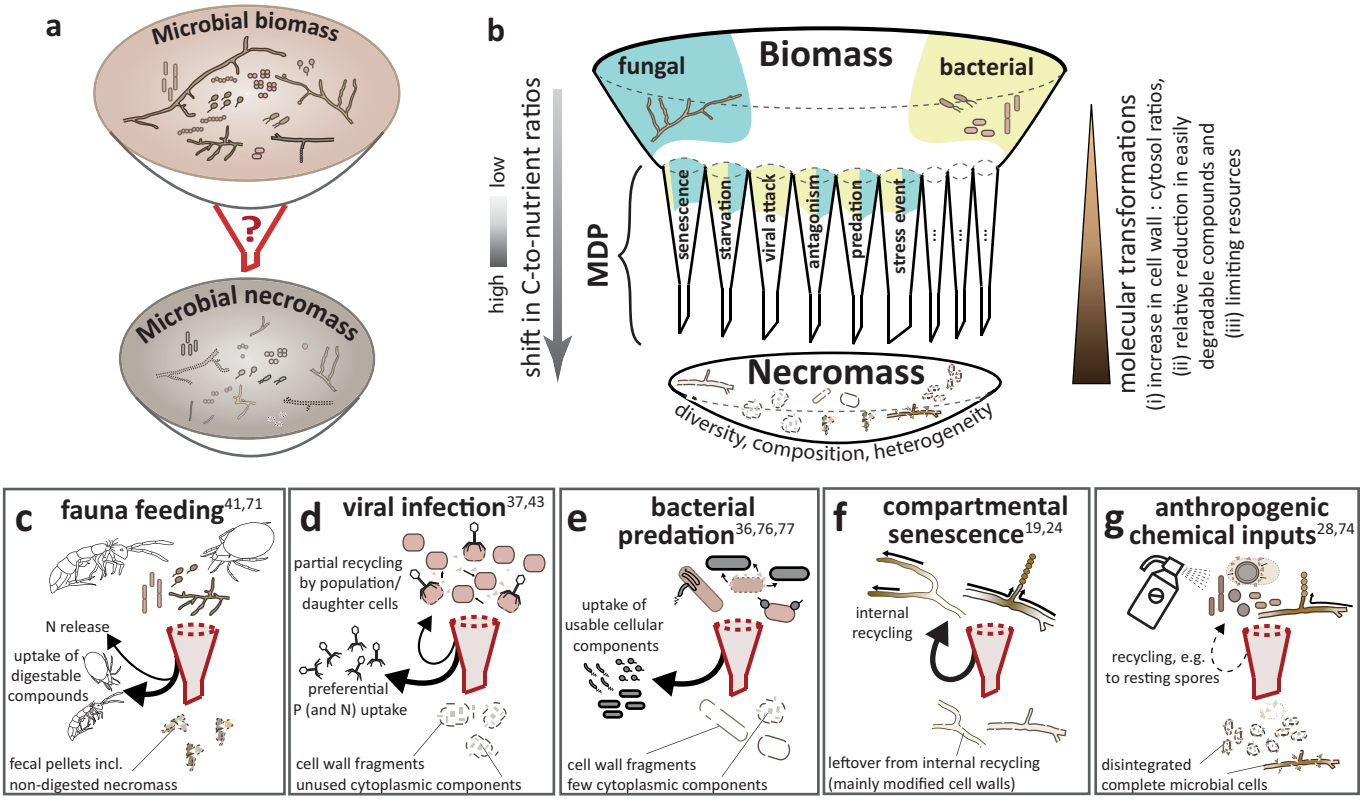
460 [2]outcomes may change depending on the limiting element in the system – selective internal recycling of limiting elements

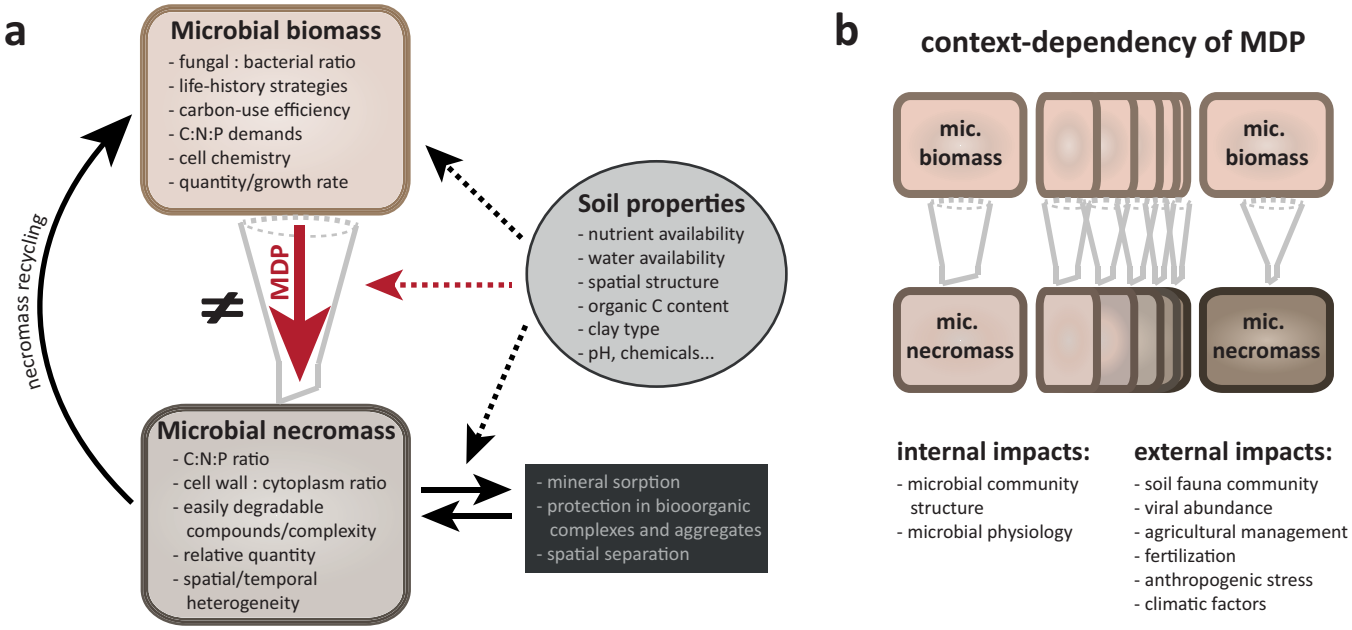
461 PCD: programmed cell death

Figure legends

Fig. 1: Visual illustration of microbial death pathways (MDP) including molecular transformations from microbial biomass to necromass. **a** The general question addressed. **b** Overview of different forms of MDP with simultaneous chemical modifications. Funnels depict the different types of MDP described, narrower lower funnel parts reflect higher internal recycling activity (Table 1). Blue and yellow colour schemes visualize the likely relevance of MDP for fungi and bacteria, respectively. **c-g** Specific examples are provided for individual types of MDP that are discussed in the literature. Known cellular and molecular transformations are illustrated, as well as directions of resource flow (black arrows).

Fig. 2: Visualizing the dependencies of microbial death pathways (MDP) on biotic and abiotic soil properties and environmental factors. **a** Processes relevant for microbial necromass composition and its fate are illustrated (solid arrows), as well as the impact of soil properties on these processes (dashed arrows). Variables of microbial biomass, necromass and the chemical and physical soil environment relevant for the respective processes are listed. **b** Simplified visualization of the strength of chemical transformations during MDP and factors relevant in this context. Following MDP, necromass may be fairly similar to the initial biomass (left); whereas certain MDP may lead to significant transformation of the initial biomass (right).


a
Microbial biomass
?
Microbial necromass
b
fungal
Biomass
bacterial
low
high
shift in C-to-nutrient ratios
MDP
senescence
starvation
viral attack
antagonism
predation
stress event
Necromass
diversity, composition, heterogeneity
molecular transformations
(i) increase in cell wall : cytosol ratios,
(ii) relative reduction in easily degradable compounds and
(iii) limiting resources
c
fauna feeding[41,71]
N release
uptake of digestable compounds
fecal pellets incl. non-digested necromass
d
viral infection[37,43]
partial recycling by population/ daughter cells
preferential P (and N) uptake
cell wall fragments unused cytoplasmic components
e
bacterial predation[36,76,77]
uptake of usable cellular components
cell wall fragments few cytoplasmic components
f
compartmental senescence[19,24]
internal recycling
leftover from internal recycling (mainly modified cell walls)
g
anthropogenic chemical inputs[28,74]
recycling, e.g. to resting spores
disintegrated complete microbial cells


a
Microbial biomass
- fungal : bacterial ratio
- life-history strategies
- carbon-use efficiency
- C:N:P demands
- cell chemistry
- quantity/growth rate
necromass recycling
≠
MDP
Soil properties
- nutrient availability
- water availability
- spatial structure
- organic C content
- clay type
- pH, chemicals...
Microbial necromass
- C:N:P ratio
- cell wall : cytoplasm ratio
- easily degradable compounds/complexity
- relative quantity
- spatial/temporal heterogeneity
- mineral sorption
- protection in biooorganic complexes and aggregates
- spatial separation
b
context-dependency of MDP
mic. biomass
mic. biomass
mic. necromass
mic. necromass
internal impacts:
- microbial community structure
- microbial physiology
external impacts:
- soil fauna community
- viral abundance
- agricultural management
- fertilization
- anthropogenic stress
- climatic factors

Box 1| Implementing microbial death pathways in the conceptual understanding of biogeochemical processes

Microbial death pathways (MDP): The distinct processes of microbial dying, including chemical transformations dependent on the cause of death and the organisms involved

Why to implement? A short summary

- Not only microbial growth, but also the process of dying represents a relevant driver of the soil *microbial carbon pump*
- The chemical composition of biomass is altered during MDP, leading to distinct characteristics of resultant necromass (see examples in Fig. 1c-g) → Microbial necromass ≠ microbial biomass
- The chemical composition of microbial necromass affects its persistence (mean residence time), having impacts on both its mineralization and stabilization rates
- Individual types of MDP affect necromass composition differently: SOC sequestration and microbial community dynamics depend on the types of MDP relevant in soil
- (Re)use of components from biomass during natural MDP - by the species itself or attacking agents - leads to efficient channeling of C, nitrogen (N) and energy in soil
- Under certain conditions C sequestration via microbial pathways is not limited by N availability, and also does not necessarily reduce plant nutrient availability → high relevance for agricultural management
- Global change factors have direct impacts on the process and rate of microbial death
- differences in MDP among functional microbial groups explain community-driven effects, especially the increased C storage in fungal dominated systems: Fungal mycelia produce large amounts of C-enriched hyphal leftovers (necromass)

In more practical terms:

- C:N estimates and conversion factors for biomarkers of microbial necromass (derived from measurements in biomass) must be corrected
- Ecological experiments with microbial necromass should use *real* necromass derived from the completed MDP (metaphorically, leaf litter decomposition experiments also do not use fresh leaves)

How to implement? Some first ideas

In experiments:

- Understand individual MDP in more detail based on controlled experiments: Microbial strains/communities may be exposed to differents stressors under laboratory conditions (agar media, artificial soil) and the chemical composition of derived necromass analyzed. Examples: inoculation with viruses or predators, exposure to sudden drought events, pesticide additions, reduction of nutrients. Necromass may be distinguished by life/death stains or isotope tracers, but also the chemical analysis of microbial biomass under stressed conditions will improve estimates of necromass chemistry (i.e., compared to biomarker conversion factors based on microbes grown on rich agar media)
- Preparing *real* necromass for experiments: (i) Microbial biomass exposed to different forms of MDP (see point above; including ^{13}C labels) can be used for subsequent experiments in soil; (ii) bacterial and fungal isolates cultivated for necromass production may not be only autoclaved, but grown over longer time spans under suboptimal conditions; (iii) spatial and temporal sampling of the dying part of the microorganisms may allow to capture microbial cells most closely resembling the *real* necromass.
- Analyze mortality rates and turnover in microbial biomass and individual groups under varying conditions[67] and determine the relevance of different MDP under varying conditions/soil types using novel techniques (i.e., stable isotope probing, microfluidic devices)[58, 66].

In biogeochemical models:

- Simple parameters to include: (i) Biomass turnover times/mortality rates → necromass quantity (relative to microbial growth); (ii) adjusted relevance of pathways in the necromass continuum[64] depending on MDP and necromass composition (e.g., stabilization vs. destabilization); (iii) *death traits* of relevant microbial groups
- theoretical models can be used to test the relevance of MDP for C sequestration and persistence