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Preservation, decomposition and stabilization of microbial necromass in biocrusts

- Microbial N demand and clay protection control
- Clay saturation with MAOC
- SOC accumulation
- MAOC formation
- Necromass accumulation coefficient
- POC formation
- Necromass recycling

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Initial soil formation by biocrusts: nitrogen demand and clay protection control microbial necromass accrual and recycling

Baorong Wang¹,², Yimei Huang³, Na Li⁴, Hongjia Yao⁴, Env Yang⁹, Andrey V. Soromotin⁵, Yakov Kuzyakov⁶,⁷, Vladimir Cheptsov⁸, Yang Yang⁹, Shaoshan An¹,⁴*

Baorong Wang: wangbaorong92@163.com; Yimei Huang: ymhuang1971@nwsuaf.edu.cn; Na Li: 1356816040@qq.com; Hongjia Yao: 963904270@qq.com; Env Yang: 2215003782@qq.com; Andrey V. Soromotin: asoromotin@mail.ru; Yakov Kuzyakov: kuzyakov@gwdg.de; Vladimir Cheptsov: cheptcov.vladimir@gmail.com; Yang Yang: yangyang@ieecas.cn; Shaoshan An: shan@ms.iswc.ac.cn

¹ State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Science and Ministry of Water Resources, Yangling 712100, China
² University of Chinese Academy of Sciences, Beijing 100049, China
³ Key Laboratory of Plant Nutrition and the Agro-environment in Northwest China, Ministry of Agriculture, College of Natural Resources and Environment, Northwest A&F University, Yangling 712100 Shaanxi, China
State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Northwest A&F University, Yangling 712100, China

Institute of Environmental and Agricultural Biology (X-BIO), Tyumen State University, 6 Volodarskogo Street, Tyumen, Russia

Department of Agricultural Soil Science, Department of Soil Science of Temperate Ecosystems, University of Gottingen, 37077, Goettingen, Germany

Agro-Technological Institute, RUDN University, 117198 Moscow, Russia

Soil Science Faculty, Lomonosov Moscow State University, 119991 Moscow, Russia

State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment, Chinese Academy of Sciences, Xi’an 710061, China

*Corresponding author: Shaoshan An
E-mail address: shan@ms.iswc.ac.cn
Tel: +86 29 87012871; Fax: +86 29 87012210.
Address: No.26, Rd. Xinong, Yangling, Shaanxi, China. 712100

Declaration of competing interest

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Abstract
Microbial biomass is increasingly considered to be the main source of organic carbon (C) sequestration in soils. Quantitative information on the contribution of microbial necromass to soil organic carbon (SOC) formation and the factors driving necromass accumulation, decomposition and stabilization during the initial soil formation in biological crusts (biocrusts) remains unavailable. To address this knowledge gap, we investigated the composition of microbial necromass and its contributions to SOC sequestration in a biocrust formation sequence consisting of five stages: bare sand, cyanobacteria stage, cyanobacteria-moss stage, moss-cyanobacteria stage, and moss stage on sandy parent material on the Loess Plateau. The fungal and bacterial necromass C content was analyzed based on the cell wall biomarker amino sugars. Microbial necromass was an important source of SOC, and was incorporated into the particulate and mineral-associated organic C (MAOC). Because bacteria have smaller and thinner cell wall fragments as well as more proteins than fungi, bacterial necromass mainly contributed to the MAOC pool, while fungal residues remained more abundant in the particulate organic C (POC). MAOC did not always increase with microbial necromass and POC more rapid accumulation than MAOC during initial soil formation suggests that the clay content was the limiting factor for stable C accumulation in this sandy soil. The necromass exceeding the MAOC stabilization level was stored in the labile POC pool (especially necromass from fungi). Activities of four enzymes (i.e., β-1,4-glucosidase, β-1,4-N-acetyl-glcosaminidase, leucine aminopeptidase, and alkaline phosphatase) increased with fungal and bacterial necromass, suggesting that the increasing activity of living microorganisms associated
with accelerated turnover and formation of necromass. Microbial N limitation raised
the production of N acquisition enzymes (e.g., β-1,4-N-acetyl-glucosaminidase and
leucine aminopeptidase) to break down necromass compounds, leading to further
increase of the nutrient pool in soil solution. The decrease of microbial N limitation
along the biocrusts formation chronosequence is an important factor for the
necromass accumulation during initial soil development. High microbial N demands
and insufficient clay protection lead to fast necromass reutilization by microorganisms
and thus, result in a low necromass accumulation coefficient, that is, the ratio of
microbial necromass to living microbial biomass (on average, 9.6). Consequently,
microbial necromass contribution to SOC during initial soil formation by biocrust is
lower (12-25%) than in fully developed soil (33%-60%, literature data). Nitrogen (N)
limitation of microorganisms and an increased ratio between N-acquiring enzyme
activity and microbial N, as well as limited clay protection, resulted in a low
contribution of microbial necromass to SOC by initial formation of biocrust-covered
sandy soil. Summarizing, soil development leads not only to SOC accumulation, but
also to increased contribution of microbial necromass to SOC, whereas the plant
biomass contribution decreases.

Keywords: extracellular enzyme activities; particulate organic carbon;
mineral-associated organic matter; initial soil formation; carbon sequestration;
microbial residues
1. Introduction

Biological soil crusts (hereafter referred to as “biocrusts”) cover approximately 12% of Earth’s terrestrial surface (Rodriguez-Caballero et al., 2018). Biocrusts are composed of photoautotrophic communities such as cyanobacteria, chlorophyte algae, lichens and mosses and co-occur with heterotrophic bacteria, fungi, and archaea (Belnap et al., 2008; Johnson et al., 2012; Pointing and Belnap, 2012). From the initial CO₂ fixation by microorganisms (Liu et al., 2018) to the later increase of soil organic carbon (SOC) content by inputs from simple vascular plants (i.e., mosses, Liu et al., 2018), biocrusts accelerate C and nutrient biogeochemical cycling in arid and semiarid regions (Grote et al., 2010; Duran et al., 2021) as well as in cold environments. For example, a global meta-analysis suggested that the SOC content under biocrusts generally increased by 71% compared to soil without crust cover (Xu et al., 2022). Biocrusts-derived organic C consists mainly of carbohydrate-C and free fatty acids (Dümig et al., 2014; Baumann et al., 2021). The high content of carbohydrate-C in well-developed biocrusts (e.g., mosses) is responsible for the high water solubility of organic C, increasing downward movement and thus induces initial soil formation (Dümig et al., 2014; Koester et al., 2021).

Microorganisms in soil biocrusts mediate C cycling processes by fixing atmospheric CO₂ (Duran et al., 2021) and degrading microbial, algae, lichen and moss residues (Pointing and Belnap, 2012). After microbial death and subsequent lysis and fragmentation, cell wall compounds contribute to the necromass pool and further to SOC sequestration (Miltner et al., 2012; Liang et al., 2017; Liang, 2020). Emerging
evidence reveals that microbial necromass contributes more than 50% of the SOC
sequestration in developed soils (Huang et al., 2019; Liang et al., 2019; Wang et al,
2021a). However, the contribution of microbial necromass to SOC accumulation
during initial soil formation in biocrust-dominated surfaces remains completely
unclear.

The balance between mineralization and stabilization of organic matter
influences the formation and decomposition of microbial necromass and its
contribution to SOC accumulation (Cui et al., 2020; Liang et al., 2020; Čapek et al.,
2021). Microbial necromass can bind to clay minerals and thus majorly forms
mineral-associated organic C or becomes microaggregates stabilized in the soil (Ye et
al., 2019; Buckeridge et al., 2020; Possinger et al., 2020; Yang et al., 2021). However,
this physically-protected necromass can be enzymatically broken down into fragments
that are released into soil solution and can be used by living microorganisms (Li et al.,
2019; Yang et al., 2021). This is mainly because microbial necromass is an optimal
substrate with high contents of N and P, that can be exploited by microorganisms to
meet their C (energy) and nutrient requirements (Buckeridge et al., 2020; Cui et al.,
2020).

Microbial necromass accumulation and decomposition are largely controlled by
stoichiometric imbalances between microbial communities and their resources
(Zechmeister-Boltenstern et al., 2015; Liang et al., 2020). The recycling of nutrients
derived from dying microbial biomass or its residues is an important mechanism, by
which microorganisms meet their nutrient requirements (López-Mondéjar et al., 2018;
Buckeridge et al., 2020; Čapek et al., 2021). The active part of the microbial community instantly recycles N from the decaying part of the community, when the available N forms are insufficient (Cui et al., 2020; Čapek et al., 2021). Microbial necromass reutilization is a more efficient strategy than obtaining N from non-readily decomposable soil organic matter (SOM) or alleviating N deficiency under labile C excess (Cui et al., 2020). Various extracellular enzymes, such as chitinases, decompose amino sugar polymers as an alternative N source for microorganisms when readily available N is limited (Li et al., 2019).

Environmental conditions and microbial nutrient demands have a strong influence on necromass recycling (Chen et al., 2019; Buckeridge et al., 2020; López-Mondéjar et al., 2020; Čapek et al., 2021). For example, high N availability accelerates microbial necromass accumulation (Ding et al., 2010; Fan et al., 2020), but only if available C is sufficient for growth (Chen et al., 2020). A nutrient-rich environment stimulates microbial growth with high biomass yield and necromass accumulation (Shao et al., 2021). Low precipitation increases microbial necromass recycling efficiency mainly because it limits nutrients and water availability, and especially drying-rewetting cycles especially contribute to the formation of mineral-associated SOM (Shahbaz et al., 2017; Buckeridge et al., 2020; Zhang et al., 2021).

Thus, nutrient deficiencies or environmental constraints stimulate the microbial degradation of necromass by extracellular enzymes (e.g., β-1,4-N-acetyl-glucosaminidase, leucine aminopeptidase, Li et al., 2019; Donhauser et al., 2020). This is mainly because microbial necromass has high N and P contents,
and consists of proteins, fungal chitin, glycoproteins, nucleic acids and bacterial peptidoglycans, whose hydrolysis greatly contributes to the formation of a bioavailable dissolved organic N and P pool (Hu et al., 2017, 2020; Warren, 2021).

Additionally, rapid abiotic retention of microbial necromass by adsorption to soil mineral particles (e.g., by drying-rewetting cycles) and localization in micro- and nanopores increases the SOC stock (Kravchenko et al., 2019; Buckeridge et al., 2020;). Consequently, a low clay content increases microbial necromass decomposition (Hu et al., 2020). Therefore, the microbial demand for C and N, environmental C and N availability, and clay protection are the major factors that determine the accumulation and decomposition of necromass. This is especially crucial during initial soil formation because of low clay and organic matter contents, and a consequently strongly limited possibility for biochemical protection and encapsulation in the pores of microaggregates. Biocrusts play a crucial role during the initial stages of soil formation and organic matter accumulation. However, biocrust-dominated soils are strongly limited by low moisture and nutrients (Johnson et al., 2012) and consequently by the formation and accumulation of microbial residues; thus, organic matter differs from that in already developed soils.

To clarify microbial necromass composition and its contribution to SOC accumulation, we investigated the biocrust formation chronosequence: bare sand, cyanobacteria, cyanobacteria-moss, moss-cyanobacteria, and moss. This is a typical sequence on the same soil, consisting of five stages that may take approximately 30 years in the semi-arid climate. Fungal and bacterial necromass was calculated based
on the microbial markers: glucosamine and muramic acid. Soil and environmental factors affecting microbial necromass formation and its contributions to SOC sequestration were explored based on the activities of extracellular enzymes, available nutrients, and organic C pools. We addressed the following questions: (1) How does microbial necromass, including fungal and bacterial necromass, contribute to SOC accumulation during initial soil formation in the biocrust formation chronosequence? (2) What are the effects of extracellular enzymes, available nutrients, particulate and mineral-associated organic C on microbial necromass accumulation? (3) Which soil properties are the most critical determinants of microbial necromass accumulation and decomposition (based on the microbial nutrient limitation and their necromass content)? Based on these questions, we developed the three hypotheses: (1) The richness of higher plant species stimulates microbial growth and biomass accumulation by increasing belowground C allocation (roots and rhizodeposition), leading to increased necromass formation and SOC accumulation (Prommer et al., 2019; Cotrufo et al., 2019; Sokol and Bradford, 2018). We hypothesized that the microbial necromass content and its contribution to SOC increase with the biocrust formation chronosequence. (2) As nutrient availability importantly determines microbial necromass accumulation (Shao et al., 2021; Wang et al., 2021b), their association with mineral particles to form MAOC contributes to stable SOC (Yang et al., 2021). We hypothesized that increased soluble nutrients during biocrusts formation facilitate live microbial populations and hence necromass formation, which contributes to increased SOC mainly in the form of MAOC. (3) As the stoichiometric
ratio of nutrients controls the necromass accumulation and its contribution to SOC (Čapek et al., 2021; Cui et al., 2020; Deng and Liang, 2021), and clay protection is the main mechanism for necromass medium- and long-term stabilization (Buckeridge et al., 2020; Hu et al., 2020). Thus, we hypothesize that extracellular enzymes released by microorganisms for nutrient mining and the clay content regulate the microbial necromass recycling and accumulation during initial soil formation.

2. Material and methods

2.1 Site description and experimental design

The field experiment was performed in Liudaogou, Shenmu (northern Loess Plateau), Shaanxi Province, China (38°46′–38°51′ N, 110°21′–110°23′). It is the center of the "wind-water erosion staggered area" of the Loess Plateau. This area has a semiarid climate, where the mean annual precipitation is 409 mm, and the potential evapotranspiration is 1337 mm. The mean annual temperature of the sites is 8.4 °C, and the elevation is between 1,081 and 1,274 m. Biocrusts comprised of cyanobacteria and moss-dominated crust are widely distributed in this region. The soil clay content (< 0.002 mm) of cyanobacteria, cyanobacteria-moss, moss-cyanobacteria, and moss in the BSC horizons and 0–2 cm soil layer is shown in Table 1. The soil was classified as an arenosol according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2015).
All sites have a similar orientation and inclination. Bare sand (~ 0–1 years old) and four biocrust formation stages, namely, cyanobacteria (~ 3–7 years old), cyanobacteria-moss (~ 8–13 years old), moss-cyanobacteria (~ 20–25 years old), and moss (~ 30 years old), were evaluated. Cyanobacteria-moss and moss-cyanobacteria stages refer to cyanobacteria-dominated biocrusts (coverage of cyanobacteria > 80% and moss < 20%) and moss-dominated biocrusts (coverage of moss > 80% and cyanobacteria < 20%), respectively. The biocrust coverage was measured with a point-intercept method using a 25 cm × 25 cm gridded square (Belnap et al., 2001).

For the soil sample collection (August 2018), we randomly selected 5 representative sites: 40 m × 40 m squares in each site with a distance over 100 m between any two sites. In each 40 × 40 m plot, an "S"-shaped random-sampling strategy with 12–15 small sampling locations was chosen. The soil samples from the 12–15 sampling points in each plot were mixed to obtain a representative sample of approximately 2 kg. Forty-five representative samples (4 stages × 5 replicates = 20 samples for the biocrust (BSC) horizon and 5 stages × 5 replicates = 25 samples for the 0–2 cm soil layer) including the BSC horizon and the 0–2 cm mineral soil layer underlying the biocrusts (Gao et al., 2017), were collected. The BSC horizon refers to the biological soil crust horizon, which consists of a colloidal complex of cryptogamic plants and associated soil microscopic organisms with surface soil particles. The measured biocrust thicknesses of cyanobacteria, cyanobacteria-moss, moss-cyanobacteria, and moss were approximately 7.2±2.2 mm, 9.6±1.9 mm, 11.1±1.9 mm, and 10.9±1.8 mm, respectively. After careful and thorough removal of moss tissues and residues from
the soil, the collected samples were put into a foam box with an ice pack and moved
to a 4 °C refrigerator within 24 h. Soil samples were separated into two parts: one part
was immediately analyzed for microbial biomass and extracellular enzyme activities
within one week. Another part of the air-dried sample was passed through a 2 mm
sieve for particle size and mineral-associated organic C determination. A part of the
soil sample was passed through the 0.15 mm size and tested for basic chemical
properties and amino sugar analysis.

2.2 Analysis

The soil organic carbon (SOC) content was determined by dichromate oxidation
(Walkley and Black, 1934). Soil total nitrogen (N) and total phosphorus (P) contents
were measured using the Kjeldahl method and vanadium molybdate yellow
colorimetric method, respectively. The soil microbial biomass contents of C, N, and P
(MBC, MBN, and MBP) were measured using the chloroform-fumigation-extraction
method according to Brookes et al. (1982, 1985) and Vance et al. (1987). Briefly, two
sample parts were subjected to fumigation with CHCl₃ for 24 h at 25 °C, and two
others were not subjected to fumigation. The fumigated and nonfumigated soil
samples were extracted in 50 ml 0.5 M K₂SO₄ at a ratio of 1:4 (W/V) for microbial
biomass C and N and 50 ml 0.5 M NaHCO₃ at a ratio of 1:20 (W/V) for P. The
microbial biomass C, N and P contents were calculated according to the difference
between fumigated and nonfumigated samples and were adjusted using the
experimentally derived conversion factor $E$, where $E_c$, $E_c'$ and $E_p = 0.45$, 0.45 and 0.40, respectively (Fanin et al., 2013; Wu et al., 1990). The dissolved organic C, N and inorganic P (DC, DN, and DP) contents were calculated from the unfumigated samples (Chen et al., 2018; Hamel et al., 2006; Li et al., 2015).

Particulate organic C and mineral-associated organic C fractions were determined according to the method of Sokol et al. (2019) and Sokol and Bradford (2018). In brief, soil samples (20 g air-dried soil) were shaken with 60 ml of the chemical dispersant sodium hexametaphosphate for 18 h and then thoroughly rinsed through a 0.053 mm sieve to separate the POC fraction (>$0.053$ mm) and the MAOC fraction (<$0.053$ mm). The collected fractions were dried at 65 °C and then crushed to 0.15 mm size for SOC determination.

The activities of four common hydrolytic enzymes associated with the microbial acquisition of C, N, and P from organic residues (including β-1,4-glucosidase (BG), β-1,4-N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP), and alkaline phosphatase (AP)) were measured in 96-well plates with appropriate fluorescently linked substrates and analyzed on a microplate fluorometer (DeForest and Moorhead, 2020; Li et al., 2019).

Amino sugar (e.g., glucosamine, galactosamine, mannosamine, and muramic acid) contents were determined according to the method of Zhang & Amelung (1996). Briefly, approximately 0.3 mg of N soil samples and standard soil samples (in which the amino sugar content is known (measured many times), used for calibration) were hydrolyzed for 8 h at 105 °C using 10 ml of 6 M HCl. After cooling, 100 μl internal
standard 1-inositol (1 mg/ml) was added. Then, the sample was filtered through Whatman qualitative circles (12.5 cm diameter), and the filtrate was dried completely by a rotary evaporator and redissolved in deionized water. The pH of the samples was adjusted to 6.6–6.8, and the samples were centrifuged and freeze-dried. Then, methanol was added to the freeze-dried residue and subsequently centrifuged to extract the amino sugars from the residues. The extracted amino sugar was dissolved in 1 ml deionized water, and then 100 μl of the internal standard N-methyl-D-glucamine (1 mg/ml) was added. A standard series of amino sugars was also established. Muramic acid was first dried under N₂ at 45 °C and then added to glucosamine, galactosamine, mannosamine, myo-inositol, N-methyl-D-glucamine, and 1 ml of deionized water, in that order. The purified samples and standard series of amino sugars were reacted with hydroxylamine hydrochloride and 4-(dimethylamino) pyridine for 30 min at 75–80 °C, and the vial was shaken 4–5 times during the heating process using a vortex instrument. The vial was cooled to room temperature, 1 ml of acetic anhydride was added, the reaction was allowed to proceed at 75–80 °C for 1 hour; the vial was shaken 5–6 times during the heating process using a vortex instrument. The vial was closed, and the recovered amino sugars were transformed into aldononitrile derivatives, which were extracted with 1.5 mL of dichloromethane from the aqueous solution. Excess anhydride was removed with 1 mol L⁻¹ HCl and deionized water. The final organic phase was dried with N₂ at 45 °C and dissolved in 200 μL of ethyl acetate-hexane (1:1). The amino sugar derivatives were separated on a gas chromatograph (GC-2014C, Shimadzu, Japan) equipped with a DB-5 column (60
Fungal and bacterial necromass were calculated based on equations (1) and (2), according to Liang et al. (2019).

\[
\text{Fungal necromass C} = \frac{\text{glucosamine}}{179.17} - 2 \times \frac{\text{muramic acid}}{251.23} \times 179.17 \times 9 \quad (1)
\]

\[
\text{Bacterial necromass C} = \text{muramic acid} \times 45 \quad (2)
\]

The coefficients 179.17 and 251.23 in equation (1) are the molecular weights of glucosamine and muramic acid, respectively; 9 is the conversion value of fungal glucosamine to fungal necromass C; and 45 is the conversion value from muramic acid to bacterial necromass C (Appuhn and Joergensen, 2006; Joergensen, 2018). The total microbial necromass C (MNC) was estimated as the sum of fungal-derived C (FNC) and bacterial-derived C (BNC). The proportion of total microbial necromass C in SOC represents the microbial necromass contribution to SOC sequestration (Liang et al., 2019).

### 2.3 Statistical analysis

One-way ANOVA followed by the post hoc Tukey HSD test (\(\alpha = 0.05\)) was carried out with IBM SPSS Statistics 21 (SPSS, Chicago, Illinois USA) software to test the significant differences in soil chemical properties, microbial biomass, extracellular enzyme activities, available nutrients, POC, MAOC, microbial necromass C and amino sugars in the biocrust formation stages. Regression analysis was performed using IBM SPSS Statistics 20 software to assess the relationships
between SOC, POC and MAOC and fungal, bacterial, and microbial necromass C. To explore the clay saturation with MAOC, regression analysis was performed to assess the relationships between SOC, clay contents, POC, MAOC, POC/MAOC and fungal and bacterial necromass C. The regressions were considered significant at a level of $p < 0.05$. Redundancy analysis (RDA) was performed to explore the relationships among microbial biomass, extracellular enzymes, available nutrients, POC and MAOC (explanatory variables), microbial necromass and amino sugars (response variables). Prior to analysis, detrended correspondence analysis (DCA) was used to select the appropriate response model for the subsequent direct gradient analyses. The length of the first DCA ordination axis was $< 3$ for the necromass and amino sugar dataset, which indicated a linear response along the axis. Forward selection in this model was used to determine the most significant factors that influenced amino sugars and microbial necromass C. The variables were incorporated stepwise into the model according to their increasing effect on the variance and their significance tested by the Monte Carlo permutation test (Legendre and Legendre, 2012).

Stoichiometric analysis of soil enzyme activities was used to identify potential C, N and P limitations in the soil. The enzymatic stoichiometric vector characteristics (including vector length and vector angle) were calculated according to Moorhead et al. (2016). These values were used as a rough reflection of microbial nutrient limitation: High vector length implies microbial C limitation, whereas a vector angle lower than 45° indicates N limitation; otherwise, P limitation dominates (Bai et al., 2021). By using 1.0 as a horizontal and vertical baseline along the axis of enzyme
activity ratios (NAG+LAP/AP as x-axis and BG/NAG+LAP as y-axis), four groups of microbial resource limitations (N limitation, P limitation, C and N colimitation and C and P colimitation) were categorized (Bai et al., 2021; Cui et al., 2020). The vector length and vector angle are calculated following Equations (1) and (2):

\[
\text{Vector length} = \sqrt{\left(\frac{\ln(BG)}{\ln(NAG+LAP)}\right)^2 + \left(\frac{\ln(BG)}{\ln(AP)}\right)^2} \quad (1)
\]

\[
\text{Vector angle} = \text{DEGREES}\{\text{ATAN2}[(\ln(BG) / \ln(AP)), (\ln(BG) / \ln(NAG+LAP))]\} \quad (2)
\]

The necromass accumulation coefficient (NAC) was calculated using Equation (3) Zhang et al. (2021).

\[
NAC = \frac{c_{\text{Necromass}}}{c_{\text{living}}}/\text{Biomass} \quad \text{Equation (3)}
\]

To test the strength of microbial homeostasis in biocrust-covered sandy soil, we calculated the degree of stoichiometric homeostasis for microorganisms using the \(1/H\) coefficient (Equation (4)). The \(1/H\) coefficient is the slope of the regression between log (resource ratio; C/N in bulk soil, dissolved matter and enzyme) and log (biomass ratio; microbial C/N ratio) and should equal a value between zero and one (Makino et al., 2003; Persson et al., 2010):

\[
\log(y) = \frac{1}{H} \log(x) + \log(c) \quad \text{Equation (4)}
\]

where \(x\) is the resource C/N stoichiometry, \(y\) is the microorganisms’ C/N stoichiometry, and \(c\) is a constant. If the regression relationship was nonsignificant (p > 0.05), \(1/H\) was set to zero, and the microorganisms were considered "strictly homeostatic." All datasets with significant regressions and \(0 < 1/H < 1\) were arbitrarily classified as follows: \(0 < 1/H < 0.25\) “homeostatic”, \(0.25 < 1/H < 0.5\) “weakly homeostatic”, \(0.5 < 1/H < 0.75\) “weakly plastic”, and \(1/H > 0.75\) “plastic”
3. Results

3.1 Microbial necromass and its contribution to organic C accumulation

Initial soil formation by biocrusts increased the fungal and bacterial necromass contents in the 0–2 cm soil layer compared to bare sand (Fig. 1a-b, $p < 0.05$). Although SOC and microbial biomass increased from the cyanobacteria to moss stage (Table 1, $p < 0.05$), there were no differences in the fungal necromass contents between formation stages in the BSC horizon (Fig. 1a, $p > 0.05$). Except for the bare sand, bacterial necromass contents remained constant in the BSC horizon but decreased in the 0–2 cm mineral soil from the cyanobacteria stage to the moss stage (Fig. 1b). Other microbial cell molecules, such as galactosamine and mannosamine contents, remained stable over the course of biocrust formation (Table 1). The microbial necromass C contribution to SOC ranged from 12% (in bare sand) to 25% in various biocrust stages (Fig. 1c). The fungal:bacterial necromass ratio at 0–2 cm increased from the cyanobacteria to the moss stage, indicating that larger fungal necromass contributed to increased SOC (Fig. 1d, $p < 0.05$).

The contribution of bacterial necromass C to SOC ranged from 2.6% (in bare sand) to ~7.7%, while the contribution of fungi at various biocrust stages ranged from 10% (bare sand) to 21% (Fig. 1e-f). Fungal necromass (> 67% of the total necromass) consistently contributed more to SOC than did bacterial necromass C (Fig. 1g).
The contribution of bacterial necromass C to SOC and microbial necromass in the BSC horizon was higher than that in the 0–2 cm soil layer, indicating that bacteria contributed more to SOC formation in the BSC than in the underlying soil (Fig. 1e). The ratio of microbial necromass C/microbial biomass C (necromass accumulation per unit of microbial biomass C: necromass accumulation coefficient) decreased from the cyanobacteria stage to the moss stage (Fig. 1h, p < 0.05). The necromass accumulation coefficient increased from the BSC horizon to the mineral soil (0–2 cm). This suggests relatively higher necromass stabilization and preservation in 0–2 cm mineral soil (Fig. 1h).

### 3.2 Extracellular enzymes and microbial nutrient limitation

The enzyme activities of NAG and AP in both layers and LAP in the 0–2 cm layer increased from bare sand to the moss stage (Fig. 2b-d, p < 0.05). The C-acquiring enzyme (BG) activity/microbial biomass C ratio (specific enzyme activity per microbial biomass unit: microbial enzyme activity coefficient) increased from the BSC horizon to the 0–2 cm layer, suggesting higher microbial C demands in the subsurface layer of biocrust-covered sandy soil (Fig. 2e). The N-acquiring enzyme (NAG+LAP) activity/microbial biomass N ratio increased from cyanobacteria to moss in the BSC horizon, suggesting higher microbial N demands with biocrust formation sequences (Fig. 2f).
The decreasing vector length of the enzyme ratio in the 0–2 cm soil depth from the bare sand to moss stage suggests a decrease in C limitation for microorganisms with biocrust formation (Fig. 3a). The vector angles of the enzyme ratio were always less than 45° (Fig. 3b), indicating that microbial N limitation was common in all biocrust stages but it tends to decrease due to the increase in N-acquisition enzymes and their enzymatic coefficients with biocrust formation (Fig. 2e-f and Fig. 3b). Microbial C and N colimitation and a higher C- and N-acquiring enzyme activity/microbial biomass C and N ratio were found in bare sand (Figs. 2e, f and 3e, f), suggesting that biocrust formation eliminates the microbial C limitation, whereas N limitation remains (Fig. 3c-d). Consequently, stronger microbial nutrient limitation leads to a higher recycling intensity of necromass, decreasing the contribution of necromass to SOC.

3.3 Effects of biocrust formation and microbial necromass accumulation on soil organic matter fractions

The POC and MAOC increased with SOC content (Fig. 4a, $p < 0.001$). The fungal, bacterial, and total microbial necromass C increased with SOC content (Fig. 4b; $p < 0.001$), indicating the critical role of microbial necromass in SOC formation in biocrust-dominated soil. Bacterial necromass was more strongly correlated with MAOC, while fungal necromass was more strongly correlated with POC (Fig. 4c-d, $p < 0.001$). Similarly, POC and MAOC increased with fungal, bacterial, and microbial
necromass C (Fig. 4c-d; \( p < 0.001 \)). Microbial necromass was more correlated with POC than MAOC (Fig. 4a-b and Table 1) and MAOC did not always increase with necromass (Fig. 5a-b), suggesting that more necromass was stabilized by forming POC due to the low clay content (only 1–2\%). The POC/MOAC ratio increased with SOC content (Fig. 5c), indicating that POC accumulated faster than MAOC.

Microbial necromass C increased with clay and silt contents (excluding bacterial necromass C in the BSC horizon; Fig. 5d-e, \( p < 0.01 \)), indicating that a higher clay and silt content could protect fungal and bacterial necromass and further benefit their accumulation.

### 3.4 Accumulation of microbial necromass depending on dissolved N content

Microbial necromass C increased with living biomass, enzyme activities and dissolved nutrients, indicating that a large living microbial biomass can stimulate necromass accumulation (Fig. S1a-i, \( p < 0.001 \)). Dissolved N was the most important factor affecting microbial necromass accumulation in BSC horizon, while other soil properties (i.e., MBN, DN, MBC, DC, clay contents, LAP, and POC) explained microbial necromass stabilization at 0–2 cm (RDA results, Fig. 6 a-d, \( p < 0.01 \)). The POC, but not MAOC, was an important factor influencing microbial necromass accumulation in the BSC horizon and 0–2 cm layer, suggesting that the POC plays an important role in necromass stabilization (Fig. 6 c-d, \( p < 0.01 \)).
4. Discussion

4.1 Microbial necromass contents and their contribution to the SOC pool

Microbial necromass formation is determined by biomass production, death of microorganisms, and cell residue decomposition (Chen et al., 2021; Shao et al., 2021). Enzyme activities increase with living microbial biomass and necromass (Fig. S1). Consequently, rapid turnover of living microbial biomass produces more necromass. A greater amount of living biomass and faster microbial growth result in higher biomass production and further necromass accumulation, thereby contributing to SOC sequestration (Prommer et al., 2019; Chen et al., 2020). The microbial necromass to SOC ratio in the BSC horizon (from cyanobacteria (3–7 years old) to moss (~30 years old)) decreased, and the fungal:bacterial necromass ratio in the 0–2 cm soil layer increased (from bare sand to moss) with biocrust formation (Fig. 1). Consequently, the contribution of undecomposed moss residues to SOC accumulation increased, and thus fungal necromass especially accumulated (Fig. 1f). This is explained by the decomposition of complex organic matter (moss residues) that is dominated by fungi (López-Mondéjar et al., 2018; Algara Gallardo et al., 2021). Fungi are better adapted to degrade persistent plant biomass and organic matter, as well as hydrophobic substances, as they produce specific carbohydrate-active hydrolases (López-Mondéjar et al., 2020) and (per)oxidases (Xia et al., 2020; Deng et al., 2021). Bacteria contributed more to SOC formation in the surface (BSC horizon) than in the subsurface (0–2 cm mineral layer) of biocrust-dominated soil (Fig. 1e) because bacteria tend to recycle microbial necromass and insufficient clay protection in the
BSC horizon, thus accelerating the turnover of living and dead biomass 
(López-Mondéjar et al., 2018; López-Mondéjar et al., 2020);

Microbial necromass is an important organic matter source in biological 
crust-dominated sandy soil that contributes to SOC sequestration (Figs. 1 and 4) by 
incorporating into the POC and MAOC (Figs. 4 and 5). However, inconsistent with 
our first hypothesis, microbial necromass content and its contribution to SOC did not 
increase with the biocrust formation chronosequence (Fig. 1). The contributions of 
microbial necromass to SOC in the BSC horizon and 0–2 cm mineral soil layer range 
from 12% to 25%, much lower than their contributions to fully developed soils: > 50%
in croplands and grasslands and ~ 35% in forests (reviewed by Khan et al., 2016; 
Liang et al., 2019; Huang et al., 2019). This phenomenon is mainly due to the (i) low 
nutrient content and availability of the initial soil formation may limit the microbial 
necromass accumulation compared to well-developed soils (Buckeridge et al., 2020; 
Shao et al., 2021; Wang et al, 2021); (ii) lower necromass accumulation coefficients 
show limiting microbial residue stabilization during the initial soil formation because 
of the very low clay content and poor microaggregation (Figs. 1 and 7). For instance, 
the necromass accumulation coefficient in biocrust-covered sandy soils of the Loess 
Plateau is 9.6 (Fig. 1), which is lower than those of the Qinghai-Tibet Plateau (16), 
Mongolian grassland soils (24), and other grasslands worldwide (30) (Zhang et al., 
2021). Consequently, the contribution of microbial necromass to SOC increases in the 
following order: Loess Plateau biocrust-covered sandy soils (22%) < Qinghai-Tibet 
Plateau (33%) < Mongolian grassland (49%) < other grasslands worldwide (58%)(Fig.
This increase corresponds to the progress of pedogenesis and formation of secondary clay particles and water stable aggregates. The necromass accumulation coefficient is regulated by biotic (microbial nutrient demands, etc.) and abiotic (soil nutrient availability, clay content, etc.) factors (Buckeridge et al., 2020; Zhang et al., 2021). The limited N content of sandy soils is mainly due to insufficient inputs and the low diversity of N fixation pathways (Steven et al., 2012; Li et al., 2020). Thus, the lower accumulation efficiency of microbial necromass C in our soil is because of the sandy texture (low clay content, poor aggregation), which is related to poor necromass preservation (Fig. 5) and/or high microbial N demands (Figs. 2 and 3).

4.2 Microbial necromass stabilization and preservation

Microbial necromass contributes to SOC sequestration by forming particulate and mineral-associated organic C (Figs. 4 and 5; Lavallee et al., 2019). The binding of microbial necromass to soil particles is the main mechanism for their medium- and long-term stabilization (Chen et al., 2020; Samson et al., 2020; Yang et al., 2021), in which organo-mineral interfaces are dominated by N-enriched residues (Buckeridge et al., 2020; Possinger et al., 2020). Although POC and MAOC increased with the microbial necromass C contents, necromass was more closely correlated with POC than with MAOC (Fig. 4). Unlike in developed soils, in which more necromass is found in the MAOC pool (Lavallee et al., 2019; Samson et al., 2020), this result
suggests that more necromass is present in biocrust-dominated soils through POC formation (Figs. 5 and 6). This is explained by (i) microbial necromass recycling or destabilization decreasing its accumulation (Figs. 4 and 7); (ii) the accumulation of MAOC may become saturation due to the limitation of clay and silt content (Fig. 5a; Craig et al., 2021); (iii) formation of POC by part of the microbial necromass (e.g., fungal hyphae), which plays an important role in SOC accrual (Lavallee et al., 2019; Samson et al., 2020); (iv) the retained thin soil water films in aggregates allowed continued microbial recycling of necromass from the MOAC (Manzoni et al., 2012), while the POC decomposition may be limited by low soil water because water-driven microbial nutrient transformations in biocrusts (Maier et al., 2021). This result may suggest that the bacterial necromass mainly contributed to the MAOC pool, while fungi contributed more to POC (Fig. 4). This is possibly because (i) bacteria are smaller and have thinner cell wall fragments compared to fungi (Schweigert et al., 2015); (ii) bacteria inhabit biofilms and soil aggregate pores (Guhra; et al., 2021; Yang et al., 2021), because the attachment of bacteria cells to mineral surfaces and their active habitat formation increases the soil aggregation potentially reducing the bacterial necromass decomposition (Krause et al., 2019).

In the BSC horizon, MAOC did not continuously increase with microbial necromass, presumably due to saturation of MAOC, because of the limited amount of clay and silt particles in biological crust dominated sandy soil (Fig. 5a; Matus, 2021). Microbial necromass stabilization might be limited by the direct sorption of exudates (e.g., released from moss, algae) outcompetes sorption of microbial residues on clay
or silt particles (Walker et al., 2003; Kubiak-Ossowska et al., 2017; Guhra; et al., 2021). Departure from our second hypothesis, the POC increased with microbial necromass (especially fungal necromass) and the POC/MAOC ratio increased with SOC, suggesting that the poor preservation conditions (e.g., limited clay content) leads to more necromass remaining as POC (Figs. 5 and 6). Microbial necromass decomposition is slow in soils with high clay content and formation of stable microaggregates (Hu et al., 2020) because adsorption on clays and protection in microaggregates are key factors controlling physico-chemical and physical stabilization (Doetterl et al., 2015). Indeed, microbial necromass-formed POC lack physico-chemical protection, which leads to their direct contact with living microorganisms and increases the likelihood of their degradation by exoenzymes (Kuzyakov and Mason-Jones, 2018; Lavallee et al., 2019). The living microbial biomass increases, but necromass remains stable, from the cyanobacteria to moss stage in BSC horizons (Fig. 1 and Table 1), suggesting a rapid necromass recycling because of the limited protection by clay or microaggregates as a result of low clay content during initial soil formation (Figs. 5 and 7). This phenomenon occurs mainly because POC generally has rapid turnover (Gunina and Kuzyakov, 2014; Poeplau et al., 2018), whereas the amounts of clay and silt particles (Fig. 5) limit necromass preservation, especially during initial soil formation (Lavallee et al., 2019).

4.3 Microbial necromass accumulation regulated by dissolved N

In line with our third hypothesis, extracellular enzyme stoichiometry revealed
that i) microbial N limitation is common in biological crust dominated sandy soil (Fig. 3), and ii) N-acquiring enzyme activities increased with biocrust formation, accelerating necromass decomposition and alleviating microbial N limitation during initial soil formation (Fig. 2). The release of extracellular enzymes by microorganisms to mineralize organic compounds is an important mechanism to maintain homeostasis of microbial C:N:P ratio and overcome nutrient limitations (Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015). Fungal and bacterial necromass increased with the soil DC and DN levels (Fig. S1), suggesting that necromass decomposition greatly contributes to the formation of a bioavailable nutrient pool. Microbial necromass is richer in N than SOM is (Cotrufo et al., 2019; Liang et al., 2019), and therefore, N mining from necromass is a more efficient strategy than N acquisition from recalcitrant soil organic matter (Buckeridge et al., 2020; Cui et al., 2020). Microbial demand for N leads to necromass mineralization when the available N forms are insufficient (Cui et al., 2020; Čapek et al., 2021).

In view of microbial N limitation and increased N-acquiring (NAG+LAP) enzyme activity/microbial biomass N ratio, as well as poor necromass preservation (Figs. 2, 5 and 7), dissolved N is the most important factor influencing necromass abundance in biocrust-covered sandy soils (Fig. 6). This is mainly because available nutrients require less energy to use than bulk SOM (Liang et al., 2017), which requires microorganisms to produce enzymes (Kaiser et al., 2014; Mooshammer et al., 2014; Čapek et al., 2021). Under N-limited conditions, microorganisms synthesize and release extracellular enzymes (e.g., NAG and LAP) to mobilize microbial
necromass for their N demand (Fig. 2). Consequently, N availability strongly affects
the accumulation or reutilization of microbial necromass during the initial formation
of biocrust-covered sandy soil. This is mainly because the homoeostatic growth of
microorganisms requires an appropriate ratio (e.g., microbial biomass C/N ratio
ranging from 2.4 to 4.3 in the BSC horizon and 0–2 cm soil layer) of nutrients (e.g., C,
N, and P) to maintain the stoichiometric balance that drives microbial metabolic
processes (Mooshammer et al., 2014; Liang et al., 2020). For example, the
homeostasis index (1/H) ranged from 0 to 0.24, indicating that the microbial C/N
stoichiometry in biocrust-covered sandy soil was homeostatic (Fig. S2). Microbial
community growth on various resources with extremely variable or low C/nutrient
ratios cannot be achieved solely via nonhomeostatic methods (Mooshammer et al.,
2014; Zechmeister-Boltenstern et al., 2015). Microorganisms require stoichiometric
homeostasis to maintain normal life activities by adjusting their own elemental
utilization efficiency or mobilizing resources by producing specific extracellular
enzymes (Mooshammer et al., 2014). Therefore, microorganisms maintain C/N
stoichiometric homeostasis by using bioavailable soluble substances or microbial
necromass (Kaiser et al., 2014; Chen et al., 2019) as well as nutrient recycling (Chen
et al., 2019; Bilyera et al., 2021). Potential shifts in microbial C/N stoichiometry and
dissolved organic C/N influence necromass accumulation (Fig. S3) because
stoichiometry controls nutrient mining from necromass (Cui et al., 2020; Čapek et al.,
2021).
5 Conclusions

Microbial biomass, extracellular enzymes, available nutrients, particulate and mineral particles determine the accumulation and recycling of necromass during initial soil formation. Microbial N limitation was common in all biocrust stages, but it decreased during biocrust development due to the increase in N-acquisition enzymes. MAOC did not always increase, but POC increased with microbial necromass, suggesting that the MAOC increase was limited by low clay content and consequently, led to stronger POC than MAOC accumulation in the BSC horizon. A potential explanation for the accumulation of POC relative to MAOC may also be that the retained thin soil water films in aggregates allowed necromass recycling from the MAOC, whereas POC decomposition might be limited by low soil water, but this needs further investigation in the future. Microbial N limitation and necromass destabilization (e.g., insufficient clay protection) lead to rapid necromass reutilization by microorganisms, resulting in a low necromass accumulation coefficient in biocrust-covered sandy soil. Microorganisms meet their nutrient demands by releasing extracellular enzymes to degrade necromass, and its decomposition forms a pool of dissolved nutrients. As a result, the microbial necromass C contributes to SOC in biocrust-covered sandy soil much lower than in fully developed soils. Consequently, the initial soil formation by biocrusts is characterized by strong N limitation and low clay content, leading to high activities of enzymes, especially of the N cycle, poor protection and stabilization of microbial necromass, leading to intensive reutilization and thus a low contribution of microbial residues to SOC sequestration.
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**Figure legends:**

**Fig. 1.** Dynamics of fungal necromass carbon (C) and bacterial necromass C, fungal:bacterial necromass ratio, microbial necromass C contribution to soil organic C, fungal and bacterial necromass C contribution to soil organic C, fungal necromass to total microbial necromass C ratio, necromass accumulation coefficient (microbial necromass C/living microbial biomass C: necromass accumulation per unit of microbial biomass C) in biological soil crusts (BSC) horizon and in the 0–2 cm of BSC-underlying mineral soil during 5 stages of BSC formation: bare sand (~0–1 years old), cyanobacteria stage (Cy, ~3–7 years old), cyanobacteria-moss stage (Cy-Mo, ~8–13 years old), moss-cyanobacteria stage (Mo-Cy, ~20–25 years old), and moss stage (Mo, ~30 years old). Total microbial necromass C was estimated as the sum of fungal and bacterial necromass C. Values are presented as the means of 5 replicates ± standard error (SE). Lowercase and uppercase letters indicate significant differences between the biological soil crust stages (p < 0.05). SOC, soil organic carbon; MNC, microbial necromass carbon.

**Fig. 2.** Activities of β-1,4-glucosidase (BG), β-1,4-N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP) and alkaline phosphatase (AP), C- and N- acquiring enzyme activity/microbial biomass C (MBC)- and N (MBN)- ratio (specific enzyme activity per microbial biomass unit: microbial enzyme activity coefficient) in the biological soil crust (BSC) horizon and in the 0–2 cm of BSC-underlying mineral soil during 5 stages of BSC formation: bare sand, cyanobacteria stage (Cy),
cyanobacteria-moss stage (Cy-Mo), moss-cyanobacteria stage (Mo-Cy), and moss stage (Mo). Values are presented as the means of 5 replicates ± standard error (SE). Letters indicate significant differences between the biological soil crust stages ($p < 0.05$).

**Fig. 3. Top:** Vector length and angle of enzymatic stoichiometry in the BSC horizon and 0–2 cm soil reflecting the microbial C and N limitations during biocrust formation (5 stages): bare sand, cyanobacteria stage (Cy), cyanobacteria-moss stage (Cy-Mo), moss-cyanobacteria stage (Mo-Cy), and moss stage (Mo). Values are the means of 5 replicates ± standard error (SE). Lowercase and uppercase letters indicate significant differences in BSC and 0–2 cm between the biological soil crust stages, respectively. A vector angle of $< 45^\circ$ denotes N limitation (red horizontal dashed line), and angles $> 45^\circ$ denote P limitation. **Bottom:** Stoichiometric analysis of soil enzyme activities to identify the C, N and P limitations in soil. By using 1.0 as a horizontal and vertical baseline along the axes of enzyme activity ratios (NAG+LAP/AP as x-axis and BG/NAG+LAP as y-axis), four groups of microbial resource limitations were categorized: N limitation, P limitation, C and N colimitation, and C and P colimitation. Nearly all stages (except Bare sand) and replicates are clearly N limited (light yellow shaded area).

**Fig. 4.** Regressions of soil organic C with particulate and mineral-associated organic C (POC and MAOC); soil organic C (SOC) a fungal, bacterial and microbial
necromass C; mineral-associated organic C with fungal, bacterial and microbial
necromass C; and particulate organic C with fungal, bacterial and microbial
necromass C. All regression lines are significant at $p < 0.001$. BNC, bacterial
necromass C; FNC, fungal necromass C; MNC, total microbial necromass C. The
black dashed line indicates a 1:1 relationship.

Fig. 5. Regressions of microbial necromass C with particulate (POC) and
mineral-associated organic C (MAOC), soil organic C (SOC) with the POC/MAOC
ratio, and soil clay and silt content with bacterial and fungal necromass C. All
regression lines are significant at $p < 0.05$. The black dashed line indicates a 1:1
relationship.

Fig. 6. Redundancy analysis (RDA) identifies the relationships between microbial
biomass, extracellular enzyme activities, available nutrients, particulate organic C,
mineral-associated organic C, clay content, and microbial necromass C. The red
arrows indicate explanatory variables, and the black arrows indicate response
variables. The points represent the stages of biocrust formation. The explanatory ratio
we use in this study is the simple effect. The light red bars represent microbial
biomass C, N, P content, the light green bars represent the four extracellular enzyme
activities and the light blue bars represent other soil properties. BNC, bacterial
necromass C; FNC, fungal necromass C; MNC, total microbial necromass C; GlcN,
glucosamine; GalN, galactosamine; ManN, mannosamine; MurA, muramic acid; F/B necromass, fungal/bacterial necromass; DC, dissolved carbon; DN, dissolved nitrogen; DP, dissolved phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus; BG, β-1,4-glucosidase; NAG, β-1,4-N-acetyl-glucosaminidase; LAP, leucine aminopeptidase; AP, alkaline phosphatase; POC, particulate organic carbon; MAOC, mineral-associated organic carbon. *p < 0.05, **p < 0.01.

Fig. 7. Conceptual framework for the preservation, decomposition and stabilization of microbial necromass in biological crust dominated sandy soils. Microbial N demand (X axis) and clay conservation (Y axis) regulate microbial necromass decomposition and accumulation, further determining particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) pool formation. Saturation of MAOC accumulation due to low clay content led to more microbial necromass being incorporated into the POC pool. However, the increase of soil clay content by weathering eliminates MAOC saturation during pedogenesis. MAOC did not always increase after saturation, but POC increased with the microbial necromass, suggesting that the limitation of MAOC increase by low clay content contributed more to POC than MAOC accumulation. The curved dotted arrow shows the accelerated POC compared to MAOC accumulation after the MAOC saturation. The N limitation of microorganisms and limited clay protection and MAOC saturation resulted in a lower necromass accumulation coefficient, which led to a much lower contribution of
microbial necromass to SOC in the initial formation of biocrust-covered sandy soil.
Table 1 The content of carbon (C), nitrogen (N), and phosphorus (P) contents in the bulk soil, microbial biomass and dissolved matter, amino sugar (glucosamine (GlcN), galactosamine (GalN), mannosamine (ManN) and muramic acid (MurA)), particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) and the mass ratio of POC to MAOC, and soil clay (%), silt (%) and sand (%) contents in biological soil crusts (BSC) horizon and in the 0–2 cm of BSC-underlying mineral soil during 5 stages of BSC formation: bare sand (~ 0–1 years old), cyanobacteria stage (Cy, ~ 3–7 years old), cyanobacteria-moss stage (Cy-Mo, ~ 8–13 years old), moss-cyanobacteria stage (Mo-Cy, ~ 20–25 years old), and moss stage (Mo, ~ 30 years old). Values are the means of 5 replicates ± standard error (SE). Lowercase and uppercase letters indicate significant differences in BSC and the 0–2 cm soil horizons between the biological soil crust stages, respectively (p < 0.05).

<table>
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<th></th>
<th>Bare sand 0-2 cm</th>
<th>BSC 0-2 cm</th>
<th>Cyanobacteria-moss 0-2cm</th>
<th>Moss-cyanobacteria 0-2cm</th>
<th>Moss 0-2cm</th>
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<td>SOC (g kg⁻¹)</td>
<td>2.44±0.29B</td>
<td>15.2±3.08B</td>
<td>6.73±1.97A</td>
<td>17.8±3.20A</td>
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<td>TN (g kg⁻¹)</td>
<td>0.10±0.01B</td>
<td>1.10±0.27B</td>
<td>0.46±0.14A</td>
<td>1.14±0.26B</td>
<td>0.42±0.11A</td>
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<td>TP (g kg⁻¹)</td>
<td>0.19±0.02B</td>
<td>0.40±0.02B</td>
<td>0.32±0.03A</td>
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<td>MBC (mg kg⁻¹)</td>
<td>12.3±6.44C</td>
<td>812±204B</td>
<td>143±60AB</td>
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<td>MBN (mg kg⁻¹)</td>
<td>6.25±2.82C</td>
<td>259±44b</td>
<td>59±27AB</td>
<td>273±25b</td>
<td>52±18B</td>
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<td>MBP (mg kg⁻¹)</td>
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<td>7.39±1.56b</td>
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<td>4.38±0.90A</td>
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<td>Clay (%)</td>
<td>0.21%±0.08%</td>
<td>1.89%±0.45%</td>
<td>1.58%±0.67%</td>
<td>47.4%±11.3%</td>
<td>6.84%±1.15%</td>
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<td>Silt (%)</td>
<td>92.9%±1.20%</td>
<td>6.84%±1.15%</td>
<td>47.4%±11.3%</td>
<td>6.84%±1.15%</td>
<td>92.9%±1.20%</td>
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<tr>
<td>Sand (%)</td>
<td>50.8%±11.7%</td>
<td>6.84%±1.15%</td>
<td>47.4%±11.3%</td>
<td>6.84%±1.15%</td>
<td>50.8%±11.7%</td>
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Note: All values are given as mean ± standard deviation. The letters indicate significant differences among treatments at p < 0.05.
Supplementary Figures

**Fig. S1.** Relationships between fungal, bacterial, and microbial necromass and C, N, and P in microbial biomass and dissolved C, N and P and enzyme activities. Only the regression lines significant at $p < 0.05$ are presented. BG, $\beta$-1,4-glucosidase; NAG, $\beta$-1,4-N-acetyl-glucosaminidase; LAP, leucine aminopeptidase; AP, alkaline phosphatase. BNC, bacterial necromass C; FNC, fungal necromass C; MNC, total microbial necromass C.

**Fig. S2.** Relationships between the log of microbial biomass carbon/nitrogen (C/N) ratio and log of ratio of the C/N in bulk soil, dissolved matter and enzymes. Only the regression lines significant at $p < 0.05$ are presented. The coefficient $1/H$ is the slope of the regression between log (resource ratio, C/N in bulk soil, dissolved matter and enzyme) and log (biomass ratio, microbial biomass C/N), which represent the degree of microbial stoichiometric homeostasis. The regression relationship was nonsignificant ($p > 0.05$), $1/H$ was set to zero, and the microorganisms were considered “strictly homeostatic”. Microorganisms maintain constant biomass C/N ratios regardless of the changes in C/N in bulk soil, dissolved matter and enzymes.

**Fig. S3.** Relationships of the log of fungal and bacterial necromass C, log of microbial biomass C/N and soil dissolved organic C/N. FNC, fungal necromass C; BNC, bacterial necromass C; MNC, microbial necromass C.
Preservation, decomposition and stabilization of microbial necromass in biocrusts

Clay saturation with MAOC

Microbial N demand and clay protection control

SOC accumulation

MAOC formation

POC formation

Necromass accumulation coefficient

Necromass recycling

Low

High

Low

Microbial N demand

High

Bare sand 0-1 years

Cyanobacteria 3-7 years

Cyanobacteria-Moss 8-13 years

Moss-Cyanobacteria-Moss 20-25 years

Moss 30 years
Highlights:

- Microbial necromass C contribution to SOC in biocrust-covered sandy soils was less than 25%.
- Biocrust-covered sandy soils have a low necromass accumulation coefficient.
- Low soil clay content leads to more microbial necromass forming particulate organic carbon.
- Microbial nitrogen limitation was common in biocrust formation sequences.
- Microbial N limitation and insufficient clay protection control the necromass dynamics.
Declaration of interests

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

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: