Received: 30 January 2022 | Revised: 28 August 2022 | Accepted: 13 September 2022

DOI: 10.1111/ejss.13307

REVIEW ARTICLE

European Journal of Soil Science WILEY

Can molecular genetic techniques improve our understanding of the role the microbial biomass plays in soil aggregation?

Rupinder Kaur[1,2] | **Kathryn L. Page**[2] | **Ram C. Dalal**[2] | **Neal W. Menzies**[2] | **Yash P. Dang**[2]

[1]Department of Botany, Panjab University, Chandigarh, India

[2]The University of Queensland, St Lucia, Queensland, Australia

Correspondence
Kathryn L. Page, The University of Queensland, St Lucia, Qld 4072, Australia.
Email: kathryn.page@uq.edu.au

Abstract

Soil structure plays an integral role in regulating the physical, chemical, and biological functioning of soil systems and is essential for maintaining agricultural productivity. Many studies have investigated soil structure formation and stability. However, the underlying mechanisms behind its formation are still often unclear, including how soil microorganisms regulate this process via the aggregation of soil particles. In this review, we seek to summarise current information regarding how microorganisms influence aggregation and explore whether the application of molecular genetic techniques has potential to increase our understanding in this important area. Specifically, we review current information regarding the exact nature and role of microbially produced soil binding agents (extracellular polymeric substances, glomalin, hydrophobins and chaplins) and how different soil microorganisms (bacteria, archaea, fungi, protists) regulate and influence the production of these substances. Molecular genetic techniques have the capacity to provide new information regarding the genetic make-up of the soil microbial biomass, which could potentially be related to their function in soil and role in the aggregation of soil particles. However, more work is required to identify the key functional genes important for studying aggregation processes. Techniques better able to study the fine scale distribution of microorganisms and microbial products are also required to fully understand microbial interactions and functioning on a scale relevant to aggregation. Future developments in this area may offer an opportunity to improve our understanding of, and potentially manipulate, soil aggregation for the benefit of soil functioning and environments.

KEYWORDS

bacteria, extracellular polymeric substances, fungi, glomalin, viruses

1 | INTRODUCTION

Healthy plant growth is intricately linked to the biological functioning of the soil system and its regulation of nutrient dynamics and biogeochemical cycling (Wilpiszeski et al., 2019). In turn, these biological functions are dependent on good soil physical structure. Soil structure refers to the size, shape, and arrangement of solid soil particles and the pore spaces between them (Letey, 1991; Sullivan et al., 2022). The individual mineral components of the soil (sand, silt, and clay) form associations with organic material and inorganic cementing agents (carbonate or iron and aluminium oxides) and aggregate together into clusters of soil particles (Figure 1) (Dalal & Bridge, 1996; Letey, 1991; Sullivan et al., 2022; Wilpiszeski et al., 2019). This aggregation creates a network of air spaces of varying size called soil pores that create a series of spatially heterogeneous habitats that support a diverse range of microorganisms (Sullivan et al., 2022; Totsche et al., 2018; Wilpiszeski et al., 2019). Greater soil structural organisation and stability is typically associated with indicators of good soil health, such as increased soil carbon (C) and nitrogen (N) storage, greater biological activity, good soil aeration, high infiltration rates and water storage, and reduced runoff, erosion, waterlogging, and emissions of greenhouse gasses (Bailey et al., 2019; Liu et al., 2019; Six et al., 2004; Tisdall & Oades, 1982; Wu et al., 2021).

Aggregation and the establishment of a stable network of soil pore spaces are intricately linked to the activities of the soil microbial biomass (Berg et al., 2020; Dalal & Bridge, 1996). The activity of the microbial biomass (bacteria, archaea, fungi, and protists) plays a key role in producing the agents and structures that bind soil particles together and create a stable soil structure. In turn, soil particle aggregation and the arrangement of pore spaces, play a critical role in regulating and protecting microbial activity due to their impact on the supply of air, water, and metabolites (Nimmo, 2005; Srivastava et al., 2019).

Soil aggregation and the formation of soil structure is a widely studied topic. There is abundant information available regarding how this is influenced by soil type, environmental conditions (precipitation and temperature), anthropogenic activities, plant type, and soil biological activity (Lavelle et al., 2020; Liu et al., 2019; Srivastava et al., 2019). However, the underlying mechanisms of aggregation and the formation of structure are often still poorly understood (Lehmann & Rillig, 2015; Trivedi et al., 2017). In particular, the interactions between aggregated soil particles, the microenvironments they create, and how the microbial communities present interact to create soil structures and regulate C and N cycling are poorly understood (Schimel & Schaeffer, 2012). We also have a poor understanding of the quantitative contribution that different groups of biota make to aggregation (Lehmann et al., 2017).

Recent advances in techniques allowing us to examine the soil microbial biomass at a genetic level have provided the soil science community with an opportunity to examine soil microbiology at a new level of detail. A better understanding of the diversity, abundance and behaviour of the soil microbial biomass, including gene expression, could help better understand the relationship between microbial activity and the formation and stability of soil structure. Knowledge of functional genes in soils will allow us to recognise how microbes respond to changing soil structure and environmental conditions and elucidate how the activities of microbes are involved in soil aggregation and stabilisation. However, while there has been an explosion in the use of genetic techniques to study various soil processes, their use to examine soil aggregation is currently limited. It is thus the aim of this current review to examine the potential for genetic techniques to improve our understanding of soil aggregation and summarise existing literature on the topic.

2 | THE ROLE OF THE MICROBIAL BIOMASS IN THE AGGREGATION OF SOIL PARTICLES

It is widely believed that the soil microbial biomass plays an integral role in the aggregation of soil particles. The stimulation of soil biological activity by the addition of organic material leads to microbial growth and the production of binding agents that stick soil particles together (Six et al., 2004). If microbial activity and the production of binding agents stops, the stability of aggregated soil particles decline, and they break down into stable silt-sized organo-mineral complexes (Márquez et al., 2019).

Given that soil microbial activity plays an important role in the formation of soil structure, a greater understanding of microbial community functioning will increase our understanding of how soil particles aggregate together and form stable networks of pore spaces (Bhattacharyya et al., 2021). It should be noted that a wide range of abiotic (e.g., clay content and mineralogy, soil pH), environmental (e.g., wetting, and drying events, anthropogenic management) and biotic (e.g., plant root growth and activity, soil fauna) factors can also have important influences on the formation of soil structure. However, detailed discussion of these factors is beyond the scope of the current review, which is focused on the influence of the microbial biomass.

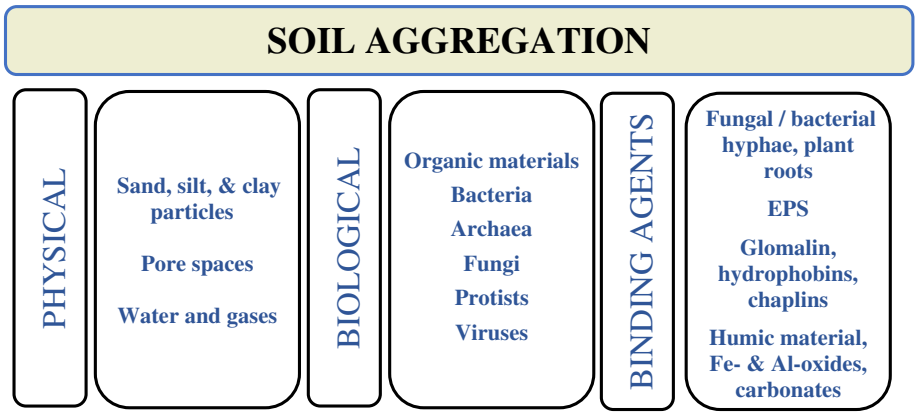

FIGURE 1 Summary of the physical and biological components involved in the aggregation of soil particles

2.1 | Binding agents involved in aggregation

The binding agents involved in aggregation can generally be categorised as either transient, temporary or persistent (Tisdall & Oades, 1982). Transient agents hold the soil particles together for a short period (weeks to months) and include organic compounds such as polysaccharides, proteins and lipids (Chenu & Cosentino, 2011; Tisdall & Oades, 1982). Temporary binding agents, which persist for months to years, are composed of plant roots and fungal hyphae, and primarily act to aggregate soil particles on a macro-scale (>250 μm) (Chenu & Cosentino, 2011; Meurer et al., 2020). Persistent binding agents include humid organic material-polyvalent metal cations complexes, iron (Fe) and aluminium (Al) oxides, and carbonates, which act as binding or cementing agents on a micro-scale (<250 μm) and strongly bind clay particles (decades to centuries) (Tisdall & Oades, 1982; Totsche et al., 2018). The soil microbial biomass is important for the production of many transient and temporary binding agents present in soil environments, and those of particular importance are described in detail below.

2.1.1 | Extracellular polymeric substances

Extracellular polymeric substances (EPS) are molecules released by a wide range of bacteria, archaea and fungi that are dominated by polysaccharides, but also include proteins, enzymes, lipids, and nucleic acids (Chenu & Cosentino, 2011; Costa et al., 2018; Deka et al., 2019; Mahapatra & Banerjee, 2013; Marvasi et al., 2010). Microorganisms produce EPSs for a variety of purposes, such as to protect themselves from predation or abiotic stressors (temperature, water deficits, pH change), and aid attachment to soil particles (Costa et al., 2018; Donot et al., 2012; Lünsdorf et al., 2000; Marvasi et al., 2010). These substances are often found to promote aggregation at a microscale (<250 μm) due to their ability to bond to mineral surfaces and bridge mineral particles together (Chenu & Cosentino, 2011; Deka et al., 2019; Lünsdorf et al., 2000; Totsche et al., 2018; Verchot et al., 2011).

While recognised as promoters of aggregation, the exact nature and role of EPS is relatively poorly understood due to their diversity and structural complexity (Costa et al., 2018). The ability of different EPSs to bind soil particles is variable and likely related to properties such as their size, hydration properties and viscosity (Akhtar et al., 2018). The EPS production by bacteria is typically greatest when C is readily available, but the build-up of microbial biomass is limited by other nutrients, such as N (Chenu & Cosentino, 2011). Drying conditions also stimulate EPS production (Chenu & Cosentino, 2011) and greater ability to produce EPSs has been observed in microorganisms adapted to living in drier environments (Lennon et al., 2012).

2.1.2 | Glomalin, melanins, hydrophobins, chaplins and rodlins

Glomalin is thought to be a glycoprotein that is produced by arbuscular mycorrhizal fungi (AMF) and widely reported to be associated with the aggregation of soil particles and soil structural stability (Agnihotri et al., 2022; Bird et al., 2002; Dai et al., 2015; Ji et al., 2019; Liu et al., 2020; Rillig et al., 2002; Wright & Upadhyaya, 1998). It is often hypothesised that glomalin is secreted by AMF and acts as a "glue" that binds soil particles together or increases their hydrophobicity (Singh et al., 2020; Wright & Upadhyaya, 1998). However, direct evidence for its role is often lacking (Irving et al., 2021; Rillig & Mummey, 2006). This is further complicated by the fact that that there is currently no methodology available to directly measure glomalin in soil. Glomalin is most commonly defined operationally by repeatedly autoclaving the soil in a citrate buffer

(of varying concentration) and then measuring the protein extracted using the non-specific Bradford protein assay (Agnihotri et al., 2022; Holatko et al., 2021; Irving et al., 2021). This process extracts a range of proteins and other compounds that can interfere with the Bradford assay, and it has long been recognised that these represent "glomalin related soil proteins" (GRSP) rather than glomalin itself (Agnihotri et al., 2022; Holatko et al., 2021; Irving et al., 2021; Rillig, 2004). Indeed, the exact chemical nature of glomalin is still largely unknown (Irving et al., 2021).

Some studies have reported that 80% of glomalin produced by AMF is tightly bound to the fungal hyphae and spores, rather than being excreted into the surrounding soil environment (Driver et al., 2005), and genetic sequencing has revealed that the gene for glomalin has homology with a class of stress-induced proteins with a cellular function (Purin & Rillig, 2007). Some researchers have thus suggested that glomalin has a primarily cellular function, likely related to helping hyphae attach to surfaces, allowing hyphae to break air-water interfaces by lowering the surface tension of water, and/or decreasing the palatability of fungal hyphae to fungal grazers (Driver et al., 2005). Its relationship to aggregation may be due to the association of fungal hypha with aggregated soil particles, and its persistence in the environment, rather than due to its active secretion (Purin & Rillig, 2007; Rillig & Mummey, 2006). Recent work has also failed to find any evidence that AMF secrete GRSP at high levels and has suggested that GRSP may simply be artefacts of the inaccurate methodology used to extract them (Murphy et al., 2020). Further work is thus required to determine which of the compounds contained in GRSP are of a fungal origin, whether they are actively extruded into the soil or only released after fungi die and are decomposed by other microorganisms, and the exact role that this plays in the aggregation of soil particles (Holatko et al., 2021).

Melanins are the cell wall proteins produced by fungi and bacteria in soil and are considered of some significance in organic C storage in soil (Siletti et al., 2017) although the role of melanins in aggregation is not clearly established. Because of their relatively high hydrophobicity, melanins may confer protection against aggregate disruption and thereby contribute to aggregation at microscale (<250–53 μm) in soil (Mugerwa & McGee, 2017).

Hydrophobins are small, cysteine-rich proteins produced by a wide range of filamentous saprophytic fungi (King, 2010; Rillig & Mummey, 2006). These are believed to have a variety of roles, such as promoting the extension of filaments across air-water boundaries by lowering water tension and facilitating mycelium attachment to surfaces (Elliot & Talbot, 2004; King, 2010; Rillig & Mummey, 2006). It has been hypothesised that these substances play a role in soil aggregation due to their role in attaching fungi to soil surfaces and their ability to increase surface hydrophobicity (Rillig & Mummey, 2006). However, there has been limited research regarding their behaviour in the soil environment to date.

Similar to hydrophobins, chaplins and rodlins are proteins with both hydrophobic and hydrophilic properties involved in the formation of aerial hyphae by filamentous *Streptomycetes* bacteria (Claessen et al., 2006; King, 2010; Rillig et al., 2007; Sawyer et al., 2011). Research into chaplin function indicates that they also act as surfactants to lower the surface tension of water and assemble into a hydrophobic layer that coats emerging hyphae (Elliot et al., 2003; Sawyer et al., 2011). Thus, similar to hydrophobins, it is believed that these structures increase the hydrophobic nature of soil surfaces, and are likely to play a role in aggregation (Rillig et al., 2007) and soil structural stability.

2.2 | Microorganisms involved in aggregation

Soil microbial communities are predominantly made up of bacteria and fungi, with these organisms making up 10^2–10^4 more biomass than other components (Fierer, 2017). However, archaea and protists are also present and can play important roles in the soil ecosystem (Fierer, 2017). One recent meta-analysis that examined the impact of animals (earthworms, nematodes, arthropods, etc), fungi, and bacteria on aggregation observed that soil biota have, on average, a positive impact on aggregation (increasing aggregation by 24%) (Lehmann et al., 2017). However, this can range from a 77% decrease (typically due to the impact of soil animals, but also some species of fungi) to over a 10-fold increase in aggregation (Lehmann et al., 2017).

Fungi are considered to be more important for the aggregation of soil particles on a macro-scale (>250 μm), while bacteria contribute to both macro- and micro- (<250 μm) aggregation (Lehmann et al., 2017). Predatory organisms such as protists (and viruses, although these are not microorganisms) may also have important indirect effects on aggregation due to their impact on bacterial and fungal community structure and functioning (Erktan, Rillig, et al., 2020; Wilpiszeski et al., 2019).

2.2.1 | Fungi

The significant influence of fungal activity on soil aggregation and stabilisation in soil has been reported in numerous studies (Bai et al., 2019; Bedini et al., 2009; Bethlenfalvay et al., 1999; Bossuyt et al., 2001; Rillig

et al., 2002; Rillig et al., 2010; Tiemann et al., 2015; Tong et al., 2020; Zheng et al., 2014). Filamentous fungi can increase aggregation by enmeshing soil particles with fungal hyphae, and via the production of products that can "glue" soil particles together (e.g. extracellular polysaccharides) (Caesar-Tonthat, 2002; Caesar-TonThat & Cochran, 2000; Chenu, 1989) and/or increase soil hydrophobicity and water repellence (e.g. glomalin, GRSP or hydrophobins) (Chenu & Cosentino, 2011; Ji et al., 2019; Rillig et al., 2010; Zheng et al., 2014). The improvements in aggregation and alteration of pore distributions created by fungal activity can also enhance the activity of bacterial communities (Rillig & Mummey, 2006). However, fungi can also act to decrease aggregation when they excrete enzymes that can degrade organic material, or when they physically destabilise aggregated soil particles by opening pathways for water influx (Lehmann et al., 2020; Lehmann & Rillig, 2015).

2.2.2 | Bacteria

Many bacteria release EPSs that glue soil particles together and are important transient binding agents (Lehmann et al., 2017). Bacterial filaments may also be important in binding soil particles at a micro-scale, similar to the way fungal filaments are important for binding particles at a macro-scale (Lehmann et al., 2017). Certain groups of bacteria are also known to produce chaplins, which increase soil hydrophobicity and stability (Singh et al., 2017). However, like soil fungi, bacteria can also decrease the stability of soil aggregation. For example, Bethlenfalvay et al. (1999) observed that the rapid proliferation of aerobic bacteria seemed to decrease the number of water stable soil aggregates, possibly due to rapid decomposition of binding agents.

2.2.3 | Protists and viruses

The activity of predatory organisms in soil environments also has the potential to impact soil microbial communities, carbon cycling, and thus soil aggregation (Erktan, Rillig, et al., 2020, Wilpiszeski et al., 2019). For example, it is known that protozoan grazing can trigger the production of aggregating agents, such as EPS, as a defence mechanism (Matz & Kjelleberg, 2005). Indeed, in a study of a simplified soil system, the addition of a free-living amoeba (*Acanthamoeba castellanii*) to a microbial community dominated by *Pseudomonas fluorescens* was observed to enhance aggregation, and this was hypothesised to be due to increased bacterial EPS production in response to attack (Erktan, Rillig, et al., 2020). Higher order predators (e.g., collembolans) were also demonstrated to influence aggregation due to their impact on fungal biomass and community composition (Erktan, Rillig, et al., 2020), although such higher order species are not part of the microbial biomass.

The impact of viruses on soil aggregation is largely unknown, although work on aquatic ecosystems suggests that they are likely to be important drivers of carbon and nutrient dynamics (Ashelford et al., 2003; Fierer, 2017; Kimura et al., 2008; Swanson et al., 2009; Williamson et al., 2005; Williamson et al., 2017), and therefore potentially aggregation. However, we currently have a very poor understanding of how viruses alter nutrient cycling, food webs and genetic variability in soils (Williamson et al., 2017), and to our knowledge, no studies have directly examined their impact on soil aggregation.

3 | MOLECULAR GENETIC TECHNIQUES AND THEIR ABILITY TO PROVIDE INSIGHTS INTO AGGREGATION

Greater understanding of the composition of the microbial biomass and its role in aggregation could potentially allow its manipulation to improve soil structure and condition (Berg et al., 2020). Genetic approaches offer a sophisticated tool that can help identify the diversity and functionality of the soil microbial biomass (Geisen et al., 2019). These can include sequencing-based approaches, whereby genes from specific taxa or functional groups of interest are searched for using specific primers (Hazen et al., 2012) and related to soil aggregation or environmental characteristics. In contrast, metagenomic, or metatranscriptomic, approaches can be applied and universal primers used to assess the range of genes present in an environment (metagenomic) and/or actively being expressed (metatranscriptomic). This can then be related to species diversity and/or soil functioning (Geisen et al., 2019). However, the diversity and complexity of the soil microbial biomass makes characterising the relationship between aggregation and microbial biomass incredibly complex (Garoutte et al., 2016; Jansson & Hofmockel, 2018; Kuzyakov & Blagodatskaya, 2015; Overy et al., 2021; Thiele-Bruhn et al., 2020), and only a limited amount of work has been conducted to date (for a selected summary, see Table 1).

3.1 | Community composition

Greater understanding of the degree to which the composition of the soil microbial biomass correlates with aggregation could help improve understanding of those

TABLE 1 Examples of studies that have used molecular genetic approaches to study soil aggregation

	Approach Applied	References
Analysis of microbial community composition	Used genetic analysis to identify soil bacteria in bulk versus rhizosphere soil and related these to soil EPS-saccharide contents and soil aggregation.	Bettermann et al. (2021), Zethof et al. (2020)
	Examined fields that had been out of cultivation for varying time periods (chronosequence) and related genetic analysis of soil bacterial and fungal communities to changes in water stable aggregates.	Duchicela et al. (2013)
	Used genetic techniques to infer the phylogenetic relationships of fungal strains that were isolated from soil and then tested for their ability to aggregate unconsolidated soil material.	Lehmann et al. (2020)
Functional genes	Disruption of specific genes known to be associated with bacterial EPS production (e.g. *SacB*, *gta*, *epsA* and *epsB*) observed to decrease aggregate formation, confirming the role of these substances in aggregation.	Bezzate et al. (2000), Deka et al. (2019), Santaella et al. (2008)
	Metagenomic approaches used to examine differences in the genes related to EPS production in different soil environments (e.g. biocrusts) or under different management (e.g. no-tillage) and related to differences in aggregation.	Cania, Vestergaard, Suhadolc, et al. (2020), Cania, Vestergaard, Kublik, et al. (2020), (2019)

organisms with a key role in aggregating soil particles (Rillig & Mummey, 2006). Genetic approaches are increasingly being used to examine community composition in soil environments and relate these to aggregation. For example, numerous studies have used genetic techniques to examine the differences in species composition or diversity between different sized aggregate classes (Bach et al., 2018; Constancias et al., 2014; Davinic et al., 2012; Han et al., 2021; Kim et al., 2008; Mummey et al., 2006; Trivedi et al., 2017). Other studies have used a combination of computed tomography (CT) scanning to characterise fine scale soil architecture including pore size distribution and connectivity of pores, followed by the dissection of macroaggregates into subsections and the characterisation of the microbial community within these subsections using 16S rRNA pyrosequencing (Kravchenko et al., 2014). This approach was able to identify the presence of different microbial communities in aggregates from different crop management types and relate species composition to differences in pore size distributions.

While these studies often identify distinct microbial populations in different aggregate sizes or pores, they do not typically provide direct evidence for the involvement of key species in aggregation, nor help highlight the distinct processes involved. To better define the relationships between species composition and aggregation, information on microbial diversity needs to be combined with indicators related to aggregation. In this regard, some studies have examined the relationship between microbial composition identified via 16S rRNA sequencing, soil EPS-saccharide content, and aggregation to identify bacterial genera that were associated with EPS production and increased aggregation (as determined by measuring aggregate stability using traditional sieving and sedimentation techniques) (Bettermann et al., 2021; Zethof et al., 2020). Similarly, a study by Duchicela et al. (2013) examined changes in aggregation following the cessation of cultivation and was able to observe a strong positive relationship between changes in fungal community composition and increases in aggregate stability (Duchicela et al., 2013). In contrast Lehmann et al. (2020) were able to analyse the DNA of fungal species isolated from the soil and tested for their ability to aggregate unconsolidated soil material to infer the phylogenetic relationships of fungal strains related to aggregation.

Testing for specific taxa that are associated with soil processes is only likely to be successful when these taxa are closely linked to specific functions (Graham et al., 2016; Thiele-Bruhn et al., 2020). Indeed, linking the taxa found in the soil to the functional capabilities of the soil microbial biomass is challenging and information about the organisms present is not always useful for predicting biochemical processes or how these may change with variations in their environment (Fierer, 2017). For example, where processes are catalysed by a diverse number of organisms, changes in community composition may have minimal impact due to the ability of alternative community members to perform that function (Geisen et al., 2019; Louca et al., 2018). The use of microbial community composition to examine aggregation processes can thus be complex due to the enormous microbial diversity in soil and the high degree of functional redundancy often present in microbial communities (Burke et al., 2011; de Graaff et al., 2015; Louca et al., 2018).

3.2 | Functional genes

An alternative approach to the use of soil microbial community composition to explain soil aggregation is the study of functional genes. To date, most of the work conducted to examine functional genes in soil environments has focused on genes for carbon degradation and nutrient cycling (King, 2010; Shi et al., 2019), rather than genes that can promote aggregation. Indeed, due to the wide range of mechanisms used by organisms to aggregate soil particles, identifying functional genes to test for soil aggregation is challenging (Caesar-TonThat et al., 2014). However, analysis of genes associated with the production of binding agents, such as EPSs, glomalin (GRSP) or hydrophobins, may be valuable to gain a greater understanding of aggregation and the impact of management strategies or abiotic variables (King, 2010; Rillig & Mummey, 2006).

3.2.1 | EPS

Primarily due to their applications in the food industry, the greatest amount of genetic information regarding EPS production is related to bacterial exopolysaccharide production (Donot et al., 2012; Schmid et al., 2015). Despite the wide range of polysaccharides produced by bacteria, the biosynthetic pathways involved in their production tend to be relatively well-conserved and common to many bacteria (Pereira et al., 2013). However, while we currently have a broad understanding of the genetic regulation of EPS in bacteria (Pereira et al., 2009; Rehm, 2010; Schmid et al., 2015) this is far from complete, and greater understanding is required of the genes involved in its synthesis and regulation in the full range of organisms found in soil (Costa et al., 2018). Indeed, for many EPS substances produced in soil environments, we still have only a relatively rudimentary understanding of the genes involved, and the structure and function of the substances resulting from their expression (Marvasi et al., 2010).

Despite our incomplete understanding, some studies have been able to demonstrate key functional genes for EPS production involved in aggregation. For example, the production of the polymer leven by *Paenibacillus polymyxa* bacteria has been associated with increased aggregation of soil around wheat roots (Bezzate et al., 2000). The infection of wheat plants with mutant bacterial strains whose *SacB* gene encoding for levansucrase had been disrupted resulted in reduced aggregation and confirmed the role of levan in the aggregation of soil particles (Bezzate et al., 2000). Similarly, twice as many water stable macroaggregates were observed around the roots of *Brassica napus* plants inoculated with a control versus mutant strain of *Rhizobium* (Santaella et al., 2008). Mutant strains were deficient in the *gta* gene, which encodes for glucosyltransferase or galactosyltransferase, and is known to be essential for the production of exopolysaccharides in the *Rhizobium* strains studied (Santaella et al., 2008). In a further example, exopolysaccharide production in *Bacillus amyloliquefaciens* is regulated by the *eps* operon containing 15 genes, with *epsA* and *epsB* being the two most important for exopolysaccharide production (Deka et al., 2019). Inactivation of these genes was observed to decrease EPS production and aggregation in inoculated soil (Deka et al., 2019).

In other studies, metagenomic approaches have been used to examine the range of genes present in the soil environment and determine which of those present are related to aggregation. For example, one study examined changes in the makeup and function of the bacterial community in bulk soil versus biocrusts and related these to aggregation (Cania, Vestergaard, Kublik, et al., 2020). In these studies, genes related to the production of exopolysaccharides and lipopolysaccharides (*wza, algE, algJ, wcaB, wcaF, wcaK, amsJ, kpsE, epsG, epsA, sacB, wzt, lptF, lptG, lptC*) were observed to increase in biocrusts compared to bulk soil samples (Cania, Vestergaard, Kublik, et al., 2020). Similar techniques were also used to demonstrate that the improved aggregate stability associated with reduced tillage practices at some sites was associated with an increase in the abundance of genes associated with exopolysaccharide and lipopolysaccharide production (Cania, Vestergaard, Suhadolc, et al., 2020), although at others no differences were observed (Cania et al., 2019; Cania, Vestergaard, Suhadolc, et al., 2020). It was noted by the authors of this study that due to the lack of gene sequence data regarding the production of EPS by other organisms, these studies are biased towards bacteria (Cania, Vestergaard, Suhadolc, et al., 2020). It was also noted that the actual production of exo- and lipo-polysaccharides could differ depending on environmental factors (carbon availability, temperature, soil pH, etc) and the application of more metatranscriptomics analysis of the soil environment would be desirable to examine these factors given that even though genes may be present, they may not always be actively expressed (see further discussion below) (Cania et al., 2019). Ideally, genetic information should also be related to direct measurements of exo- and lipopolysaccharides in the soil, although the methods available to extract these substances require development and are currently unable to differentiate between substances produced by different organisms (Cania et al., 2019).

3.2.2 | Glomalin, hydrophobins, chaplins and rodlins

Given the association of GRSP with aggregation observed by many studies (as discussed above), work to examine the expression of genes for glomalin production could assist in identifying the role that its production plays in aggregation and how its expression is affected by environmental conditions. Evidence indicates that genes coding for homologues of heat shock protein (hsp) 60 are responsible for glomalin production (Purin & Rillig, 2007). However, we are unaware of any studies that have related glomalin genes to aggregation or soil structure. Recently, work has been conducted to create PCR primers that can amplify a variety of glomalin gene lineages as markers for AMF diversity (Magurno et al., 2019). This work has also noted that the gene sequences for glomalin partially differ between different species of AMF, which may also indicate differences in the nature of the glomalin produced (Magurno et al., 2019) and provide scope for more detailed work on the relationship between fungal diversity, glomalin production and aggregation. However, the use of Hsp60 as a gene target for glomalin production has been called into question by some authors who consider it unlikely that Hsp60 are critical components of glomalin, and greater research in this area is required (Holatko et al., 2021; Irving et al., 2021; Murphy et al., 2020).

Research has also been carried out to identify the gene sequences involved in hydrophobin production, with one study reporting on the involvement of over 70 genes from a variety of fungal species, many of which contain multiple genes for hydrophobin (Linder et al., 2005). However, limited research has been conducted regarding the behaviour of hydrophobins in soil environments (Rillig, 2005) and we are unaware of any studies that have examined the relationship between hydrophobin gene expression and soil aggregation. The presence of multiple genes within organisms also makes the study of hydrophobin function via techniques such as gene inactivation more challenging, as other genes may compensate for the activity of genes that are deleted (Linder et al., 2005; Whiteford & Spanu, 2002).

Genes for chaplin formation in *Streptomycetes* bacteria have been identified (*chpA-H*). Two short chaplins (*chpE* and *chpH*) are known to be expressed during both vegetative and aerial mycelial phases, while the remainder are only expressed during aerial hyphae formation (Elliot et al., 2003; Sawyer et al., 2011). Similarly, genes for rodlin formation in *Streptomyces* produce Rod1A and Rod1B proteins, which are present on the outer surface of aerial hyphae (Claessen et al., 2006). However, we are unaware of any studies that have related the expression of chaplin and rodlin genes in soil to the process of aggregation.

3.2.3 | Carbon catabolism

While genes associated with carbon degradation are typically associated with a decline in aggregation, in some instances analysis of these genes can also be useful for understanding the changes in aggregation in response to environmental conditions or management change. For example, some studies have observed that nitrogen addition decreases decomposition by down-regulating the expression of ligninolytic (lcc) (Edwards et al., 2011) or cellobiohydrolases (cbhI) genes (Fan et al., 2012). The potential impact of such processes on aggregation are clear, although very few studies have related the changes in genes of carbon catabolism to changes in aggregation.

4 | CHALLENGES ASSOCIATED WITH GENETIC TECHNIQUES

While genetic techniques clearly have the capacity to provide significant insight into the aggregation of soil particles, there are several challenges associated with their use. Firstly, because these techniques have only been available for a relatively short period of time, they can suffer from variability due to the variety of methodologies or protocols used by different laboratories, which can make comparison between studies difficult (Geisen et al., 2019; Hazen et al., 2012; Hirsch et al., 2010; Thiele-Bruhn et al., 2020). In addition, full extraction of the DNA present in soil environments can be difficult, with some authors estimating that most metagenomic data from soil covers <20% of the diversity present (Nannipieri et al., 2020).

Databases that contain genetic sequencing information [e.g. the Kyoto Encyclopaedia of Genes and Genomes (KEGG), the National Centre for Biotechnology Information (NCBI)] are currently used to relate the genes extracted from a soil with particular types of microorganisms or functions. These databases can differ significantly (Nannipieri et al., 2020), and while they are rapidly evolving, they are currently dominated by sequences from fast-growing bacteria (Cania et al., 2019; Cania, Vestergaard, Suhadolc, et al., 2020; Thiele-Bruhn et al., 2020), partly due to the relative ease with which these organisms can be cultured and analysed. However, the soil microbial biomass also contains slow-growing bacteria, archaea, fungi and protists that play important roles in aggregation. Similarly, tools such as functional

gene arrays, which target the extraction of genes involved in various functional processes, also do not represent the full diversity of microbial functions (Shi et al., 2019) and particularly those related to aggregation (e.g., EPS production). The use of genetic techniques to understand the impact of viruses on soil processes is also limited, largely because there is no universal marker gene for viruses and it is difficult to recover enough viral DNA from soil to use metagenomic approaches to examine viral community ecology (Emerson, 2019).

Many genetic approaches also only provide information regarding the relative abundance of different organisms or genes, rather than actual abundance, which can complicate interpretation of their ecological significance (Fierer, 2017; Geisen et al., 2019). Techniques such as real time quantitative PCR (RT-qPCR) analysis can provide some quantitative indication of the organisms present (Hirsch et al., 2010). However, these techniques may provide no differentiation between active, dormant or dead microorganisms, which may overestimate potential function or diversity (Graham et al., 2016; Thiele-Bruhn et al., 2020). Metagenomic approaches can also detect genes that are not actively being expressed, and many genes can have more than one function, depending on the context of the gene in the genome of the microorganism (Jansson & Hofmockel, 2018; Nannipieri et al., 2020). For example, the *nirK* gene, which codes for the nitrate reductase enzyme, is involved in ammonia oxidation, nitrite oxidation, denitrification and aerobic ammonia oxidation (Prosser, 2015). In addition, quantitative approaches do not provide any information regarding process rates, which can be relatively insensitive to gene abundance, meaning that these approaches may not always be reliable predictors of soil functions (Prosser, 2015).

Because RNA is only synthesised by actively growing cells, and degrades relatively rapidly once produced (particularly mRNA, although rRNA can survive for months in moribund or dead cells), its measurement can be used to identify metabolically active communities (Hirsch et al., 2010) and studies have used extraction and measurements of these components to try and identify active organisms (Chen et al., 2013; Garoutte et al., 2016). However, some authors suggest that this approach may be flawed and that the correlation between organism activity and rRNA in environmental samples is inconsistent (Blazewicz et al., 2013; Geisen et al., 2019). The low phylogenetic resolution of the rRNA region also limits its use for species identification (Lindahl et al., 2013).

A further problem with the use of genetic techniques to study aggregation is the scale at which they need to be applied to provide useful information. The distribution of carbon and other resources (oxygen, water) is highly heterogeneous in soil, and physical constraints on the movement of the microbial community can limit the cross-species interactions and contribute to the large number of niches and high species diversity within soil environments (Jansson & Hofmockel, 2018; Nannipieri et al., 2020). Given the spatial arrangement and interaction between microbial communities have a direct role in their functional relationships and the expression of functional genes, understanding of the fine scale spatial distribution of microorganisms and how they function is desirable to fully understand soil processes (Crawford et al., 2007; Jansson & Hofmockel, 2018; Kuzyakov & Blagodatskaya, 2015), including soil architecture and aggregation. However, current genetic approaches often use relatively large amounts of soil and are incapable of defining this fine scale resolution (Baveye, 2021; Crawford et al., 2007; Nannipieri et al., 2020; Prosser, 2012).

The most common approach used to study the distribution and activity of organisms on a spatial scale relevant to the aggregation of soil particles is to separate soil into different aggregate classes using sieving techniques before analysis (Sainju, 2006; Wilpiszeski et al., 2019). However, the manner in which this sieving is carried out (e.g. wet or dry) can influence aggregate properties and microbial activity or diversity (Blaud et al., 2017; Liao et al., 2021; Sainju, 2006; Vos et al., 2013). These techniques also do not preserve pore structures, which are key habitats for microorganisms (Vos et al., 2013; Wilpiszeski et al., 2019), or enable the spatial distribution of soil taxa to be examined (Geisen et al., 2019).

Aggregate dissection techniques followed by the examination of the microbial communities associated with classes of soil aggregates can help identify the spatial associations between microbes on a fine scale (Vos et al., 2013). Some studies have also combined X-Ray μ-CT scanning using synchrotron with molecular techniques to better determine the spatial distribution of organisms, as discussed above (Bailey et al., 2013; Kravchenko et al., 2014). The isolation and sequencing of DNA and RNA from individual cells extracted from georeferenced locations is also an approach that could be used to better characterise the distribution of organisms within soil pores (Erktan, Or, & Scheu, 2020), and the evolution of such techniques has been recently described (Baveye et al., 2018). However, given there is likely to be a wide range of organisms capable of binding and aggregating soil particles, and thus a degree of functional redundancy in aggregation processes, the usefulness of such characterisation to understand aggregation may be limited. In addition, this approach is technically challenging and difficult to apply.

5 | CONCLUSIONS AND DIRECTIONS FOR FUTURE RESEARCH

Aggregation and soil structural stability are vital for many aspects of physical, chemical, and biological health. However, we still have an incomplete understanding of the exact mechanisms involved in aggregation and the role the microbial biomass plays in these processes. While it has long been recognised that microbial binding agents, such as EPS, are intricately associated with soil aggregation and stabilisation, the exact nature and behaviour of these substances in the soil environment and how their production differs depending on environmental conditions and/or the organisms present is poorly understood. Molecular genetics could potentially increase understanding of the diversity and functionality of the soil microbial biomass and its relationship to aggregation, although the challenges associated with this approach are significant. The diversity and complexity of the soil microbial biomass make characterising relationships between it and aggregation complex, particularly given the gaps in understanding the key organisms and functional genes important for aggregation and the challenges associated with studying the fine scale distribution of the microbial biomass and its activity.

Current approaches to the study of aggregation have overwhelmingly used methodologies that destroys the soil structure and relies on correlations between different sized aggregates classes and soil compositional or functional traits to draw conclusions regarding the organisms and genes responsible for binding soil particles. While this approach has provided some useful information, the destruction of soil architecture is a significant barrier to obtaining a complete understanding of how different organisms interact and behave to induce aggregation. The reliance on observational and correlational studies also fails to provide direct evidence of the exact mechanisms involved in aggregation.

To advance our understanding, future work should concentrate on combining techniques capable of examining microbial behaviour within the inherent soil architecture and seeking to manipulate experimental conditions to gain greater evidence of the mechanisms responsible for aggregation. This will require work to better determine the exact nature of binding agents involved in aggregation. In addition, the identification of functional genes associated with the production of binding agents is required and likely to be a more promising area of research than the study of the organisms responsible for aggregation given the high degree of functional redundancy among microbial communities. Further advancements will likely to be made when functional gene expression, for example, the specific enzymes and their products, can directly be studied in situ within the surfaces of aggregated soil particles and associated pores using a combination of synchrotron-based X-Ray μ-CT scanning, nano-secondary ion mass spectrometry (nano-SIMS) and 3-D-nuclear magnetic resonance spectrometry 3-D-NMR, for ^{13}C, ^{15}N and ^{31}P. While studying the process of aggregation at the molecular level is no doubt complex, if successful this future research may increase our ability to manipulate environmental conditions to enhance aggregation for the benefit of soil health, agricultural productivity and environmental benefits, including carbon sequestration.

AUTHOR CONTRIBUTIONS

Rupinder Kaur: Writing – original draft (lead). **Kathryn Louise Page:** Writing – original draft (equal). **Ram C Dalal:** Conceptualization (equal); writing – review and editing (equal). **Neal Menzies:** Writing – review and editing (equal). **Yash P Dang:** Conceptualization (equal); writing – review and editing (equal).

ACKNOWLEDGEMENTS

None. Open access publishing facilitated by The University of Queensland, as part of the Wiley - The University of Queensland agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Kathryn L. Page https://orcid.org/0000-0001-8994-6561

REFERENCES

Agnihotri, R., Sharma, M. P., Prakash, A., Ramesh, A., Bhattacharjya, S., Patra, A. K., Manna, M. C., Kurganova, I., & Kuzyakov, Y. (2022). Glycoproteins of arbuscular mycorrhiza for soil carbon sequestration: Review of mechanisms and controls. *Science of the Total Environment*, *806*, 150571. https://doi.org/10.1016/j.scitotenv.2021.150571

Akhtar, J., Galloway, A. F., Nikolopoulos, G., Field, K. J., & Knox, P. (2018). A quantitative method for the high throughput screening for the soil adhesion properties of plant and microbial polysaccharides and exudates. *Plant & Soil*, *428*(1), 57–65. https://doi.org/10.1007/s11104-018-3670-1

Ashelford, K. E., Day, M. J., & Fry, J. C. (2003). Elevated abundance of bacteriophage infecting bacteria in soil. *Applied Environmental Microbiology*, *69*(1), 285–289. https://doi.org/10.1128/AEM.69.1.285-289.2003

Bach, E. M., Williams, R. J., Hargreaves, S. K., Yang, F., & Hofmockel, K. S. (2018). Greatest soil microbial diversity found in micro-habitats. *Soil Biology & Biochemistry*, *118*, 217–226. https://doi.org/10.1016/j.soilbio.2017.12.018

Bai, N., Zhang, H., Li, S., Zheng, X., Zhang, J., Zhang, H., Zhou, S., Sun, H., & Lv, W. (2019). Long-term effects of straw and straw-derived biochar on soil aggregation and fungal community in a rice-wheat rotation system. *PeerJ*, *6*, e6171. https://doi.org/10.7717/peerj.6171

Bailey, V. L., McCue, L. A., Fansler, S. J., Boyanov, M. I., DeCarlo, F., Kemner, K. M., & Konopka, A. (2013). Micrometer-scale physical structure and microbial composition of soil macroaggregates. *Soil Biology & Biochemistry*, *65*, 60–68. https://doi.org/10.1016/j.soilbio.2013.02.005

Bailey, V. L., Pries, C. H., & Lajtha, K. (2019). What do we know about soil carbon destabilization? *Environmental Research Letters*, *14*(8), 83004. https://doi.org/10.1088/1748-9326/ab2c11

Baveye, P. C. (2021). Bypass and hyperbole in soil research: Worrisome practices critically reviewed through examples. *European Journal of Soil Science*, *72*(1), 1–20. https://doi.org/10.1111/ejss.12941

Baveye, P. C., Otten, W., Kravchenko, A., Balseiro-Romero, M., Beckers, É., Chalhoub, M., Darnault, C., Eickhorst, T., Garnier, P., Hapca, S., Kiranyaz, S., Monga, O., Mueller, C. W., Nunan, N., Pot, V., Schlüter, S., Schmidt, H., & Vogel, H.-J. (2018). Emergent properties of microbial activity in heterogeneous soil microenvironments: Different research approaches are slowly converging, yet major challenges remain. *Frontiers in Microbiology*, *9*, 1–48. https://doi.org/10.3389/fmicb.2018.01929

Bedini, S., Pellegrino, E., Avio, L., Pellegrini, S., Bazzoffi, P., Argese, E., & Giovannetti, M. (2009). Changes in soil aggregation and glomalin-related soil protein content as affected by the arbuscular mycorrhizal fungal species *glomus mosseae* and *glomus intraradices*. *Soil Biology & Biochemistry*, *41*(7), 1491–1496. https://doi.org/10.1016/j.soilbio.2009.04.005

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C. C., Charles, T., Chen, X., Cocolin, L., Kellye, E., Smidt, H., Van Overbeek, L. S., & Sloter, M. (2020). Microbiome definition revisited: Old concepts and new challenges. *Microbiome*, *8*(1), 103–122. https://doi.org/10.1186/s40168-020-00875-0

Bethlenfalvay, G. J., Cantrell, I. C., Mihara, K. L., & Schreiner, R. P. (1999). Relationships between soil aggregation and mycorrhizae as influenced by soil biota and nitrogen nutrition. *Biology and Fertility of Soils*, *28*(4), 356–363. https://doi.org/10.1007/s003740050504

Bettermann, A., Zethof, J. H. T., Babin, D., Cammeraat, E. L. H., Sole-Benet, A., Lazaro, R., Luna, L., Nesme, J., Sorensen, S. J., Kalbitz, K., Smalla, K., & Vogel, C. (2021). Importance of microbial communities at the root-soil interface for extracellular polymeric substances and soil aggregation in semiarid grasslands. *Soil Biology & Biochemistry*, *159*, 108301. https://doi.org/10.1016/j.soilbio.2021.108301

Bezzate, S., Aymerich, S., Chambert, R., Czarnes, S., Berge, O., & Heulin, T. (2000). Disruption of the *Paenibacillus polymyxa* levansucrase gene impairs its ability to aggregate soil in the wheat rhizosphere. *Environmental Microbiology*, *2*(3), 333–342. https://doi.org/10.1046/j.1462-2920.2000.00114.x

Bhattacharyya, R., Rabbi, S. M. F., Zhang, Y., Young, I. M., Jones, A. R., Dennis, P. G., Menzies, N. W., Kopittke, P. M., & Dalal, R. C. (2021). Soil organic carbon is significantly associated with the pore geometry, microbial diversity and enzyme activity of the macro-aggregates under different land uses. *Science of the Total Environment*, *778*, 146286. https://doi.org/10.1016/j.scitotenv.2021.146286

Bird, S. B., Herrick, J. E., Wander, M. M., & Wright, S. F. (2002). Spatial heterogeneity of aggregate stability and soil carbon in semi-arid rangeland. *Environmental Pollution*, *116*(3), 445–455. https://doi.org/10.1016/S0269-7491(01)00222-6

Blaud, A., Menon, M., van der Zaan, B., Lair, G. J., & Banwart, S. A. (2017). Effects of dry and wet sieving of soil on identification and interpretation of microbial community composition. *Advances in Agronomy*, *142*, 119–142. https://doi.org/10.1016/bs.agron.2016.10.006

Blazewicz, S. J., Barnard, R. L., Daly, R. A., & Firestone, M. K. (2013). Evaluating rRNA as an indicator of microbial activity in environmental communities: Limitations and uses. *The ISME Journal*, *7*(11), 2061–2068. https://doi.org/10.1038/ismej.2013.102

Bossuyt, H., Denef, K., Six, J., Frey, S. D., Merckx, R., & Paustian, K. (2001). Influence of microbial populations and residue quality on aggregate stability. *Agriculture, Ecosystems & Environment*, *16*(3), 195–208. https://doi.org/10.1016/S0929-1393(00)00116-5

Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S., & Thomas, T. (2011). Bacterial community assembly based on functional genes rather than species. *Proceedings of the National Academy of Science USA*, *108*(34), 14288–14293. https://doi.org/10.1073/pnas.1101591108

Caesar-TonThat, T. C., & Cochran, V. L. (2000). Soil aggregate stabilization by a saprophytic lignin-decomposing basidiomycete fungus I. microbiological aspects. *Biology and Fertility of Soils*, *32*(5), 374–380. https://doi.org/10.1007/s003740000263

Caesar-TonThat, T. C., Stevens, W. B., Sainju, U. M., Caesar, A. J., West, M., & Gaskin, J. F. (2014). Soil-aggregating bacterial community as affected by irrigation, tillage, and cropping system in the northern Great Plains. *Soil Science*, *179*(1), 11–20. https://doi.org/10.1097/SS.0000000000000036

Caesar-Tonthat, T. C. (2002). Soil binding properties of mucilage produced by a basidiomycete fungus in a model system. *Mycological Research*, *106*(8), 930–937. https://doi.org/10.1017/S0953756202006330

Cania, B., Vestergaard, G., Kublik, S., Koehne, J. M., Fischer, T., Albert, A., Winkler, B., Schloter, M., & Schulz, S. (2020). Biological soil crusts from different soil substrates harbor distinct bacterial groups with the potential to produce exopolysaccharides and lipopolysaccharides. *Microbial Ecolology*, *79*(2), 326–341. https://doi.org/10.1007/s00248-019-01415-6

Cania, B., Vestergaard, G., Suhadolc, M., Mihelic, R., Krauss, M., Fliessbach, A., Mader, P., Szumelda, A., Schloter, M., & Schulz, S. (2020). Site-specific conditions change the response of bacterial producers of soil structure-stabilizing agents such as exopolysaccharides and lipopolysaccharides to tillage intensity. *Frontiers in Microbiology*, *11*, 1–14. https://doi.org/10.3389/fmicb.2020.00568

Cania, B., Vestergaard, G., Krauss, M., Fliessbach, A., Schloter, M., & Schulz, S. (2019). A long-term field experiment demonstrates the influence of tillage on the bacterial potential to produce soil structure-stabilizing agents such as

exopolysaccharides and lipopolysaccharides. *Environmental Microbiome*, *14*(1), 1. https://doi.org/10.1186/s40793-019-0341-7

Chen, X., Yirong, S., He, X., Liang, Y., & Jinshui, W. (2013). Comparative analysis of basidiomycetous laccase genes in forest soils reveals differences at the cDNA and DNA levels. *Plant and Soil*, *366*(1), 321–331. https://doi.org/10.1007/s11104-012-1440-z

Chenu, C. (1989). Influence of a fungal polysaccharide, scleroglucan, on clay microstructures. *Soil Biology & Biochemistry*, *21*(2), 299–305. https://doi.org/10.1016/0038-0717(89)90108-9

Chenu, C., & Cosentino, D. (2011). Microbial regulation of soil structural dynamics. In K. Ritz & I. Young (Eds.), *The architecture and biology of soils: Life in inner space* (pp. 37–70). CABI.

Claessen, D., de Jong, W., Dijkhuizen, L., & Wosten, H. A. B. (2006). Regulation of *Streptomyces* development: Reach for the sky! *Trends in Microbiology*, *14*(7), 313–319. https://doi.org/10.1016/j.tim.2006.05.008

Constancias, F., Prévost-Bouré, N. C., Terrat, S., Aussems, S., Nowak, V., Guillemin, J.-P., Bonnotte, A., Biju-Duval, L., Navel, A., Martins, J. M. F., Maron, P.-A., & Ranjard, L. (2014). Microscale evidence for a high decrease of soil bacterial density and diversity by cropping. *Agronomy for Sustainable Development*, *34*(4), 831–840. https://doi.org/10.1007/s13593-013-0204-3

Costa, O. Y. A., Raaijmakers, J. M., & Kuramae, E. E. (2018). Microbial extracellular polymeric substances: Ecological function and impact on soil aggregation. *Frontiers in Microbiology*, *9*, 1636. https://doi.org/10.3389/fmicb.2018.01636

Crawford, J. W., O'Donnell, A. G., Young, I. M., Shirley, M. D., & Rushton, S. P. (2007). Visualization, modelling and prediction in soil microbiology. *Nature Reviews Microbiology*, *5*(9), 689–699. https://doi.org/10.1038/nrmicro1714

Dai, J., Hu, J., Zhu, A., Bai, J., Wang, J., & Lin, X. (2015). No tillage enhances arbuscular mycorrhizal fungal population, glomalin-related soil protein content, and organic carbon accumulation in soil macroaggregates. *Journal of Soils and Sediments*, *15*(5), 1055–1062. https://doi.org/10.1007/s11368-015-1091-9

Dalal, R. C., & Bridge, B. J. (1996). Aggregation and organic matter storage in sub-humid and semi-arid soils. In M. R. Carter & B. A. Stewart (Eds.), *Structure and organic matter storage in agricultural soils* (pp. 263–308). CRC Press.

Davinic, M., Fultz, L. M., Acosta-Martinez, V., Calderón, F. J., Cox, S. B., Dowd, S. E., Allen, V. G., Zak, J. C., & Moore-Kucera, J. (2012). Pyrosequencing and mid-infrared spectroscopy reveal distinct aggregate stratification of soil bacterial communities and organic matter composition. *Soil Biology & Biochemistry*, *46*, 63–72. https://doi.org/10.1016/j.soilbio.2011.11.012

de Graaff, M. A., Adkins, J., Kardol, P., & Throop, H. L. (2015). A meta-analysis of soil biodiversity impacts on the carbon cycle. *The Soil*, *1*(1), 257–271. https://doi.org/10.5194/soil-1-257-2015

Deka, P., Goswami, G., Das, P., Gautom, T., Chowdhury, N., Boro, R. C., & Barooah, M. (2019). Bacterial exopolysaccharide promotes acid tolerance in *bacillus amyloliquefaciens* and improves soil aggregation. *Molecular Biology Reports*, *46*(1), 1079–1091. https://doi.org/10.1007/s11033-018-4566-0

Donot, F., Fontana, A., Baccou, J. C., & Schorr-Galindo, S. (2012). Microbial exopolysaccharides: Main examples of synthesis, excretion, genetics and extraction. *Carbohydrate Polymers*, *87*(2), 951–962. https://doi.org/10.1016/j.carbpol.2011.08.083

Driver, J. D., Holben, W. E., & Rillig, M. C. (2005). Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi. *Soil Biology & Biochemistry*, *37*(1), 101–106. https://doi.org/10.1016/j.soilbio.2004.06.011

Duchicela, J., Sullivan, T. S., Bontti, E., Bever, J. D., & Wan, S. (2013). Soil aggregate stability increase is strongly related to fungal community succession along an abandoned agricultural field chronosequence in the Bolivian Altiplano. *The Journal of Applied Ecology*, *50*(5), 1266–1273. https://doi.org/10.1111/1365-2664.12130

Edwards, I. P., Zak, D. R., Kellner, H., Eisenlord, S. D., & Pregitzer, K. S. (2011). Simulated atmospheric N deposition alters fungal community composition and suppresses ligninolytic gene expression in a northern hardwood forest. *PLoS One*, *6*(6), e20421. https://doi.org/10.1371/journal.pone.0020421

Elliot, M. A., Karoonuthaisiri, N., Huang, J., Bibb, M. J., Cohen, S. N., Kao, C. M., & Buttner, M. J. (2003). The chaplins: A family of hydrophobic cell-surface proteins involved in aerial mycelium formation in *Streptomyces coelicolor*. *Genes and Development*, *17*(14), 1727–1740. https://doi.org/10.1101/gad.264403

Elliot, M. A., & Talbot, N. J. (2004). Building filaments in the air: Aerial morphogenesis in bacteria and fungi. *Current Opinion in Microbiology*, *7*(6), 594–601. https://doi.org/10.1016/j.mib.2004.10.013

Emerson, J. B. (2019). Soil viruses: A new hope. *mSystems*, *4*(3), e00120-19. https://doi.org/10.1128/mSystems.00120-19

Erktan, A., Rillig, M. C., Carminati, A., Jousset, A., & Scheu, S. (2020). Protists and collembolans alter microbial community composition, C dynamics and soil aggregation in simplified consumer–prey systems. *Biogeosciences*, *17*(20), 4961–4980. https://doi.org/10.5194/bg-17-4961-2020

Erktan, A., Or, D., & Scheu, S. (2020). The physical structure of soil: Determinant and consequence of trophic interactions. *Soil Biology & Biochemistry*, *148*, 107876. https://doi.org/10.1016/j.soilbio.2020.107876

Fan, F., Li, Z., Wakelin, S. A., Yu, W., & Liang, Y. (2012). Mineral fertilizer alters cellulolytic community structure and suppresses soil cellobiohydrolase activity in a long-term fertilization experiment. *Soil Biology & Biochemistry*, *55*, 70–77. https://doi.org/10.1016/j.soilbio.2012.06.008

Fierer, N. (2017). Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*, *15*(10), 579–590. https://doi.org/10.1038/nrmicro.2017.87

Garoutte, A., Cardenas, E., Tiedje, J., & Howe, A. (2016). Methodologies for probing the metatranscriptome of grassland soil. *Journal of Microbiolical Methods*, *131*, 122–129. https://doi.org/10.1016/j.mimet.2016.10.018

Geisen, S., Briones, M. J. I., Gan, H., Behan-Pelletier, V. M., Friman, V.-P., de Groot, G. A., Hannula, S. E., Lindo, Z., Philippot, L., Tiunov, A. V., & Wall, D. H. (2019). A methodological framework to embrace soil biodiversity. *Soil Biology & Biochemistry*, *136*, 107536. https://doi.org/10.1016/j.soilbio.2019.107536

Graham, E. B., Knelman, J. E., Schindlbacher, A., Siciliano, S., Breulmann, M., Anthony, Y., Beman, J. M., Abell, G., Philippot, L., Prosser, J., Foulquier, A., Yuste, J. C.,

Glanville, H. C., Jones, D. L., Angel, R., Salminen, J., Newton, R. J., Bürgmann, H., Ingram, L. J., ... Nemergut, D. R. (2016). Microbes as engines of ecosystem function: When does community structure enhance predictions of ecosystem processes? *Frontiers in Microbiology*, *7*, 214. https://doi.org/10.3389/fmicb.2016.00214

Han, S., Delgado-Baquerizo, M., Luo, X., Liu, Y., Van Nostrand, J. D., Chen, W., Zhou, J., & Huang, Q. (2021). Soil aggregate size-dependent relationships between microbial functional diversity and multifunctionality. *Soil Biology & Biochemistry*, *154*, 108143. https://doi.org/10.1016/j.soilbio.2021.108143

Hazen, T. C., Rocha, A. M., & Techtmann, S. M. (2012). Advances in monitoring environmental microbes. *Current Opinion in Biotechnology*, *24*(3), 526–533. https://doi.org/10.1016/j.copbio.2012.10.020

Hirsch, P. R., Mauchline, T. H., & Clark, I. M. (2010). Culture-independent molecular techniques for soil microbial ecology. *Soil Biology & Biochemistry*, *42*(6), 878–887. https://doi.org/10.1016/j.soilbio.2010.02.019

Holatko, J., Brtnicky, M., Kucerik, J., Kotianova, M., Elbl, J., Kintl, A., Kynicky, J., Benada, O., Datta, R., & Jansa, J. (2021). Glomalin - truths, myths, and the future of this elusive soil glycoprotein. *Soil Biology & Biochemistry*, *153*, 108116. https://doi.org/10.1016/j.soilbio.2020.108116

Irving, T. B., Alptekin, B., Kleven, B., & Ané, J.-M. (2021). A critical review of 25 years of glomalin research: A better mechanical understanding and robust quantification techniques are required. *New Phytologist*, *232*(4), 1572–1581. https://doi.org/10.1111/nph.17713

Jansson, J. K., & Hofmockel, K. S. (2018). The soil microbiome—From metagenomics to metaphenomics. *Current Opinion in Microbiology*, *43*, 162–168. https://doi.org/10.1016/j.mib.2018.01.013

Ji, L., Tan, W., & Chen, X. (2019). Arbuscular mycorrhizal mycelial networks and glomalin-related soil protein increase soil aggregation in Calcaric Regosol under well-watered and drought stress conditions. *Soil & Tillage Research*, *185*, 1–8. https://doi.org/10.1016/j.still.2018.08.010

Kim, J.-S., Dungan, R. S., & Crowley, D. (2008). Microarray analysis of bacterial diversity and distribution in aggregates from a desert agricultural soil. *Biology and Fertility of Soils*, *44*(8), 1003–1011. https://doi.org/10.1007/s00374-008-0291-5

Kimura, M., Jia, Z.-J., Nakayama, N., & Asakawa, S. (2008). Ecology of viruses in soils: Past, present and future perspectives. *Soil Science and Plant Nutrition*, *54*(1), 1–32. https://doi.org/10.1111/j.1747-0765.2007.00197.x

King, G. M. (2010). Enhancing soil carbon storage for carbon remediation: Potential contributions and constraints by microbes. *Trends Microbiology*, *19*(2), 75–84. https://doi.org/10.1016/j.tim.2010.11.006

Kravchenko, A. N., Negassa, W. C., Guber, A. K., Hildebrandt, B., Marsh, T. L., & Rivers, M. L. (2014). Intra-aggregate pore structure influences phylogenetic composition of bacterial community in macroaggregates. *Soil Science Society of America Journal*, *78*(6), 1924–1939. https://doi.org/10.2136/sssaj2014.07.0308

Kuzyakov, Y., & Blagodatskaya, E. (2015). Microbial hotspots and hot moments in soil: Concept & review. *Soil Biology & Biochemistry*, *83*, 184–199. https://doi.org/10.1016/j.soilbio.2015.01.025

Lavelle, P., Spain, A., Fonte, S., Bedano, J. C., Blanchart, E., Galindo, V., Grimaldi, M., Jimenez, J. J., Velasquez, E., & Zangerlé, A. (2020). Soil aggregation, ecosystem engineers and the C cycle. *Acta Oecologica*, *105*, 103561. https://doi.org/10.1016/j.actao.2020.103561

Lehmann, A., & Rillig, M. C. (2015). Understanding mechanisms of soil biota involvement in soil aggregation: A way forward with saprobic fungi? *Soil Biology & Biochemistry*, *88*, 298–302. https://doi.org/10.1016/j.soilbio.2015.06.006

Lehmann, A., Zheng, W., & Rillig, M. C. (2017). Soil biota contributions to soil aggregation. *Nature Ecology and Evolution*, *1*(12), 1828–1835. https://doi.org/10.1038/s41559-017-0344-y

Lehmann, A., Zheng, W., Ryo, M., Soutschek, K., Roy, J., Rongstock, R., Maaß, S., & Rillig, M. C. (2020). Fungal traits important for soil aggregation. *Frontiers in Microbiology*, *10*, 2904. https://doi.org/10.3389/fmicb.2019.02904

Lennon, J. T., Aanderud, Z. T., Lehmkuhl, B. K., & Schoolmaster, D. R. (2012). Mapping the niche space of soil microorganisms using taxonomy and traits. *Ecology*, *93*(8), 1867–1879. https://doi.org/10.1890/11-1745.1

Letey, J. (1991). The study of soil structure - science or art. *Soil Research*, *29*(6), 699–707. https://doi.org/10.1071/SR9910699

Liao, H., Gao, S., Hao, X., Qin, F., Ma, S., Chen, W., & Huang, Q. (2021). Soil aggregate isolation method affects interpretation of protistan community. *Soil Biology & Biochemistry*, *161*, 108388. https://doi.org/10.1016/j.soilbio.2021.108388

Lindahl, B. D., Henrik Nilsson, R., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., Kõljalg, U., Pennanen, T., Rosendahl, S., Stenlid, J., & Kauserud, H. (2013). Fungal community analysis by high-throughput sequencing of amplified markers – A user's guide. *New Phytologist*, *199*(1), 288–299. https://doi.org/10.1111/nph.12243

Linder, M. B., Szilvay, G. R., Nakari-Setälä, T., & Penttilä, M. E. (2005). Hydrophobins: The protein-amphiphiles of filamentous fungi. *FEMS Microbiology Reviews*, *29*(5), 877–896. https://doi.org/10.1016/j.femsre.2005.01.004

Liu, H., Wang, X., Liang, C., Ai, Z., Wu, Y., Xu, H., Xue, S., & Liu, G. (2020). Glomalin-related soil protein affects soil aggregation and recovery of soil nutrient following natural revegetation on the Loess Plateau. *Geoderma*, *357*, 113921. https://doi.org/10.1016/j.geoderma.2019.113921

Liu, M., Han, G., & Zhang, Q. (2019). Effects of soil aggregate stability on soil organic carbon and nitrogen under land use change in an erodible region in Southwest China. *International Journal of Environmental Research and Public Health*, *16*(20), 3809. https://doi.org/10.3390/ijerph16203809

Louca, S., Polz, M. F., Mazel, F., Albright, M. B. N., Huber, J. A., O'Connor, M. I., Ackermann, M., Hahn, A. S., Srivastava, D. S., Crowe, S. A., Doebeli, M., & Parfrey, L. W. (2018). Function and functional redundancy in microbial systems. *Nature Ecology and Evolution*, *2*(6), 936–943. https://doi.org/10.1038/s41559-018-0519-1

Lünsdorf, H., Erb, R. W., Abraham, W. R., & Timmis, K. N. (2000). 'Clay hutches': A novel interaction between bacteria and clay minerals. *Environmental Microbiology*, *2*(2), 161–168. https://doi.org/10.1046/j.1462-2920.2000.00086.x

Magurno, F., Malicka, M., Posta, K., Wozniak, G., Lumini, E., & Piotrowska-Seget, Z. (2019). Glomalin gene as molecular marker for functional diversity of arbuscular mycorrhizal fungi

in soil. *Biology and Fertility of Soils*, *55*(4), 411–417. https://doi.org/10.1007/s00374-019-01354-x

Mahapatra, S., & Banerjee, D. (2013). Fungal exopolysaccharide: Production, composition and applications. *Microbiology Insights*, *2013*(6), 1–16. https://doi.org/10.4137/MBI.S10957

Márquez, C. O., García, V. J., Schultz, R. C., & Isenhart, T. M. (2019). A conceptual framework to study soil aggregate dynamics. *European Journal of Soil Science*, *70*(3), 466–479. https://doi.org/10.1111/ejss.12775

Marvasi, M., Visscher, P. T., & Martinez, L. C. (2010). Exopolymeric substances (EPS) from *Bacillus subtilis*: Polymers and genes encoding their synthesis. *FEMS Microbiology Letters*, *313*(1), 1–9. https://doi.org/10.1111/j.1574-6968.2010.02085.x

Matz, C., & Kjelleberg, S. (2005). Off the hook – How bacteria survive protozoan grazing. *Trends in Microbiology*, *13*(7), 302–307. https://doi.org/10.1016/j.tim.2005.05.009

Meurer, K., Barron, J., Chenu, C., Coucheney, E., Fielding, M., Hallett, P., Herrmann, A. M., Keller, T., Koestel, J., Larsbo, M., Lewan, E., Or, D., Parsons, D., Parvin, N., Taylor, A., Vereecken, H., & Jarvis, N. (2020). A framework for modelling soil structure dynamics induced by biological activity. *Global Change Biology*, *26*(10), 5382–5403. https://doi.org/10.1111/gcb.15289

Mugerwa, M. T. O., & McGee, P. A. (2017). Potential effect of melanised endophytic fungi on levels of organic carbon within an Alfisol. *Soil Research*, *55*, 245–252. https://doi.org/10.1071/SR16006

Mummey, D., Holben, W., Six, J., & Stahl, P. (2006). Spatial stratification of soil bacterial populations in aggregates of diverse soils. *Microbial Ecology*, *51*(3), 404–411. https://doi.org/10.1007/s00248-006-9020-5

Murphy, C. L., Youssef, N. H., Hartson, S., & Elshahed, M. S. (2020). The extraradical proteins of Rhizophagus irregularis: A shotgun proteomics approach. *Fungal Biology*, *124*(2), 91–101. https://doi.org/10.1016/j.funbio.2019.12.001

Nannipieri, P., Ascher-Jenull, J., Ceccherini, M. T., Pietramellara, G., Renella, G., & Schloter, M. (2020). Beyond microbial diversity for predicting soil functions: A mini review. *Pedosphere*, *30*(1), 5–17. https://doi.org/10.1016/S1002-0160(19)60824-6

Nimmo, J. R. (2005). Aggregation: Physical aspects. In D. Hillel (Ed.), *Encyclopedia of soils in the environment* (pp. 28-35). Elsevier. https://doi.org/10.1016/B0-12-348530-4/00532-4

Overy, D. P., Bell, M. A., Habtewold, J., Helgason, B. L., & Gregorich, E. G. (2021). "Omics" technologies for the study of soil carbon stabilization: A review. *Frontiers in Environmental Science*, *9*(116), 617952. https://doi.org/10.3389/fenvs.2021.617952

Pereira, S. B., Mota, R., Santos, C. L., De Philippis, R., & Tamagnini, P. (2013). Assembly and export of extracellular polymeric substances (eps) in cyanobacteria: A phylogenomic approach. *Advances in Botanical Research*, *65*, 235–279. https://doi.org/10.1016/B978-0-12-394313-2.00007-X

Pereira, S., Zille, A., Micheletti, E., Moradas-Ferreira, P., De Philippis, R., & Tamagnini, P. (2009). Complexity of cyanobacterial exopolysaccharides: Composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. *FEMS Microbiology Reviews*, *33*(5), 917–941. https://doi.org/10.1111/j.1574-6976.2009.00183.x

Prosser, J. I. (2012). Ecosystem processes and interactions in a morass of diversity. *FEMS Microbiology Ecology*, *81*(3), 507–519. https://doi.org/10.1111/j.1574-6941.2012.01435.x

Prosser, J. I. (2015). Dispersing misconceptions and identifying opportunities for the use of 'omics' in soil microbial ecology. *Nature Reviews Microbiology*, *13*(7), 439–446. https://doi.org/10.1038/nrmicro3468

Purin, S., & Rillig, M. C. (2007). The arbuscular mycorrhizal fungal protein glomalin: Limitations, progress, and a new hypothesis for its function. *Pedobiologia*, *51*(2), 123–130. https://doi.org/10.1016/j.pedobi.2007.03.002

Rehm, B. H. A. (2010). Bacterial polymers: Biosynthesis, modifications and applications. *Nature Reviews Microbiology*, *8*(8), 578–592. https://doi.org/10.1038/nrmicro2354

Rillig, M. C. (2004). Arbuscular mycorrhizae, glomalin, and soil aggregation. *Canadian Journal of Soil Science*, *84*(4), 355–363. https://doi.org/10.4141/S04-003

Rillig, M. C. (2005). A connection between fungal hydrophobins and soil water repellency? *Pedobiologia*, *49*(5), 395–399. https://doi.org/10.1016/j.pedobi.2005.04.004

Rillig, M. C., Caldwell, B. A., Wösten, H. A. B., & Sollins, P. (2007). Role of proteins in soil carbon and nitrogen storage: Controls on persistence. *Biogeochemistry*, *85*(1), 25–44. https://doi.org/10.1007/s10533-007-9102-6

Rillig, M. C., Mardatin, N. F., Leifheit, E. F., & Antunes, P. M. (2010). Mycelium of arbuscular mycorrhizal fungi increases soil water repellency and is sufficient to maintain water-stable soil aggregates. *Soil Biology & Biochemistry*, *42*(7), 1189–1191. https://doi.org/10.1016/j.soilbio.2010.03.027

Rillig, M. C., & Mummey, D. L. (2006). Mycorrhizas and soil structure. *New Phytologist*, *171*(1), 41–53. https://doi.org/10.1111/j.1469-8137.2006.01750.x

Rillig, M. C., Wright, S. F., & Eviner, V. T. (2002). The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: Comparing effects of five plant species. *Plant and Soil*, *238*(2), 325–333. https://doi.org/10.1023/A:1014483303813

Sainju, U. M. (2006). Carbon and nitrogen pools in soil aggregates separated by dry and wet sieving methods. *Soil Science*, *171*(12), 937–949. https://doi.org/10.1097/01.ss0000228062.30958.5a

Santaella, C., Schue, M., Berge, O., Heulin, T., & Achouak, W. (2008). The exopolysaccharide of rhizobium sp. YAS34 is not necessary for biofilm formation on Arabidopsis thaliana and Brassica napus roots but contributes to root colonization. *Environmental Microbiology*, *10*(8), 2150–2163. https://doi.org/10.1111/j.1462-2920.2008.01650.x

Sawyer, E. B., Claessen, D., Haas, M., Hurgobin, B., & Gras, S. L. (2011). The assembly of individual chaplin peptides from *Streptomyces coelicolor* into functional amyloid fibrils. *PLoS One*, *6*(4), e18839. https://doi.org/10.1371/journal.pone.0018839

Schimel, J. P., & Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Frontiers in Microbiology*, *3*, 348. https://doi.org/10.3389/fmicb.2012.00348

Schmid, J., Sieber, V., & Rehm, B. (2015). Bacterial exopolysaccharides: Biosynthesis pathways and engineering strategies. *Frontiers in Microbiology*, *6*, 496. https://doi.org/10.3389/fmicb.2015.00496

Shi, Z., Yin, H., Van Nostrand, J. D., Voordeckers, J. W., Tu, Q., Deng, Y., Yuan, M., Zhou, A., Zhang, P., Xiao, N., Ning, D., He, Z., Wu, L., & Zhou, J. (2019). Functional gene array-based ultrasensitive and quantitative detection of microbial populations in complex communities. *mSystems*, *4*(4), e00296-19. https://doi.org/10.1128/mSystems.00296-19

Siletti, C. E., Zeiner, C. A., & Bhatnagar, J. M. (2017). Distribution of fungal melanins across species and soils. *Soil Biology and Biochemistry*, *113*, 285–293. https://doi.org/10.1016/j.soilbio.2017.05.030

Singh, A. K., Zhu, X., Chen, C., Wu, J., Yang, B., Zakari, S., Jiang, X. J., Singh, N., & Liu, W. (2020). The role of glomalin in mitigation of multiple soil degradation problems. *Critical Reviews in Environmental Science and Technology*, *52*, 1–35. https://doi.org/10.1080/10643389.2020.1862561

Singh, G., Sangwan, S., Narwal, E., & Annapurna, K. (2017). Microbial protiens and soil carbon sequestration. In A. Rakshit, P. C. Abhilash, H. B. Singh, & S. Ghosh (Eds.), *Adaptive soil management: From theory to practices* (pp. 131–139). Springer Singapore.

Six, J., Bossuyt, H., Degryze, S., & Denef, K. (2004). A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil & Tillage Research*, *79*(1), 7–31. https://doi.org/10.1016/j.still.2004.03.008

Srivastava, P., Singh, R., Bhadouria, R., Tripathi, S., Singh, H., & Raghubanshi, A. S. (2019). Chapter 13 - understanding soil aggregate dynamics and its relation with land use and climate change. In K. K. Choudhary, A. Kumar, & A. K. Singh (Eds.), *Climate change and agricultural ecosystems* (pp. 331–354). Woodhead Publishing.

Sullivan, P. L., Billings, S. A., Hirmas, D., Li, L., Zhang, X., Ziegler, S., Murenbeeld, K., Ajami, H., Guthrie, A., Singha, K., Giménez, D., Duro, A., Moreno, V., Flores, A., Cueva, A., Koop, E., Aronson, L., Barnard, H. R., Banwart, S. A., … Wen, H. (2022). Embracing the dynamic nature of soil structure: A paradigm illuminating the role of life in critical zones of the Anthropocene. *Earth-Science Reviews*, *225*, 103873. https://doi.org/10.1016/j.earscirev.2021.103873

Swanson, M. M., Fraser, G., Daniell, T. J., Torrance, L., Gregory, P. J., & Taliansky, M. (2009). Viruses in soils: Morphological diversity and abundance in the rhizosphere. *Annals of Applied Biology*, *155*(1), 51–60. https://doi.org/10.1111/j.1744-7348.2009.00319.x

Thiele-Bruhn, S., Schloter, M., Wilke, B. M., Beaudette, L. A., Martin-Laurent, F., Cheviron, N., Mougin, C., & Rombke, J. (2020). Identification of new microbial functional standards for soil quality assessment. *The Soil*, *6*(1), 17–34. https://doi.org/10.5194/soil-6-17-2020

Tiemann, L. K., Grandy, A. S., Atkinson, E. E., Marin-Spiotta, E., & McDaniel, M. D. (2015). Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecology Letters*, *18*(8), 761–771. https://doi.org/10.1111/ele.12453

Tisdall, J. M., & Oades, J. M. (1982). Organic matter and water-stable aggregates in soils. *Journal of Soil Science*, *33*, 141–163. https://doi.org/10.1111/j.1365-2389.1982.tb01755.x

Tong, L., Zhu, L., Lv, Y., Zhu, K., Liu, X., & Zhao, R. (2020). Response of organic carbon fractions and microbial community composition of soil aggregates to long-term fertilizations in an intensive greenhouse system. *Journal of Soils and Sediments*, *20*(2), 641–652. https://doi.org/10.1007/s11368-019-02436-x

Totsche, K. U., Amelung, W., Gerzabek, M. H., Guggenberger, G., Klumpp, E., Knief, C., Lehndorff, E., Mikutta, R., Peth, S., Prechtel, A., Ray, N., & Kögel-Knabner, I. (2018). Microaggregates in soils. *Journal of Plant Nutrition and Soil Science*, *181*(1), 104–136. https://doi.org/10.1002/jpln.201600451

Trivedi, P., Delgado-Baquerizo, M., Jeffries, T. C., Trivedi, C., Anderson, I. C., Lai, K., McNee, M., Flower, K., Singh, B. P., Minkey, D., & Singh, B. K. (2017). Soil aggregation and associated microbial communities modify the impact of agricultural management on carbon content. *Environmental Microbiology*, *19*(8), 3070–3086. https://doi.org/10.1111/1462-2920.13779

Verchot, L. V., Dutaur, L., Shepherd, K. D., & Albrecht, A. (2011). Organic matter stabilization in soil aggregates: Understanding the biogeochemical mechanisms that determine the fate of carbon inputs in soils. *Geoderma*, *161*(3), 182–193. https://doi.org/10.1016/j.geoderma.2010.12.017

Vos, M., Wolf, A. B., Jennings, S., & Kowalchuk, G. A. (2013). Micro-scale determinants of bacterial diversity in soil. *FEMS Microbioly Reviews*, *37*(6), 936–954. https://doi.org/10.1111/1574-6976.12023

Whiteford, J. R., & Spanu, P. D. (2002). Hydrophobins and the interactions between fungi and plants. *Molecular Plant Pathology*, *3*(5), 391–400. https://doi.org/10.1046/j.1364-3703.2002.00129.x

Williamson, K. E., Fuhrmann, J. J., Eric Wommack, K., & Radosevich, M. (2017). Viruses in soil ecosystems: An unknown quantity within an unexplored territory. *Annual Reviews in Virology*, *4*(1), 201–219. https://doi.org/10.1146/annurev-virology-101416-041639

Williamson, K. E., Radosevich, M., & Eric Wommack, K. (2005). Abundance and diversity of viruses in six Delaware soils. *Applied Environmental Microbiology*, *71*(6), 3119–3125. https://doi.org/10.1128/AEM.71.6.3119-3125.2005

Wilpiszeski, R. L., Aufrecht, J. A., Retterer, S. T., Sullivan, M. B., Graham, D. E., Pierce, E. M., Zablocki, O. D., Palumbo, A. V., & Elias, D. A. (2019). Soil aggregate microbial communities: Towards understanding microbiome interactions at biologically relevant scales. *Applied Environmental Microbiology*, *85*(14), e00324-19. https://doi.org/10.1128/AEM.00324-19

Wright, S. F., & Upadhyaya, A. (1998). A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil*, *198*(1), 97–107. https://doi.org/10.1023/A:1004347701584

Wu, X. J., Peng, J. J., Liu, P. F., Bei, Q. C., Rensing, C., Li, Y., Yuan, H. M., Liesack, W., Zhang, F. S., & Cui, Z. L. (2021). Metagenomic insights into nitrogen and phosphorus cycling at the soil aggregate scale driven by organic material amendments. *Science of the Total Environment*, *785*, 147329. https://doi.org/10.1016/j.scitotenv.2021.147329

Zethof, J. H. T., Bettermann, A., Vogel, C., Babin, D., Cammeraat, E. L. H., Sole-Benet, A., Lazaro, R., Luna, L., Nesme, J., Woche, S. K., Sorensen, S. J., Smalla, K., & Kalbitz, K. (2020). Prokaryotic community composition and extracellular polymeric substances affect soil microaggregation in carbonate containing semiarid grasslands. *Frontiers in Environmental Science*, *8*, 1–19. https://doi.org/10.3389/fenvs.2020.00051

Zheng, W., Kathryn Morris, E., & Rillig, M. C. (2014). Ectomycorrhizal fungi in association with Pinus sylvestris seedlings promote soil aggregation and soil water repellency. *Soil Biology & Biochemistry*, *78*, 326–331. https://doi.org/10.1016/j.soilbio.2014.07.015

How to cite this article: Kaur, R., Page, K. L., Dalal, R. C., Menzies, N. W., & Dang, Y. P. (2022). Can molecular genetic techniques improve our understanding of the role the microbial biomass plays in soil aggregation? *European Journal of Soil Science*, *73*(5), e13307. https://doi.org/10.1111/ejss.13307