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Review

Microbial extracellular polymeric substances (EPS) in soil: From interfacial behaviour to ecological multifunctionality

Ming Zhang, Yichao Wu, Chenchen Qu, Qiaoyun Huang and Peng Cai

National Key Laboratory of Agricultural Microbiology, College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China

Abstract

Extracellular polymeric substances (EPS) are high molecular weight polymers that microorganisms secrete into their extracellular environment. EPS serves as the carrier of the structural integrity of microbial biofilms, determining the physicochemical properties and the functional complexity of biofilms. EPS creates an ideal environment for interfacial reactions and nutrient trapping around microbial cells, while also acting as a buffer zone against environmental stresses. EPS in soil can contribute to soil health through its own properties such as adhesion, hygroscopicity and complexing ability. Here, we first introduce the concept, components, properties and controlled factors of EPS in the soil environment, and outline current advances in extraction methods and characterization techniques for soil EPS. EPS form a dynamic biophysical-chemical interface between microbes and the soil matrix. We explore the role of EPS in the colonization and survival of microorganisms, aggregation and weathering of soil minerals, and cross-linking with soil organic matter. We then summarize the soil ecological functions of microbial EPS: 1) promoting aggregate formation and stabilization; 2) enhancing water retention and holding capacity; 3) mediating nutrient storage and trapping; and 4) regulating contaminant sequestration and transformation. Finally, we propose several future research interests for microbial EPS in soil, thereby calling for more attention and research on microbial EPS and its functions in soil ecosystems, and exploring their potential applications in the development of environment-friendly agriculture.

Keywords: ecological functions; extracellular polymeric substances (EPS); interfacial reactions; soil aggregate; soil biofilm; soil health

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Introduction

Soil is the product of terrestrial biogeochemical processes and an essential foundation for human survival. Microorganisms endue soil the property of life and drive the biogeochemical cycles within it. Meanwhile, microorganisms play a vital role in soil structure improvement, soil fertility enhancement, soil pollution control, response to global climate change, and contributing to the Earth's habitability. Microorganisms in soil mainly adhere to the surface of soil minerals and organic matter in the form of microcolonies or biofilms (Burmølle *et al.*, 2011; Flemming and Wuertz, 2019). Biofilms are microbial colonies (bacteria, algae, fungi and/or archaea) embedded in self-produced extracellular polymeric substances (EPS) and attached to the organic-inorganic interface (Flemming and Wingender, 2010). The synthesis and secretion of EPS are costly and energy-consuming processes for cells (Flemming and Wingender, 2010). From the perspective of biological evolution, biofilm formation is a life strategy for microorganisms to enhance their survival ability in the environment (Burmølle *et al.*, 2011). Morphology and properties of microbial biofilms in soil are rarely considered in current studies. For example, Bystrianský *et al.* (2019) used glass microfibre filters to create separation traps in an attempt to separate biofilm communities and planktonic communities in soil bacteria, revealing significant differences between the bacterial communities of the two life strategies. Wu *et al.*, (2019) found that high nutrient inputs favoured biofilm formation in artificial soil, and microbial community diversity, evenness index and metabolic activity were enhanced. Deepening research on soil biofilms is a critical next step to better understand and manage biologically mediated nutrient turnover and soil health (Burns *et al.*, 2013; Cai *et al.*, 2019).

Biofilms are composed of microbial cells and EPS, where the cells generally account for 10–20% of the biofilm's dry mass, while EPS can make up as much as 80–90% (Flemming and Wingender 2010). In soil environments, EPS acts as a bridging zone between cells and the soil matrix, mediating interfacial reactions between microbes and soil minerals or organic substances (Schulz and Manies 2022). EPS secreted by microbes provides effective protection for the cells, such as supplying nutrients under starvation conditions, maintaining moisture during drying processes, resisting the toxicity of pollutants to cells, and alleviating the damage to cells caused by rapid changes in temperature, pH and salinity in soils (Costa *et al.*, 2018). EPS also endows microbes with various ecological advantages, including enhancing colony adhesion, maintaining habitat heterogeneity, altering genetic material transfer, and

Corresponding authors: Yichao Wu and Peng Cai; Emails: wuyichao@mail.hzau.edu.cn; cp@mail.hzau.edu.cn

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providing extracellular enzyme storage and nutrient capture (Flemming *et al.*, 2023). The many benefits of microbial EPS in soil have yet to receive sufficient attention for maintaining soil health. For instance, the adhesive properties of EPS can enhance the stability of soil aggregates (Guhra *et al.*, 2022), and the retention of extracellular enzymes by EPS may contribute to the metabolic stability of soil (Burns *et al.*, 2013). Quantifying the composition and structure of soil EPS can be used to reveal the microscopic mechanisms of soil microbes' response to environmental changes and clarify the role of microbial EPS in soil functions, thereby contributing to ensuring soil health and achieving sustainable green agriculture. Here, we focus on outlining the research progress of microbial EPS from the perspective of soil, elaborating on the aspects of interfacial reactions and ecological functions (Fig. 1). We explore points of interest for future research on soil EPS, with a view to exploring its potential application value in environmentally friendly agricultural development.

Microbial EPS in the soil environment

Physical and chemical properties of EPS

Extracellular polymeric substances are a class of macromolecular biopolymers released by microorganisms (bacteria, fungi and archaea) during their growth and metabolism (Wingender *et al.*, 1999). Biopolymers in EPS provide structural support for microbial biofilms to resist shearing forces through five non-covalent forces (hydrogen bonds, ionic interactions, electrostatic interactions, hydrophobic interactions and entanglement) (Flemming *et al.*, 2023). The molecular weight of bacterial EPS has been reported to range from 10^5 to 10^6 Dalton, and high molecular weight EPS contributes to strong bridging and flocculation activities (More *et al.*, 2014). The main components of EPS are polysaccharides, proteins, lipids, and extracellular DNA (Flemming and Wingender, 2010). EPS-polysaccharides are the most studied matrix components, which can be grouped into homopolysaccharides (dextran, curdlan, cellulose, etc.) and heteropolysaccharides (alginate, xanthan, gellan, hyaluronic acid, etc.). EPS-polysaccharides vary widely in composition and structure based on the reported EPS secreted by different strains. The physical properties of heteropolysaccharides are determined by the bonding degree between the monosaccharide units and side chain branches. The presence of uronic acids and their derivatives regulates the charge properties of EPS macromolecules (More *et al.*, 2014). EPS-protein components have functions as structural proteins and extracellular enzymes. Structural or non-enzymatic proteins are involved in the formation of the extracellular matrix network and contribute to the connection of microorganisms with their surroundings. Glycoproteins are produced when sugar moieties are covalently cross-linked on proteins, which perform various functions such as promoting bacterial aggregation via lectin-like proteins (Park and Novak, 2009). The extracellular enzyme can hydrolyze exogenous substrates such as water-soluble/insoluble polymers and organic particles, and target the hydrolysis of EPS from homologous or heterologous bacteria (Costa *et al.*, 2020b). eDNA in EPS matrix is released through active secretion or controlled lysis by microorganisms. eDNA regulates the early spatial shaping and later structural stability of biofilms, and plays important roles in bacterial adhesion to solid surfaces and horizontal gene transfer (Okshevsky and Meyer, 2015; Peng *et al.*, 2020). Lipids and lipid derivatives in the EPS matrix are involved in the adhesion process and act as biosurfactants. Lipopolysaccharide promotes the adhesion of *Thiobacillus ferrooxidans* onto pyrite

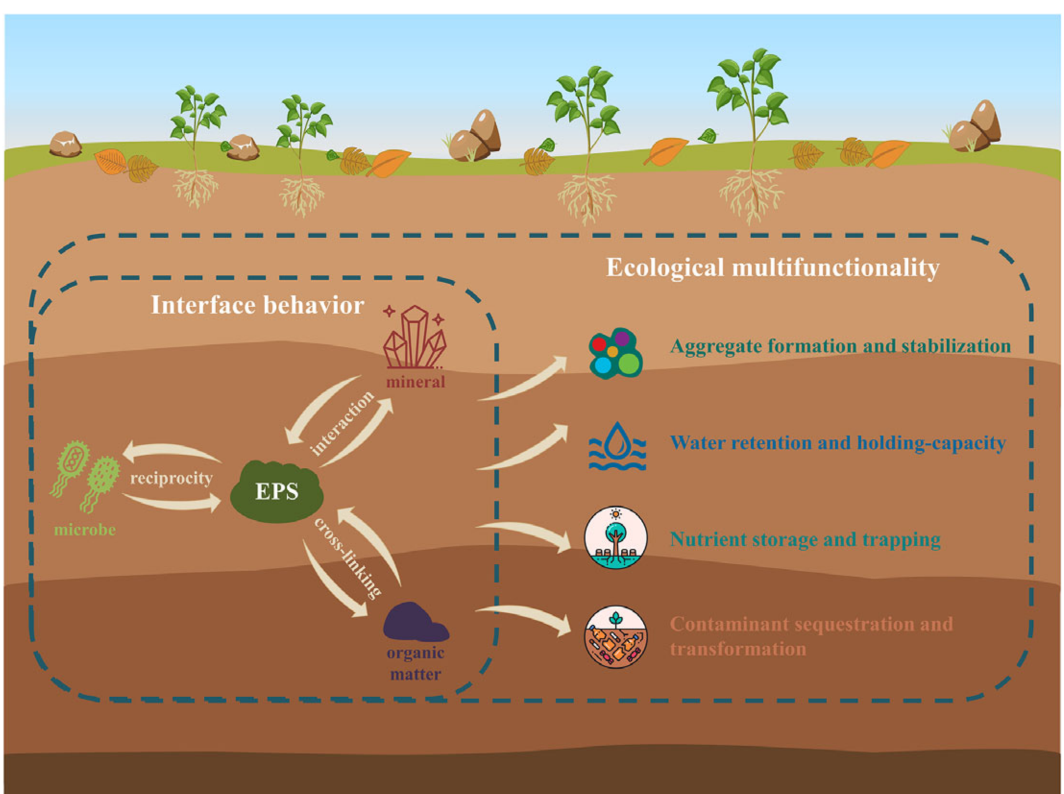

Figure 1. Conceptual framework for the interfacial reactions and ecological functions of microbial extracellular polymeric substances (EPS) in soils.

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surfaces. The biosurfactants rhamnolipid and lipopeptide are found to be present in the EPS matrix of *Pseudomonas aeruginosa* and *Bacillus subtilis*, respectively (More *et al*., 2014).

EPS composition as a function of the surrounding environment

The secretion and composition of microbial EPS are affected by strain type, growth stage, substrate availability and physicochemical environmental parameters. *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* showed EPS synthesis in the stationary phase of growth, while *Azotobacter vinelandii* continuously produced EPS-polysaccharides such as alginate, cellulose, and gellan throughout its growth process. *Pseudomonas* sp. secretes EPS during the exponential and stationary phases, but produces EPS with different chemical structures (Saha *et al*., 2020). Most microorganisms maximize EPS production at near-neutral pH. However, researchers have found that the EPS composition of native biofilms exhibits conservatism under extreme pH environments, indicating that different microorganisms secrete similar EPS components to resist environmental stressors (Blanco *et al*., 2019). The optimal temperature for EPS production by microorganisms depends mainly on the strain type and its natural environmental temperature, and most microorganisms have been reported to produce higher amounts of EPS in the temperature range of 25–30°C. The availability of oxygen is also one of the triggering mechanisms for EPS production. The increase or decrease in EPS production induced by oxygen concentration is strain-dependent (More *et al*., 2014). Stress factors can stimulate microorganisms to secrete EPS. Roberson and Firestone (1992) found that desiccation stress induced microbes to secrete more EPS in a simulated soil system. Kazy *et al*. (2002) found that *Pseudomonas aeruginosa* showed a four-fold increase in EPS production in response to heavy metal Cu stress. Microorganisms have different preferences for carbon and nitrogen sources, and in turn the concentration and type of substrates greatly affect the production efficiency and chemical properties of the corresponding EPS. The easily available carbon substrate glycerol can stimulate the production of EPS-polysaccharides in oligotrophic soils (Redmile-Gordon *et al*., 2014), whereas the insoluble carbon substrate chitin can stimulate the microbial community to produce EPS with better water retention properties (Bhattacharjee *et al*., 2020). Nutrient supply with appropriate carbon-to-nitrogen ratios significantly stimulates microbial EPS secretion and biofilm formation in artificial soil (Wu *et al*., 2019), while excessive inorganic nitrogen input hinders the production efficiency of soil EPS (Redmile-Gordon *et al*., 2015b).

Extraction and characterization of soil EPS

The specific extraction of soil EPS and its subsequent analysis are the key to understanding its ecological function in soil. The cation exchange resin (CER) method was initially used to extract EPS from activated sludge (Frølund *et al*., 1996) and was subsequently widely recommended for extracting EPS from various environmental objects, such as microbial biofilms, algae, sludge and sediments (Zhang *et al*., 2023). Redmile-Gordon *et al*. (2014) first demonstrated that the CER method was suitable for extracting EPS from sandy soil, which can minimize the intracellular contamination caused by microbial lysis and the co-extraction of extracellular non-target organic matter. The CER method was further proven to be the best conservative method for extracting EPS from Fe- and Ca-rich soils (Wang *et al*., 2019; Bérard *et al*., 2020). The extracellular specificity of this method has also been validated in silty-clay loams by stable isotope probing, which showed that newly synthesized EPS is preferentially extracted over potentially contaminating organic matter (Redmile-Gordon *et al*., 2015b). Redmile-Gordon *et al*. (2013) optimized the Lowry assay to estimate protein in soil EPS, and subsequently confirmed the reliability of colorimetric protein quantification using mass spectrometry (Redmile-Gordon *et al*., 2015a). The polysaccharide and uronic acid contents in soil EPS extracts were quantified using the phenol-sulfuric acid method and *meta*-hydroxydiphenyl assay, respectively (Zhang *et al*., 2023). The bicinchoninic acid (BCA) method was recently proposed to quantify polysaccharides in extracts of soil EPS (Bublitz *et al*., 2023). However, while the BCA method uses less-toxic reagents, Bublitz et al.'s paper (2023) contains no comparison of results to the commonly applied method of Dubois *et al*. (1956) and contains misleading guidance with regard to EPS extraction from soil. Bublitz *et al*. (2023) interpreted an absence of measurable carbohydrates in their initial extract as supporting their claim that the 'Step 1' extraction with $CaCl_2$ was unnecessary in agricultural soils. However, this overlooks the requirement to remove soluble interferences which can vary greatly due to the timing of fertilizer applications, plant expression and rainfall at the site before sampling (Redmile-Gordon *et al*., 2014). While Bublitz *et al*. (2023) claimed that most agricultural soils will not contain sufficient labile carbon to interfere, the rhizosphere is a well-recognized hotspot of both EPS and labile carbon availability (Redmile-Gordon *et al*., 2020). Moreover, by application of advanced spectral-chemometrics and analytical procedures, Zhang *et al*. (2023) showed that Step 1 extraction with 0.1 M $CaCl_2$ removed significant amounts of dissolved organic carbon, soluble nitrogen compounds, polysaccharide and uronic acids from an agricultural soil that would otherwise have contributed to false measures of EPS in the second step. Zhang *et al*. (2023) applied excitation-emission-matrix fluorescence spectroscopy and demonstrated that $CaCl_2$ maintained the integrity of EPS for extraction using CER in the second step. The insight provided by spectral chemometrics serves as a cautionary tale against ad-hoc modification of techniques specifically designed to remove variable and ephemeral artefacts in soils.

EPS as dynamic biophysical-chemical interfaces between microbes and the soil matrix

Colonization and survival of microorganisms

Extracellular polymeric substances act as a physical barrier between the cell membrane and its surrounding matrix, and play an important role in microbial physiological characteristics and ecological adaptation (Flemming *et al*., 2023). Microbial EPS regulates the adhesion behaviour of its cells onto solid surfaces. Strains with low EPS secretion inhibit bacterial adhesion through electrostatic repulsion, while strains with high EPS secretion enhance cell adhesion through interactions between EPS functional groups such as glyoxylate groups and acetyl groups (Tsuneda *et al*., 2003). The removal of EPS from the bacterial surfaces decreased the adhesion between bacteria and clay minerals or soil particles, but increased the adhesion to goethite by forming P–O–Fe bonds (Hong *et al*., 2013; Nkoh *et al*., 2020). Zhao *et al*., (2015) found that the removal of EPS inhibited the adhesion of *Streptococcus suis* to soil particles and enhanced the adhesion of *Escherichia coli.* Chemical bond formation and electrostatic interactions are the main mechanisms controlling bacterial adhesion to soil particle surfaces (Ren *et al*., 2018b). The outer membrane *c*-type cytochromes OmcA and MtrC

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of *Shewanella oneidensis* MR-1 play dominant roles in the initial and late stages of its colonization on iron minerals, respectively (Jing *et al.*, 2020). The EPS matrix provides an ideal microenvironment for microorganisms to survive even under adverse external conditions. Escudero *et al.* (2018) verified the presence of active biofilms in oligotrophic porous subsurface rocks by catalyzed reporter deposition-FISH (CARD-FISH) combined with fluorescence lectin-binding assay (FLBA) (Fig. 2). Traces of EPS components were observed around all colonies containing bacteria and archaea. However, in soils where the living conditions are more favourable, the porous three-dimensional structure and compositional complexity hinder the visualization of its biofilms.

Aggregation and weathering of minerals

Microbial EPS has been referred to as a 'slime' or 'glue', and its rich functional groups make it easy to adhere to mineral surfaces to form mineral-organic associations (Flemming, 2011; Kleber *et al.*, 2015). The main minerals forming organic-mineral associations involve clay minerals, iron/aluminium/manganese oxides and carbonates (Totsche *et al.*, 2018). The formation of associations directly affects the fate of microbial-derived organic matter and the reactivity of minerals (Fig. 3). The EPS-proteins were adsorbed predominantly on the surfaces of montmorillonite and kaolinite by electrostatic interaction and hydrogen bonding, while the EPS-nucleic acids were adsorbed preferentially on the surface of goethite by ligand exchange (Cao *et al.*, 2011; Lin *et al.*, 2016b). The coverage of EPS components on mineral surfaces was observed as heterogeneous patches under dehydrated conditions (Liu *et al.*, 2013). The different formation pathways of organic-mineral associations (adsorption and coprecipitation) significantly affect the selective retention of EPS components on iron and aluminium oxides (Mikutta *et al.*, 2011; Zhang *et al.*, 2021b). Ren *et al.*, (2018a) explored the adsorption capacity of bacterial EPS to soil colloids under different pH and ionic strengths, and proved that EPS-proteins and phosphate groups contribute to the adsorption of EPS onto soil colloids. The adsorbed EPS also changes the mineral surface charge and its aggregation behaviour. EPS-polysaccharides promote nanoparticle aggregation through intermolecular bridging, while EPS-proteins contribute to nanoparticle stabilization (Lin *et al.*, 2016a). The aggregation extent of mineral particles is affected by the pH, ionic strength and EPS concentration in the system (Lin *et al.*, 2018). EPS can serve as a binding agent or dispersant according to the surface characteristics of soil minerals, which are mainly driven by electrostatic interaction and steric hindrance (Guhra *et al.*, 2019). In addition, the retention of moisture and organic acids within the EPS matrix may allow mineral weathering at higher rates than those experienced during soil drying (Finlay *et al.*, 2020). Gazzè *et al.*, (2013) used atomic force microscopy to demonstrate the presence of EPS halos around *Paxillus involutus* hyphae colonizing mineral surfaces. Microbial EPS halos may enhance mineral weathering by promoting the accumulation of weathering agents such as organic acids and acidic polysaccharides (Fig. 3b), but further quantification of localized concentrations of these molecules is necessary.

Cross-linking and enzymatic activity of organic matter

The EPS matrix can serve as a protective carrier for extracellular enzymes, and facilitates efficient cleavage of exogenous organic matter with high molecular weight prior to nutrient uptake. Extracellular enzyme retention in the EPS matrix promotes the

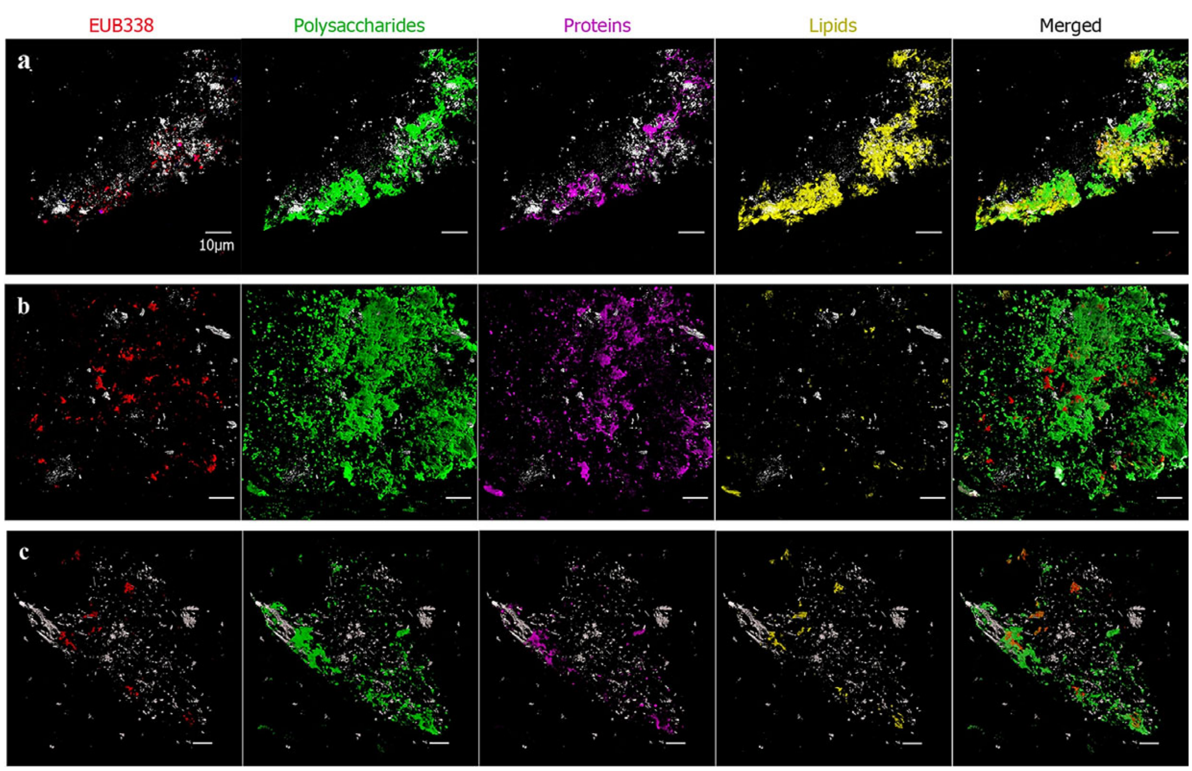

Figure 2. Bacterial biofilms and their EPS components in subsurface rocks at different depths detected by CARD-FISH and FLBA analysis. Bacteria (red), EPS-polysaccharides (green), EPS-proteins (violet) and EPS-lipids (yellow) at 3557 mbs (a), 420 mbs (b) and 5191 mbs (c). Scale bar 10 μm. Reproduced with permission from Escudero *et al.* (2018). Copyright 2018 Springer Nature.

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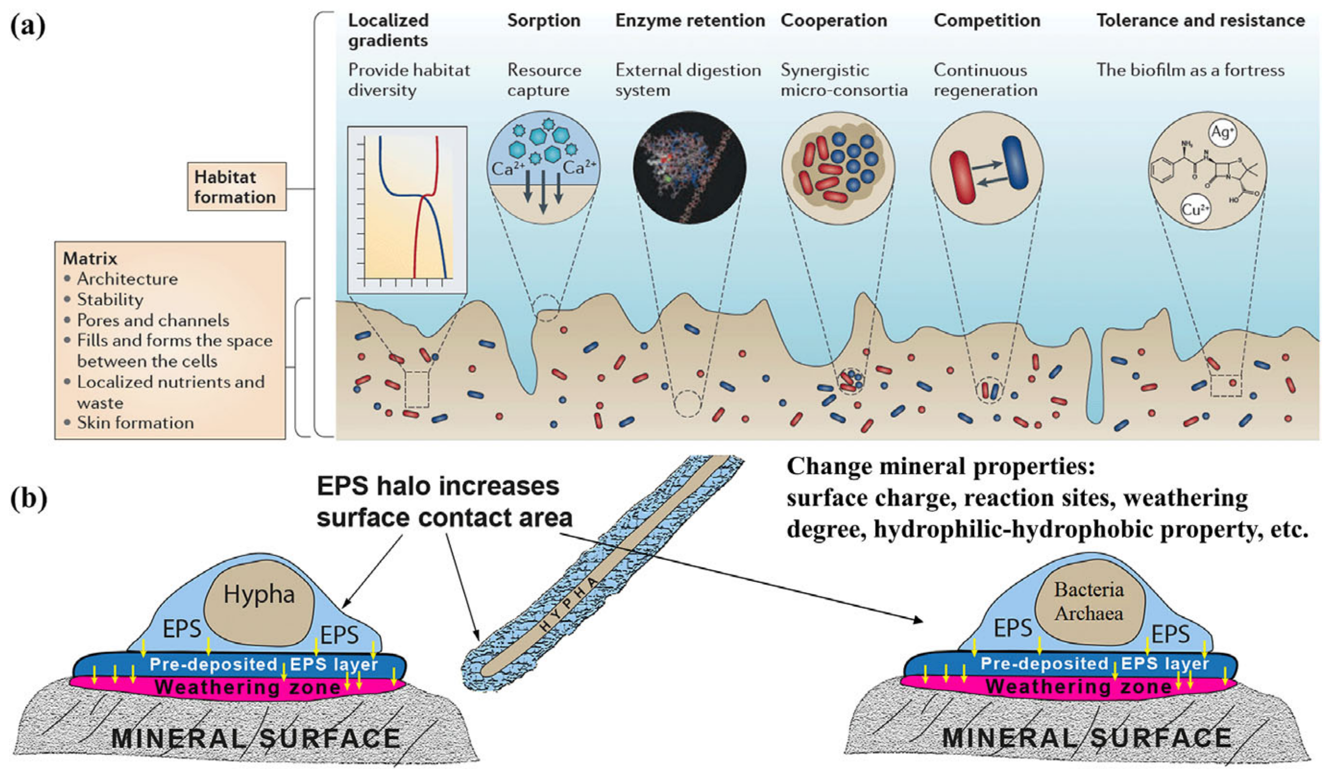

Figure 3. (a) Schematic diagram showing the structure and function of biofilms and the biological and chemical processes affected by them. (b) EPS halos of microorganisms (fungal hypha, bacteria and archaea) and their possible effects on mineral surfaces. Adapted with permission from Finlay *et al.* (2020). Copyright 2020 European Geosciences Union.

formation of an extracellular digestive system (Fig. 3a) (Burns *et al.*, 2013; Op De Beeck *et al.*, 2021). EPS promotes close proximity between extracellular enzymes and organic matter, which helps to keep the cost of microbial metabolism at a low level. EPS can deliver extracellular enzymes to distant substrates along the movement of soil moisture, and capture the nutrients decomposed by the extracellular enzymes and transfer them back to the biofilm during contraction. The sharp contraction/expansion properties of EPS also help to maintain soil pore space, promote gas diffusion, and preserve soil heterogeneity. Extracellular enzymes can be stabilized by their interaction with the EPS matrix. The catalytic process of extracellular enzymes on soil organic matter is considered to have 'enzymatic memory' (Dilly and Nannipieri, 2001), and Kemmitt *et al.* (2008) have proposed the 'Regulatory Gate' hypothesis for abiotic processes controlling soil organic matter mineralization. The protective effect of soil EPS on extracellular enzymes may be a key link to unravelling the mystery of soil organic matter dynamics.

Roles of microbial EPS for soil health

Aggregate formation and stabilization

Soil microorganisms secreting EPS are important for improving soil aggregates, which are key parameters for sustainable soils. Microorganisms have a clear positive impact on soil aggregates. Bacteria have a strong contribution to both macroaggregates and microaggregates, while fungi have a strong impact on macroaggregates. Non-motile bacteria have a greater impact on soil aggregates than motile bacteria, especially on microaggregates. This may be attributed to the production of EPS by non-motile bacteria and the subsequent formation of biofilms (Lehmann *et al.*, 2017).
The relationship between EPS and aggregate stability was explored by adding EPS-producing bacteria or EPS components to the soil. The genera *Bacillus* and *Pseudomonas* are well-known soil bacteria that can secrete EPS and form biofilms (Costa *et al.*, 2018). Inoculation of bacteria with high EPS production can significantly improve the stability of soil aggregates, such as *Pseudomonas putida* GAP-P45, *Bacillus amyloliquefaciens* HYD-B17, *Bacillus licheniformis* HYTAPB18, *Bacillus subtilis* RMPB44, *Pseudomonas chlororaphis* A20 and *Bacillus proteolyticus* A27 (Sandhya and Ali, 2015; Cheng *et al.*, 2020). The ability of bacteria to improve soil aggregates formation is influenced by the ability of strains to secrete EPS, the composition and structure of EPS, and soil type (Costa *et al.*, 2018).

Revealing the role of soil EPS in aggregate turnover based on natural soils has been explored due to having overcome some methodological difficulties. Soil EPS components can sensitively respond to indirect effects of environmental factors such as soil texture, land use patterns and vegetation types (Bérard *et al.*, 2020). Some studies have reported a positive correlation between EPS polysaccharides and soil aggregates (Fig. 4a) (Zethof *et al.*, 2020; Bettermann *et al.*, 2021; Hale *et al.*, 2021). Redmile-Gordon *et al.*, (2020) found that extractable EPS in soil is mainly affected by current land use patterns, and there is a good correlation between EPS-proteins content and aggregate stability (Fig. 4b). The cultivation of switchgrass can promote the production of EPS-polysaccharides by soil microorganisms, which in turn increases aggregate stability. This phenomenon provides a potential explanation for how the planting of switchgrass can improve the structure of poor soil (Sher *et al.*, 2020). The root exudates of semiarid grassland plants provide easily accessible energy and nutrient substances, which stimulate the growth of rhizosphere microorganisms and their EPS secretion. The degree of soil aggregation and EPS-

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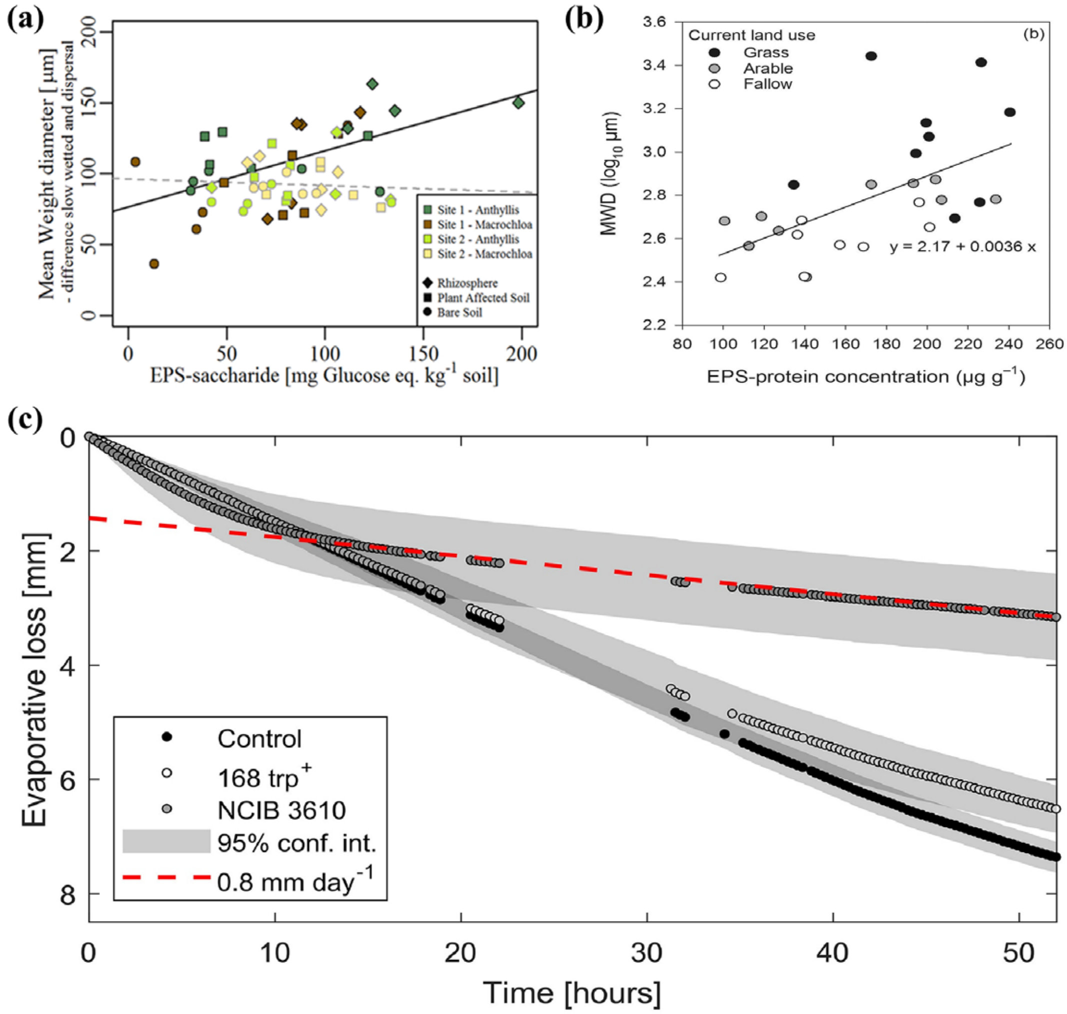

Figure 4. Stable aggregate mean weight diameter (MWD) as a linear function of (a) EPS- polysaccharide and (b) EPS-protein. Reproduced with permission from Bettermann *et al*. (2021) and Redmile-Gordon *et al*. (2020). Copyright 2021 and 2020 Elsevier BV. (c) Derived water losses from sand microcosms monitored with time-series neutron radiography. Control treatment (sterile, black dots), treatment inoculated with *Bacillus subtilis* 168 trp^{+} (Mutants with low EPS production, blank dots), treatment inoculated with *Bacillus subtilis* NCIB 3610 (wildtype with high EPS production, grey dots), and the corresponding 0.8 mm day^{-1} evaporation rate (red dashed line) are shown. Reproduced with permission from Benard *et al*. (2023). Copyright 2023 Elsevier BV.

polysaccharides content both decrease with distance from the rhizosphere (Zethof *et al*., 2020). In addition, the high abundance of polyvalent cations in carbonate-rich soils promotes the stabilizing effect of EPS on soil microaggregates by increasing EPS production and altering the EPS structure (Zethof *et al*., 2020).

Water retention and holding capacity

Extracellular polymeric substances are one of the important strategies for microorganisms to cope with environmental stresses. Microbial cells need to maintain a hydrated environment around them. EPS acts like a water-absorbing sponge to protect microorganisms from desiccation stress, thereby allowing microorganisms to adjust their metabolism. An EPS-overproducing mutant strain of *Pseudomonas protegens* CHA0 increased the survival of culturable cells under desiccation conditions by five-fold compared to the wild-type strain (Krause *et al*., 2019). One of the key features of EPS is that it induces hydraulic decoupling during the moisture fluctuation contexts, which may be a microbial survival strategy to manipulate water retention to protect microorganisms embedded within the biofilm (Bérard *et al*., 2015). Soils often suffer from frequent drying-wetting events, and the repeated osmotic adjustment of microbial cytoplasm may damage cell functions, suggesting that the hygroscopic regulation of EPS may be more efficient in the long-term resistance of microorganisms to water fluctuations.

The hygroscopic nature of EPS has received continuous attention in improving soil water retention and holding capacity (Bhattacharjee *et al*., 2020). The water-holding capacity of EPS can be as high as 15 to 20 times its own mass (Or *et al*., 2007a). The EPS-polysaccharides that have been reported to have high water retention properties are high molecular weight compounds such as xanthan gum, colanic acid and alginate. The addition of 1% xanthan gum or EPS produced by *Pseudomonas* sp. can significantly increase the porosity and water-holding capacity of sandy soils (Roberson and Firestone, 1992; Rosenzweig *et al*., 2012). Furthermore, microbial EPS can increase the viscosity of soil solutions, reduce their surface tension, and decrease soil hydraulic conductivity, which ultimately slows down the rate of soil drying (Benard *et al*., 2023). Soil water evaporation was quantified and spatially resolved using time-series neutron radiography, and

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Bacillus subtilis NCIB 3610 with high EPS production could significantly delay soil drying compared with the uninoculated control treatment and the inoculation with the low EPS-producing mutant strain 168 trp^+ (Fig. 4c) (Benard *et al*., 2023). The synergistic effects of microbial EPS and pore structure on soil water retention have been explored using microfluidic systems that simulate the physical structure of the soil (Deng *et al*., 2015; Guo *et al*., 2018). Different carbon substrates and their accessibility have obvious effects on the chemical properties and water-retention capabilities of microbial EPS. The insoluble substrate chitin stimulates microbial communities to produce EPS with better water retention properties compared to the soluble substrate N-acetylglucosamine (Bhattacharjee *et al*., 2020). Different carbon source substrates indirectly alter the nature and water retention capabilities of EPS by affecting the structure of microbial communities, which in turn influences the adaptability of soil microbial communities to drought. Water scarcity, as the most serious abiotic environmental stress, can seriously affect crop productivity. Appropriate field management practices indirectly stimulate soil microorganisms to secrete EPS to improve the water retention capacity of the soil, which may be one of the important approaches for green development in agriculture.

Nutrient storage and trapping

Microbial communities in unsaturated soil environments tend to reside in biofilms, where EPS can capture and store nutrients (Or *et al*., 2007b). The small molecular substances produced by degrading EPS can be used as carbon and energy sources for cell growth under nutrient limitation. However, the molecular complexity of EPS necessitates the involvement of multiple, distinct enzymes for their complete degradation (Flemming and Wingender, 2010). *Rhizobium NZP* 2037 can use the self-secreted EPS as the only carbon source when carbon sources are limited (Patel and Gerson, 1974). When nitrogen source availability is low, soil bacteria can perform 'N mining' from soil organic matter through secreted EPS-proteins (Redmile-Gordon *et al*., 2015b). Microbial secretion of EPS can serve as an extracellular strategy for carbon storage, but few studies have focused on the role of EPS in nutrient supply or cross-feeding between microorganisms. Wang *et al*., (2015) used the ^{13}C isotope to label EPS produced by *Beijerinckia indica* and observed that EPS can be mainly assimilated by bacteria with low genetic relationships, especially phylum *Planctomycetes*. Costa *et al*. (2020b) used stable isotope probes combined with metagenomics targeting to study the microbial communities and functions involved in EPS degradation in soil, and identified the microbial communities that produce glycoside hydrolases. The EPS produced by *Acidobacteria Granulicella* sp. strain WH15 was mainly assimilated by microorganisms from *Planctomycetes*, *Verrucomicrobia*, *Ascomycota* and *Basidiomycota* (Costa *et al*., 2020a).

Contaminant sequestration and transformation

Extracellular polymeric substances secreted by microorganisms contain rich functional groups that can adsorb and trap heavy metals, thereby obviously affecting the environmental behaviour of heavy metals. Many studies have explored the potential of EPS for the biosorption of metal ions (Joshi and Juwarkar 2009; Nkoh *et al*., 2019a), with a view to providing theoretical support for microbial remediation of heavy metal-contaminated soils. The binding site and complexing ability of EPS are related to its protein, polysaccharide and lipid content (Wei *et al*., 2017). Biosorption involves various mechanisms between EPS functional groups and metals, including physical adsorption, ion exchange, complexation and precipitation (Fang *et al*., 2014; More *et al*., 2014). Moreover, EPS secreted by microorganisms can be adsorbed onto the surface of soil minerals, which affects the ability of minerals to bind heavy metals. Mikutta *et al*. (2012) found that bentonite selectively adsorbs low-molecular weight and N-containing components from EPS secreted by *Bacillus subtilis*, which further increases the adsorption extent and rate of bentonite for heavy metals (Pb^{2+}, Cu^{2+}, Zn^{2+}). However, ferrihydrite selectively retains high-molecular weight and P-rich components, which leads to a decrease in the adsorption of heavy metals by ferrihydrite (Mikutta et al., 2012). The effect of EPS on Zn adsorption by γ-alumina is pH-dependent, in which the carboxyl and phosphoryl groups of EPS play a crucial role in this process (Li *et al*., 2017). Nkoh *et al*. (2019b) found that the addition of bacterial EPS increased the negative charge on the surface of soil colloids, which led to an increase in the adsorption of heavy metals Cu^{2+} and Cd^{2+} by variable charge soils. EPS can also serve as an electron transfer medium and electron donor (Xiao *et al*., 2017). EPS can reduce metal ions (Ag^+ and Au^{3+}) to elemental nanoparticles through hemiacetal groups, thereby reducing the bioavailability of metal ions (Kang *et al*., 2014; 2017). However, Zhang *et al*. (2020) found that EPS can interfere with the precipitation of mercury sulfide and lead to the formation of metacinnabar, thus increasing the environmental risk of the neurotoxin methylmercury. Soil EPS can also function as a biochemical indicator of pollution. Redmile-Gordon and Chen (2017) found that bacteria in acidic soil secrete EPS-polysaccharides and soluble uronic acids as a tolerance mechanism in response to Zn^{2+} stress. Bacteria in heavy metal-contaminated soil respond to Cr stress by secreting EPS-polysaccharides and EPS-proteins as detoxification pathways (Zhang *et al*., 2021a).

EPS secreted by microorganisms is also effective in alleviating the stress of organic pollutants in the soil environment. EPS contains hydrophilic and hydrophobic groups, which facilitates the adsorption of positively/negatively charged organic pollutants. Protein fractions in soil EPS increased the adsorption of polybrominated diphenyl ethers by soil particles, whereas the effect of polysaccharide fractions showed concentration dependence (Liu *et al*., 2017). Meanwhile, many hydrocarbon-degrading bacteria have the ability to produce EPS with emulsifying activity. EPS as a biosurfactant can reduce surface tension and interfacial tension, thus improving the dispersion, emulsification and bioavailability of organic pollutants. EPS secreted by rhizobia exhibits better emulsifying activity compared to common surfactants such as Tween 80. EPS produced by nitrogen-fixing bacteria increases the dispersion of insoluble organic pollutants, which contributes to the degradation of the pollutants by enhancing their bioaccessibility (Gauri *et al*., 2012). Microbial degradation of polycyclic aromatic hydrocarbons is considered to be an effective bioremediation technology, where EPS enhances the bioavailability of polycyclic aromatic hydrocarbons and accelerates their biodegradation (Zhang *et al*., 2011). Han *et al*. (2021) established biofilm communities on carriers of model soil components montmorillonite and humic acid, and found that the biofilm on the organic carrier had a dense EPS matrix to accelerate the biodegradation of benzo[a]pyrene. Wei *et al*. (2024) sorted out the possible mechanisms of microbial EPS on the degradation of persistent organic pollutants (POPs) through external electron transfer, photodegradation and enzyme catalysis.

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Overall, microbial EPS in soil can provide a cost-effective and ecologically minimal disruptive approach to remediate organic-contaminated environments, thus possessing future prospects and application potential.

Perspectives and future prospects

Soil microorganisms have developed a range of survival strategies to adapt to their surrounding environment. The secretion of microbial EPS is an important strategy for maintaining moist conditions, trapping nutrients, facilitating interfacial chemical reactions, and responding to environmental stress. Microbial EPS contain highly diverse biopolymers, and the functionality of EPS mainly depends on its composition and structure. Our understanding has deepened with the development of microbiomics and instrumental characterization methods such as metaproteomics, atomic force microscopy-based infrared spectroscopy (AFM-IR), correlative Raman imaging and scanning electron microscopy (RISE), environmental scanning electron microscope coupled with an X-ray energy dispersive system (ESEM-EDS), and nano-scale secondary ion mass spectrometry coupled with stable isotope probing (NanoSIMS-SIP). This will hopefully reveal the compositional structure and interfacial behavior of EPS more comprehensively, and accelerate the discovery of the functional potential and turnover mechanism of EPS in soil ecosystems.

The research of soil EPS is in the booming stage. Microbial EPS can promote soil aggregation, enhance soil fertility and improve soil quality. EPS in soil and the rhizosphere can also improve the utilization of nutrients and water by microorganisms and plants, thereby benefiting the soil-microbe-plant system as a whole. Soil microorganisms have great potential for environmental functions. Promoting the formation of soil biofilms and improving the composition of soil EPS through agronomic measures may be potential approaches to support the development of environmentally friendly agriculture. Some interesting points await further clarification to enhance our understanding of soil EPS:

(1) The vague delineation of the sources of soil biochemical substances hinders the current development of soil science. The extraction and analysis of total sugars, peptides and amino acids in soil provide rich information on soil biochemical substances, but the inability to accurately distinguish the sources of these substances makes it difficult to answer many key questions in soil science (Gunina and Kuzyakov, 2015; Marchus *et al.*, 2018). Specific extraction and precise analysis of soil EPS may provide a pathway to understanding related issues (Redmile-Gordon *et al.*, 2015b). Quantifying soil EPS and its components can elucidate the adaptability of the biofilm phenotype of microbial communities to environmental conditions and reveal the correlation between soil EPS and soil functions.

(2) Microbial EPS is at the interface between microbial cells and soil matrix, and soil EPS has been considered as one of the ideal soil biochemical indicators, *e.g.* to assess the extracellular response of soil microorganisms to heatwave events (Bérard *et al.*, 2015). Profiling of soil EPS helps to uncover the hidden mechanisms of microbial extracellular responses and clarify the interfacial processes between microbial cells and mineral/organic substances in soil. Soil EPS deserves to be evaluated for its relative importance and intercorrelation with other soil health indicators, and to promote its development as one of a class of sensitive biological indicators of soil health.

(3) Microbial inoculants have been studied for decades, but further improvements are needed to achieve high biomass and high colonization survival rates of the inoculated microorganisms. Considering the beneficial effects of EPS on microorganisms and soil structure, EPS can be used to encapsulate microbial strains to prepare new biofilm biofertilizers (Saha *et al.*, 2020; Velmourougane *et al.*, 2023). EPS is expected to enhance the colonization and survival of inoculated microorganisms in the soil, while improving soil structure and nutrients. In addition, agricultural waste materials can serve as substrates for large-scale production of microbial EPS. This not only increases the economic benefits for related industries, but also addresses the environmental issues caused by the accumulation or incineration of agricultural waste materials.

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