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SUMMARY

The formation of rhizosheaths, a protective layer surrounding plant roots, is a fascinating phenomenon that has been attributed to various factors, one of which is the presence of mucilage. However, to our knowledge there is no evidence of influence of mucilage concentration on rhizosheath development. Therefore, I evaluated the rhizosheath formation under various mucilage concentrations together with numerous soil moisture contents and alternative dry and wet cycles. In a lab study with artificial root system I investigated (i) the effect of chia seed mucilage concentration on rhizosheath formation under in different soils, (ii) compared chia and flax seed mucilage in terms of rhizosheath formation, (iii) tested the effect of various chia seed mucilage on rhizosheath development under various volumetric water contents, (iv) effect of drying and wetting cycles on rhizosheath formation, (v) evaluated the particle size distribution and stability of rhizosphere soil under dry and wet conditions in the presence of mucilage, and finally (vi) a new model is presented to describe the pore distribution of dry mucilage.

In a lab study, (i) jute cords modelled as plant roots were disposed in a sandy loam and quartz sand under various chia seed mucilage concentration. Soil was poured into the PVC cylinders and then the artificial root at the top of the soil layer was inserted. A wet mucilage solution was prepared at five concentrations: 0.0g (control), 0.02g, 0.04g, 0.12g, and 0.2g dry mucilage g^{-1} water. To prepare the desired mucilage concentrations, freeze-dried mucilage was diluted with deionized water and kept in a sealed container for 15 min to swell. The wet mucilage was then uniformly injected by syringe onto the artificial root model to resemble the exudation of mucilage into soil. Finally, rest of soil was uniformly poured over the sample and soil samples were kept for 48 h at $25^{o}C \pm 1^{o}C$ room temperature. At the end artificial roots were removed from the soil system and rhizosheaths development were weighed on balance (ii) I checked the rhizosheath development under flax seed mucilage with five different concentrations 0.0g (control), 0.02g, 0.04g, 0.12g, and 0.2g dry mucilage g^{-1} water) in a quartz sandy soil, I again followed the same method of sample preparation (iii) In a subsequent study following the same method of sample preparation I amended the oven dried soil with five volumetric water contents: control (without moisture), $0.5 cm^3 cm^{-3}$, $0.15 cm^3 cm^{-3}$, $0.30 cm^3 cm^{-3}$, and $0.35 cm^3 cm^{-3}$ to check the rhizosheath formation in a quartz sandy soil under the influence of chia seed mucilage (iv) Finally, I conducted a lab study treated with 0.12 g dry mucilage g^{-1} water, to check the rhizosheath formation in a sterilized and unsterilized soils with high and low clay contents under dry and wet cycles to75% of the water holding capacity (WHC) with constant wet conditions (reference samples) (v) I checked the particle size distribution (PSD) and stability of aggregates from rhizosphere soils with laser diffraction method. Additionally, I also performed the scan electron microscopy (SEM) to obtain the images aggregates from rhizosheath.

I found that (i) in dry soil, rhizosheath formation peaked at an intermediate mucilage concentration. This behavior was supported by our conceptual model of mucilage spreading and rhizosheath formation, which relies on a radial diffusion equation and assumes that at low mucilage concentration, molecule numbers are insufficient to support polymer-like networks that stick soil

particles together. In a very concentrated gel, however, mucilage is too sticky to diffuse far into the soil (ii) No significant effect of flax seed mucilage on rhizosheath formation (iii) Increasing soil moisture promotes rhizosheath formation both in a low and a high mucilage concentration range, although only up to an intermediate volumetric water content of 0.15cm^3 cm^{-3} (iv) The presence of mucilage doubled the amount of rhizosheath relative to the soil that did not receive mucilage additions. However, an even stronger enhancement was found by the application of constant wet conditions, which significantly enhanced rhizosheath development, particularly in unsterilized soil with only 22% clay. The application of drying and wetting cycles, in turn, reduced the amount of rhizosheath significantly. (v)Noteworthy, there were also little interactions to the aggregation of particles outside the rhizosheath, which all exhibited an average size diameter < 10 µm but hardly impacts from the water regime

In summary both water and mucilage concentration are important drivers of rhizosheath formation. The effects are not additive but can combine to an optimum range, with a maximum formation of rhizosheaths observed in this study at 0.12 g mucilage g^{-1} rhizosphere water. Also, water regime is a key parameter controlling the contribution of mucilage to rhizosheath formation. The effects of clay content or of microbial activity were of minor importance, at least for this experiment performed under laboratory conditions.

ZUSAMMENFASSUNG

Die *Rhizosheath*, eine schützende Schicht um Pflanzenwurzeln, wurde in dieser Studie untersucht. Meine Hypothese lautete, dass die Bildung und Dicke der *Rhizosheath* von den Wurzelexsudaten (*Mucilage*) und dem Wassergehalt abhängen. Um diese Hypothese zu überprüfen, wurden Laborstudien mit einem künstlichen Wurzelsystem durchgeführt. Dabei wurden folgende Aspekte untersucht: (i) Die Auswirkungen der *Mucilage*-Konzentration von Chiasamen auf die *Rhizosheath*-Bildung in verschiedenen Bodenarten (ii) Die Rolle der *Mucilage*-Qualität von Chia- und Leinsamen bei der *Rhizosheath*-Bildung (iii) Die Abhängigkeit dieser Auswirkungen vom volumetrischen Wassergehalt (iv) Die Auswirkungen von Trocken- und Nasszyklen auf Veränderungen der Aggregatgröße und -stabilität im umgebenden Boden (v) Die Entwicklung eines Modells zur Beschreibung dieser Effekte.

Im Detail wurden (i) Flachskordeln als Modell für Pflanzenwurzeln verwendet. Sie wurden in sandigem Lehm und Quarzsand unter verschiedenen Konzentrationen von Chiasamenmucilage appliziert. Die Erde wurde in PVC-Zylinder gefüllt, bevor die künstlichen Wurzeln oben auf den Boden gelegt wurden. Es wurden *Mucilage*-Lösungen in fünf Konzentrationen hergestellt: 0,0 g (Kontrolle), 0,02 g, 0,04 g, 0,12 g und 0,2 g gefriergetrocknete *Mucilage* pro Gramm deionisiertem Wasser. Die Lösung wurde 15 Minuten lang quellen gelassen und dann gleichmäßig auf die künstlichen Wurzeln aufgebracht. Anschließend wurde der restliche Boden über die Flachskordeln verteilt, und die Bodenproben wurden 48 Stunden lang bei 25 ± 1 °C im Dunkeln gelagert. Schließlich wurden die künstlichen Wurzeln aus dem Bodensystem entfernt, und die anhaftende der *Rhizosheath* gewogen (ii) die Entwicklung der *Rhizosheath* unter Leinsamenmucilage mit ähnlichen *Mucilage*- und Wasserkonzentrationsbereichen überprüft (iii) Des Weiteren wurde die Studie mit fünf verschiedenen volumetrischen Wassergehalten durchgeführt: lufttrocken (Kontrolle, keine Wasserzugabe), 0,5 cm^3 cm^{-3}, 0,15 cm^3 cm^{-3}, 0,30 cm^3 cm^{-3} und 0,35 cm^3 cm^{-3}, um die Bildung der *Rhizosheath* in Quarzsandboden unter dem Einfluss von Chiasamenmucilage zu untersuchen. Gleichzeitig wurde (iv) Schließlich habe ich eine weitere Laborstudie mit ähnlichem Design durchgeführt, bei der die Modellwurzel mit 0,12 g trockener *Mucilage* pro Gramm Wasser zu sterilisiertem und unsterilisiertem Boden mit jeweils hohem und niedrigem Tongehalt gegeben wurde. Dabei wurden die Proben entweder unter konstant feuchten Bedingungen (als Referenz) oder unter Trocken- und Nasszyklen bis zu einem Wassergehalt von jeweils 75 % der Wasserhaltekapazität ausgesetzt (v) Die Partikelgrößenverteilung (PSD), die Stabilität und die Größe der Aggregate aus den umgebenden Boden wurden schließlich mit Hilfe der Laserbeugung und der Rasterelektronenmikroskopie (REM) bewertet; während (vi) das konzeptionelle Modell der *Rhizosheath*-Entwicklung auf radialen Diffusionsgleichungen beruhte.

Ich fand heraus, dass (i) in trockenem Boden die *Rhizosheath*-Bildung bei einer mittleren *Mucilage*-Konzentration ihren Höhepunkt erreicht. Dieses Verhalten wurde durch unser Modell der Wurzelexsudatausbreitung und der *Rhizosheath*-Bildung unterstützt, das davon ausgeht, dass bei niedriger *Mucilage*-Konzentration die Anzahl der Moleküle nicht ausreicht, um polymerartige Netzwerke zu bilden, die Bodenpartikel zusammenkleben. In einem sehr konzentrierten Gel ist die

Mucilage jedoch zu klebrig, um weit in den Boden zu diffundieren. (ii) Interessanterweise förderte Leinsamenmucilage die Bildung von *Rhizosheath* nicht. (iii). Eine Erhöhung der Bodenfeuchte förderte die Bildung von *Rhizosheath* sowohl im Bereich niedriger als auch hoher Mucilage-Konzentrationen, allerdings nur bis zu einem mittleren volumetrischen Wassergehalt von 0,15 cm^3 cm^{-3} (iv) Das Vorhandensein von *Mucilage* verdoppelte jedoch die Menge an Rhizosheath im Vergleich zu dem Boden, dem keine *Mucilage* zugesetzt wurde. Eine noch stärkere Steigerung wurde jedoch durch die Anwendung von konstanten feuchten Bedingungen festgestellt, die Entwicklung der *Rhizosheath* signifikant förderten, insbesondere in nicht sterilisierten Böden mit nur 22 % Ton, während Trocknungs- und Nasszyklen die *Rhizosheath* signifikant reduzierten. (v) Bemerkenswert ist, dass die Aggregate außerhalb der *Rhizosheath* nicht betroffen waren und alle einen durchschnittlichen Durchmesser von <10 µm aufwiesen, unabhängig vom Wasserregime.

Zusammenfassend lässt sich sagen, dass sowohl die Wasser- als auch die Mucilage-Konzentration wichtige Faktoren für die Bildung von Rhizosheath sind. Die Auswirkungen sind nicht additiv, sondern können in einem optimalen Bereich kombiniert werden, wobei die maximale Bildung von Rhizosheath bei 0,12 g *Mucilage* g^{-1} Rhizosphärenwasser erreicht wird. Die Auswirkungen des Tongehalts oder der mikrobiellen Aktivität waren von geringer Bedeutung, zumindest für die gewählten Laborbedingungen.

LIST OF FIGURES

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Al	Aluminum
ANOVA	Analysis of variance
C	Carbon
CEC	Cation exchange capacity
CFD	Computational fluid dynamics
Cd	Cadmium
Cu	Cupper
DLVO	Derjaguin, Landau, Verwey and Overbeek
Dv	Volume based diameter
DW	Dry wet
EDL	Electric double layer
EPS	Extracellular polymeric substances
ESEM	Environmental scanning electron microscope
LBM	Lattice Boltzmann model
KPa	Kilo Pascal
KeV	Kilo electric volt
LSD	Least square distance
LSM	Lattice spring model
lu	Lattice unit
MPa	Mega Pascal
P	Phosphorus
ρ_b	Soil bulk density
ρ_l	Water density
PSD	Particle size distribution
pH	Potential hydrogen
USA	United states of America
Vol.WC	Volumetric water contents
WHC	Water holding capacity

CONTENTS

CONTENTS

CONTENTS

CHAPTER I
GENERAL INTRODUCTION

1.1 Rationale

The convergence of water scarcity, a projected global population surge to approximately 9 billion by 2050, increasing drought forecasts from global climate models (Seneviratne et al., 2011) , and the current state of unsustainable agricultural practices has reached a crucial juncture, demanding a transformative change in farming methodologies. This transformation is imperative to secure future food sustainability and address the impending food security crisis. With the growing need for food production from existing resources, understanding the fundamental responses of plants to drought conditions without compromising crop yields has become paramount (De Oliveira Nascimento et al., 2020).

In this context, it is crucial to identify and harness beneficial plant root traits that enable plants to thrive in drought conditions while maintaining high yields. The rhizosheath, a structure that has been proposed as one of the most vital mechanisms for mitigating drought stress (Price, 1911), plays a pivotal role. It enables the ideal interaction between the soil and root interface, a crucial factor for effective absorption of nutrients and water. Comprising soil particles adhering to roots through the assistance of root hairs and mucilage, the rhizosheath has a rich history dating back to the 19th century when it was first observed as soil particles clinging to grass roots in arid regions (Volkens, 1887). Its designation as a "rhizosheath" was formalized later (Wullstein et al., 1979). Under arid conditions, rhizosheaths are commonly found in cereals and wild grasses, underscoring their significance.

In recent times, there has been a growing interest in rhizosheath formation, largely driven by the role of mucilage. Mucilage, a polymeric gel, is primarily composed of polysaccharides derived from both plants and microorganisms, with a minor lipid component (Read et al., 2003; Vermeer & McCully, 1982). The process of soil aggregation that leads to rhizosheath formation is intricately

linked to the interaction between mucilage and moisture conditions within the rhizosphere. Mucilage's hygroscopic properties play a vital role in nutrient mobilization and uptake. When fully hydrated, mucilage can contain up to 105 percent of its fresh weight in water, surpassing its dry weight. This remarkable water-holding capacity of mucilage, as elucidated by McCully & Boyer., (1997), serves as a reservoir in the rhizosphere, safeguarding roots from the perils of drought.

Mucilage's adhesiveness and hydrophilic properties enhance soil structure, mitigating erosion risks (Carminati et al., 2010). It serves as a carbon (C) source, fostering microbial activity (X. Zhang et al., 2020), leading to improved nutrient mobilization (Jones et al., 2004). The diverse microbial community it attracts bolsters nutrient cycling and disease resistance while actively participating in rhizosheath development (Canarini et al., 2019). Mucilage's hydrophilic nature also aids in moisture retention, conferring drought resistance to plants and promoting sustainable agriculture (George et al., 2014a). Understanding mucilage's central role in rhizosheath dynamics is pivotal for innovative strategies in plant science and soil ecology, offering insights into enhanced soil stability, nutrient availability, and ecosystem services (Dakora & Phillips, 2002; Rovira, 1959).

Scientific studies have demonstrated that mucilage in the rhizosheath improves water availability for plants, promotes nutrient acquisition, enhances soil aggregation, stimulates microbial activity, and contributes to plant resilience under drought conditions. These functions have significant ecological relevance, as they influence plant growth, nutrient cycling, soil stability, and overall ecosystem productivity. As mucilage in dry soil dehydrates, its surface tension rises, and the increased viscosity further aids in stabilizing both aggregates and the encompassing rhizosheath. (Read & Gregory, 1997). While the significance of mucilage in rhizosheath formation is increasingly acknowledged, there remains a need for comprehensive investigations that integrate

these factors with mucilage concentration, soil variability, mucilage quality, water contents and environmental dynamics to provide a holistic understanding of this intricate phenomenon.

Hence the overarching goals of this research encompass understanding the multiple relationships between mucilage, water content, soil types, and environmental dynamics in the context of rhizosheath formation. This investigation involves evaluating how varying chia seed mucilage (CSM) concentrations influence rhizosheath development across different soil types, assessing the role of mucilage quality derived from chia and flax seeds, and exploring interactions with drying and wetting cycles. Additionally, the study aims to elucidate changes in aggregate size and soil stability in the surrounding environment and ultimately to construct a predictive model that integrates these factors. These objectives collectively strive to enhance our comprehension of plant-soil interactions, offering potential insights for sustainable agricultural practices and soil management.

1.2 State of the Art

Rhizosheaths are aggregates of soil particles held together by root exudates, creating a protective structure around the root system. The process of rhizosheath formation, characterized by the aggregation of soil particles and root exudates around plant roots, was initially noted in grasses within the Egyptian desert by Volkens in (1887). Subsequently, it was documented in South Africa by Price, in (1911), eventually becoming a recognized occurrence in a wide range of plant species. Rhizosheath formation is observed in the majority of angiosperms, approximately 81% according to (Brown et al., 2017a), including major cereal crops like wheat, maize, barley, oat, rye, sorghum, and pearl millet, as reported by Duell & Peacock., (1985) and Ndour et al., (2017). There's a distinct contrast between the rhizosheath, referring to soil adhering to the root upon excavation, and the rhizosphere, typically recognized as the soil region surrounding plant roots that is impacted

by root exudates, fostering the proliferation of microorganisms (Hassan & Mathesius, 2015). To address the persistent confusion surrounding various root-soil terminologies and facilitate better comparison and reproducibility in experiments, it is essential to establish clear definitions for each term and provide visual representations. One common source of confusion, identified by both York et al., (2016) and Pang et al., (2017), pertains to the interchangeable, yet incorrect, usage of the terms "rhizosheath" and "rhizosphere," particularly mislabeling rhizosheath soil as rhizosphere soil (Brown et al., 2017a; Delhaize et al., 2012; Smith et al., 2004). To mitigate this issue and promote consistency across studies, the adoption of a standardized method for rhizosheath collection and measurement is recommended, as proposed by Brown et al., (2017). This approach offers ease of implementation and applicability across various plant clades, enhancing comparability in the scientific literature (Pang et al., 2017b). It is well-documented for its adhesive properties, contributing to soil particle aggregation and the formation of the rhizosheath—a protective structure that surrounds and shields plant roots (Marasco et al., 2022). The rhizosheath is crucial for nutrient uptake, water retention, and microbial interactions (Aslam et al., 2022; Mo et al., 2023). While the role of mucilage in rhizosheath formation is recognized, **what remains a significant gap in our understanding is how varying concentrations of mucilage affect the development and stability of the rhizosheath**. This knowledge is essential for optimizing the use of mucilage-rich plants like chia in agriculture and for elucidating the full extent of mucilage's potential in soil management and ecosystem sustainability.

Fig 1.1 A well-developed rhizosheath surrounding the roots https://soils.vidacycle.com/blog/how-do-rhizosheaths-tell-the-story-of-soil-health/

Although the significance of root exudates in the formation of rhizosheaths is widely recognized, **a critical gap in our understanding pertains to the effect of the concentration of specific root exudates, such as mucilage**, on the development and stability of the rhizosheath. Mucilage, a viscid and gelatinous substance produced by plant roots, plays a substantial part in soil consolidation and water retention (Galloway et al., 2020; Williams et al., 2021). **However, the concentration-dependent impact of mucilage on rhizosheath formation remains an understudied aspect.**

Some plant species may release exudates with a strong affinity for binding soil particles, leading to the formation of a more pronounced rhizosheath. Others may release exudates that primarily attract specific microbial communities. Therefore, the composition and structure of the rhizosheath can differ significantly among plant species (Kuzyakov & Razavi, 2019). The formation of a rhizosheath, characterized by the gelatinous mucilage adhering to soil particles, results from intricate polysaccharides and glycoproteins originating from both microbial and root sources. Mucilage comprises a wide range of organic compounds such as organic acids, sugars, carbohydrates, amino acids, and secondary metabolites, collectively influencing the

physicochemical conditions in the vicinity of plant roots (Badri & Vivanco, 2009). Organic acids in mucilage can chelate cations, increasing nutrient availability and promoting microbial activity, ultimately enhancing soil aggregation (Carminati et al., 2010; Neumann & Römheld, 1999). Sugars and carbohydrates serve as an energy source for soil microorganisms, stimulating microbial-mediated processes that influence soil structure and rhizosheath development (Dennis et al., 2010). Amino acids can impact soil pH and microbial activity, contributing to rhizosheath formation (Phillips et al., 2011). Additionally, secondary metabolites in mucilage may exert allelopathic effects on neighboring plants and soil microorganisms, influencing soil microbial communities and rhizosheath development (Bais et al., 2006; Mazzola & Manici, 2012). **Furthermore, variability in mucilage composition among plant species can lead to differences in rhizosheath formation potential** (Brown et al., 2017b; Watt et al., 1994b). Plant-derived mucilage, observed at root caps and on root surfaces as they enter the soil (Vermeer & McCully, 1982), contributes to the adhesion and cohesion of maize rhizosheaths. Watt et al. (1993) demonstrated that both plant and bacterial mucilage play roles in this process, using different mechanisms for root-cap mucilage. The root cap mucilage comprises polysaccharide molecules with complex oligosaccharide twigs, featuring neutral sugars at their ends. This mucilage drives soil adhesion primarily through hydrogen bonds formed between these neutral sugars' hydroxyl groups and soil particles, as emphasized by Watt et al. (1993). In contrast, bacterial mucilage, with greater protein content, employs distinct binding mechanisms. Moreover, the study by Watt et al. (1993) noted that bacterial mucilage exhibits higher hydrophilicity compared to root-cap mucilage. Additionally, Brown et al., (2017a) made an intriguing observation that the hydrophobicity of roots varies among different species. **Hence there is still need to check how different type of mucilage with different concentration influence rhizosheath formation?** Understanding how these

diverse compositions of mucilage interact with the rhizosphere environment is essential for unraveling the intricate mechanisms governing soil adhesion and rhizosheath formation. Young, actively growing roots may release more exudates, whereas older roots may have a more established and structured rhizosheath (Bais et al., 2006).

The presence of root hairs is a vital factor in the formation of rhizosheaths, as demonstrated by studies conducted by Brown et al. in 2012, Burak et al. in 2021, Haling et al. in 2010, and Watt et al. in 1994. Root hairs exert their influence on various aspects, including physically ensnaring soil particles, as noted by De León-González et al., in 2007 and Watt et al., in 1993. They also enhance root penetration, a phenomenon highlighted by Bengough et al., in 2016, and lead to increased root exudation and mucilage production, as evidenced by research conducted by Holz et al., in 2018 and Watt et al., in 1993. Additionally, root hairs play a role in modifying rhizosphere water content, an effect documented in studies by Albalasmeh & Ghezzehei, in 2014 and Carminati et al., in 2017. Longer root hairs often result in larger rhizosheaths, as observed in various studies. For example, Delhaize and colleagues in 2012 discovered a significant correlation between the density of rhizosheaths and the length of root hairs across different wheat traits. This connection was reinforced in later-generation intercross wheat populations, maintaining a consistent correlation across diverse soil types without any chemical limitations as shown in Delhaize et al. 's 2015 study. Yet, contradictory results have surfaced regarding the link between rhizosheath size and root hair length. For barley, a feeble correlation between rhizosheath size and root hair length implies that factors other than root hair length significantly contribute to rhizosheath formation, as indicated by George et al., in 2014b. In 2010, Haling et al. explored this connection utilizing a restricted set of barley and wheat lines. They found that when they analyzed rhizosheath size against the volume of the root hair cylinder (defined as the annulus around roots determined by root hair length), the

variability in root hair cylinder volume explained 52% of the variability in rhizosheath size in wheat and 66% in barley. In the presence of root hairs, it was found that enhanced root hair development had a more significant impact on rhizosheath formation than the adhesive properties of root exudates. Conversely, without root hairs, the adhesiveness of root exudates emerged as the predominant factor.

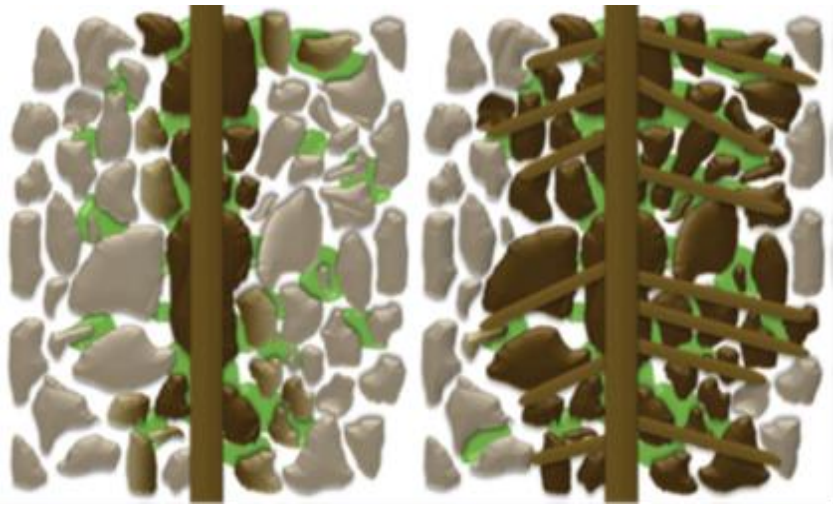

Fig 1.2 A conceptual representation illustrating root hair and mucilage contributions to rhizosheath formation. In **(a)**, roots without root hairs bind only directly contacting soil particles, while **(b)** with root hairs exhibit increased soil binding, resulting in a more substantial rhizosheath (Burak et al., (2021).

Soil properties, including texture and nutrient content, can influence rhizosheath formation. In soils with high clay content, the rhizosheath may exhibit better particle adhesion and aggregation due to the higher cation exchange capacity (CEC) of clay particles. Sandy soils may exhibit a less dense rhizosheath, limiting nutrient retention (Carminati et al., 2010). Various environmental factors such as temperature, moisture levels, and soil pH can significantly influence the formation of the rhizosheath. In regions characterized by arid conditions and scarce water availability, plants tend to increase exudate release. This aids in retaining soil moisture and enhancing nutrient availability, resulting in a thicker and stronger rhizosheath (Neumann & Römheld, 2012). The development of a rhizosheath serves as a protective measure against the formation of air gaps around roots, particularly in desert plant species. These roots contract radially when moisture is

lacking, as observed in studies conducted by Carminati et al. (2017) and North & Nobel (1997). These gaps in the soil surrounding the roots generally exhibit high hydraulic resistance, but the presence of a rhizosheath effectively reduces their impact. This protective effect ensures continuous contact between roots and soil, maintaining efficient water uptake potential (North & Nobel, 1997).

The process of rhizosheath formation is intricate and ever-changing, encompassing the gathering of soil particles and organic substances around plant roots. Although literature dedicated solely to rhizosheath formation is limited, studies on rhizosphere processes, root exudates, and interactions between soil and microbes offer insights. The formation of rhizosheaths displays diverse patterns influenced by factors like plant species, soil makeup, environmental conditions, and the presence of specific microorganisms. Different plant species release varying types and quantities of root exudates. The nutrient-rich environment of the rhizosheath promotes efficient nutrient absorption by plant roots, especially in nutrient-poor soils (Lambers et al., 2006). The presence of beneficial microbes in the rhizosheath can further enhance nutrient cycling and mineralization, benefiting plant nutrition (Bais et al., 2006) and contribute to soil structure and erosion control, ultimately benefiting plant growth and ecosystem health (Carminati et al., 2010). They serve several crucial functions, such as improving soil stability, enhancing nutrient acquisition such as phosphorus (P) and nitrogen (N) (Othman et al., 2003; Wullstein et al., 1979), and providing a habitat for beneficial soil microorganisms (Carminati et al., 2010; York et al., 2016).

1.2.1 Root exudates

Recent advancements in root exudate research have shed light on the complex and dynamic interactions occurring within the rhizosphere. Root exudates comprise an intricate blend of organic substances, encompassing sugars, organic acids, amino acids, and phenolic compounds

(Carvalhais et al., 2011; Dakora & Phillips, 2002; Dey & Sengupta, 2020). They play a crucial role in modifying the physicochemical properties of the soil, making it more adhesive and conducive to root-surface adhesion (Carminati et al., 2010). Microbes in the rhizosphere are attracted to the root exudates, including bacteria, fungi, and mycorrhizal fungi (Marschner et al., 2001). Microbial activities, such as the secretion of extracellular polymeric substances (EPS), contribute to soil particle aggregation and rhizosheath formation (Haichar et al., 2008; Ma et al., 2016; Oades, 1984). Recent research has increasingly recognized the critical function of root exudates, particularly focusing on mucilage, in shaping the rhizosphere environment and impacting plant well-being and ecosystem functioning (Hartmann et al., 2009; M. K. Hassan et al., 2019; Walker et al., 2003). These exudates, composed of a complex mixture of organic compounds, play a multifaceted role in shaping the rhizosphere environment. They serve as a communication channel between plants and soil microorganisms, modulating nutrient acquisition, disease resistance, and overall plant health (Bais et al., 2006; Bertin et al., 2003; Walker et al., 2003). Root exudates also influence soil physical properties, including soil structure and aggregation, which in turn impact water infiltration and root penetration (Carminati et al., 2010). This makes root exudates essential components of rhizosphere processes pivotal for sustainable agriculture and ecosystem functioning.

Root exudates can be categorized into several types based on their composition and functions. Low and High-Molecular-Weight Compounds encompass basic sugars (e.g., glucose, fructose), organic acids (e.g., citric, malic acid), amino acids, and phenolic compounds. These compounds are readily soluble in water and serve as an energy source for soil microorganisms. In a comprehensive examination conducted by Akhtar and colleagues (2018), it was observed that the adhesive capacity of high molecular weight components within root exudates varies significantly across

different plant species, encompassing both cereals and legumes. This research highlights the diversity in the adhesive properties of these compounds, shedding light on the plant-specific variations in their interactions with soil. Plants can release secondary metabolites such as flavonoids, alkaloids, and terpenoids. These compounds can have allopathic effects, inhibiting the growth of competing plants and protecting the plant from herbivores and pathogens.

1.2.2 Mucilage

Mucilage primarily consists of polysaccharides, as indicated by Chaboud in 1983 and Sasse et al., in 2018 , originating from both plants and microorganisms. Additionally, it contains minor quantity of lipids (Read et al., 2003; Vermeer & McCully, 1982). It represents a prevalent exudate with a high molecular weight originating from the tips of plant roots. (Morrél et al., 1967). The examination demonstrated that a significant portion of mucilage makeup consists of polysaccharides (78.4%), proteins (7.3%), minerals (5.6%), and lipids (3.1%), collectively constituting 94.3% of the mucilage (Nazari, 2021). It helps in water retention, soil aggregation (Morel et al., 1991; Watt et al., 1993), C source for microorganisms, and root lubrication, aiding in root penetration through the soil (Czarnes et al., 2000). However, it was suggested that mucilage can enhance drought tolerance of plants through sustaining a better liquid connectivity via mucilage bridges between soil particles and thus a better hydraulic conductivity in the rhizosphere at rather dry soil conditions (Ahmed et al., 2014; Carminati et al., 2010, 2017). Mucilage consists of long polymers that build up crosslinks forming a network-like structure in soil, which can attach to soil surfaces. The applicability of the typical diffusion equation that was originally developed for spreading of low-molecular solutes in soils is limited. When freshly exuded, mucilage, as a mixture of polymers, water and further components has a certain concentration. At typical concentrations of exudation, mucilage is a fluid of high viscosity. When released into either dry or

moist soil, the liquid exudate may undergo a process of concentration or dilution, leading to a shift from a solid-like state at elevated mucilage concentrations to a more Newtonian liquid exhibiting lower viscosity at lower concentrations. The impact of soil volumetric water content on rhizosheath formation in the presence of mucilage remains a pivotal research area with significant implications for plant-soil interactions. While numerous studies have explored the influence of either soil moisture or mucilage on rhizosheath dynamics separately, **there exists a research gap in comprehensively addressing their combined effects**. Understanding how varying water contents interact with mucilage is essential, as it may reveal critical insights into rhizosheath development, nutrient acquisition, and plant adaptation to changing environmental conditions (Carminati et al., 2010; Vetterlein et al., 2020). **Therefore, it still needs to be tested how various soil volumetric waters contents influence rhizosheath formation in the presence of chia seed mucilage?** Furthermore, this knowledge can have practical applications in optimizing crop performance and soil management (Brown et al., 2017b). Future research in this domain can bridge this gap, shedding light on the intricate interplay between soil moisture, mucilage, and rhizosheath formation.

1.2.3 Functions of root exudates

Root exudates serve several important functions in plant-microbe-soil interactions. Root exudates release organic acids that can solubilize mineral nutrients, making them more available for plant uptake. For instance, citric acid and malic acid in root exudates can help mobilize P in the soil (Lambers et al., 2006). Lipids found in mucilage play a role in enhancing plant water absorption and facilitating the release of bound P from soil particles in the rhizosphere. (Brown et al., 2017a; Jones & Oburger, 2011). Root exudates attract beneficial microorganisms, including mycorrhizal fungi and rhizobia, which form symbiotic relationships with plants. These microbes assist in

nutrient uptake and enhance plant growth (Bais et al., 2006). Recent investigations mentioned in the research conducted by Van Deynze et al., (2018) and Bennett et al., (2020) have revealed an intriguing finding: mucilage produced by the crown roots of a Mexican landrace maize (Zea mays L.) functions as a habitat for N-fixing bacteria. These bacteria have remarkable capacity to fulfil a significant proportion, ranging from 29% to 82% of the plant N requirements. This revelation about mucilages N fixing ability is currently a subject of great interest and attention within scientific community. It holds the potential to substantially increase maize yields, particularly in regions plagued by low soil fertility, offering a sustainable alternative to chemical N fertilizers. Mucilage, a specific type of root exudate, holds particular importance within the rhizosphere (Carminati et al., 2010; Vetterlein et al., 2020). Mucilage minerals, including monovalent and divalent cations, exhibit the capacity to exchange with other cations in the rhizosphere.

Some root exudates allelopathic compounds that inhibit the growth of competing plant and potential herbivores and pathogens (Inderjit & Dakshini, 1994). Furthermore, mucilage mitigates rhizosphere toxicity induced by aluminum (Al) as well as heavy metals like cadmium (Cd) and copper (Cu). Proteins that play essential roles in alleviating abiotic stress include diverse groups such as heat shock proteins, chaperonins, temperature-sensitive histone proteins, water stress proteins, and salt stress proteins. Additionally, significant protein categories engaged in responding to both biotic and abiotic stresses comprise chitinases and peroxidases. Chitinases and peroxidases are particularly vital for combating soil-borne plant pathogenic fungi and their biocontrol, as emphasized in various studies by Ordentlich et al., (1988), (Mittler, 2002), and (Gohel et al., 2005).

1.2.4 Dry Wet Cycles

The alternation between soil drying and wetting, known as dry-wet cycles (DW), significantly impacts the development and behavior of rhizosheaths. These rhizosheaths refer to the soil aggregates and organic matter (OM) that cling to plant roots within the rhizosphere. Soil aggregates form through the associations between abiotic components of the soils like clay minerals and oxides with plant-derived OM, and microbial substances (Totsche et al., 2018). Elevated soil microbial activity may both enhance generation of binding substances like microbial exudates and hyphae (Rahman et al., 2017), while also potentially degrading them, particularly in the absence of additional C sources from plants (Amelung et al., 2023). Soil sterilization can affect the growth and community structure of newly established bacterial populations, potentially altering root growth as well (K. Li et al., 2019; Wertz et al., 2007). Additionally, plant roots significantly promote aggregate formation through their physical enmeshment, alterations of water tension during growth, and excretion exudates from root tips. As a result, there is an enhanced formation of soil aggregates within the rhizosphere when compared to the surrounding bulk soil environment. (J. Li et al., 2020; Amelung et al., 2023). Mucilage, one of the most efficient plant exudate that supports soil aggregation (Monnier, 1965), consists of high-molecular-weight polysaccharides, forming a polymeric gel. The specific chemical compounds of root mucilage readily attaches to clay minerals, assisting in aggregate formation and stabilization (Mench et al., 1988; Morel et al., 1991). Particularly, freshly released mucilage from maize roots aids in aggregating the rhizosheath and preventing its disintegration (Morel et al., 1991). Under dry conditions, soil particles are tightly bound due to stronger water menisci and final coagulation of particles at low distance, as suggested by the DLVO theory (Osipov, 2015; Pashley & Karaman, 2021). In contrast, wetting the soil not only leads to the expansion of the electric double layer

(EDL) and related particle dispersion but also results in a rapid incorporation of free water, trapping air in pores, causing swelling and finally also causing inflation of soil aggregates (Kemper et al., 1985; Stewart & Hartge, 1995). Microbial activity can be boosted by DW cycles due to cell death during drought and microbial reconsumption after rewetting (Bell et al., 2014; Clark et al., 2009; Placella et al., 2012). Based on this, it's reasonable to hypothesize **that aggregate formation, sizes, and stability in the rhizosphere are influenced by DW cycles, therewith potentially modulating the role of mucilage as key bonding partner.**

Plant exudates, in combination with mucilage, exert a significant influence on particle size distribution (PSD) and aggregate stability within the rhizosphere, particularly under alternating dry and wet cycles. During dry periods, root exudates can enhance soil particle aggregation, forming stable aggregates held together by OM (Carminati et al., 2010; Tisdall, 2020; Tisdall & Oades, 1982; Totsche et al., 2018). However, as the soil dries, these aggregates can undergo shrinkage and become susceptible to disintegration. Upon rewetting, root exudates and mucilage contribute to the reformation of soil aggregates by acting as binding agents, facilitating the restoration of aggregate stability. The constant process of aggregation and disaggregation triggers variations in PSD, profoundly influencing soil structure, water retention, and nutrient accessibility. The specific exudate composition, presence of mucilage, and plant species involved collectively dictate the extent of these impacts, highlighting the intricate relationship between rhizosphere dynamics and soil attributes. **While prior studies have delineated the individual roles of exudates and OM as binding agents, there's a pressing need to thoroughly explore how these CSM components interact amidst shifting moisture conditions across diverse soils. Understanding these interactions is crucial in deciphering their influence on PSD and aggregate stability**. This research endeavors to bridge this knowledge gap by investigating the

joint effects of CSM and various soil types within rhizosphere contexts undergoing cycles of drying and wetting.

Hydrological processes and the diffusion of mucilage are interrelated dynamics. As mucilage polymers accumulate at high concentrations, they increase the viscosity of the soil solution, leading to reduced water molecule mobility. This enhanced viscosity facilitates the formation of crosslinks and adhesion to the soil surface, resulting in the creation of a spider-web-like structure within the pore space (Brax et al., 2017; Kroener et al., 2018). Nonetheless, water content and hydraulic dynamics significantly impact diffusion, convection, adsorption, and the breaking and establishment of crosslinks. **Currently, there exists a considerable gap not only in terms of parameters but also a lack of dedicated models for describing these individual physical processes specific to mucilage**. While there is an urgent need for such models, their development and the systematic experimental determination of all the necessary parameters extend beyond the scope of a single research endeavor. **In this study, we aimed to offer a qualitative overview of the influence of mucilage concentration in dry soil conditions by incorporating all the previously mentioned interrelated processes into the model, utilizing a simplified diffusion equation.**

1.3 Objectives

The present study aims to test the potential role of CSM in rhizosheath formation under various concentrations. I examined the rhizosheath formation under various soil water contents. On the other hand I also compared the two different plant derived mucilage in terms of rhizosheath formation. Together with this, I aim to investigate the rhizosheath formation under alternative DW cycles. For this I conducted a lab study using an artificial system consisting of artificial plant root

made of jute cord to mimic real plant roots. Influence of CSM was considered as real plant exudates and their role in rhizosheath formation. The specific questions to be addressed are as follows:

1. **Does mucilage concentration influence rhizosheath formation under different soils?**

   In this study, I aimed to offer a qualitative overview of the influence of mucilage concentration in dry soil conditions by incorporating all the previously mentioned interrelated processes into the model, utilizing a simplified diffusion equation.

2. **How is rhizosheath formation and stability influenced by mucilage from different plants?**

   Rhizosheath formation is a complex process**.** Different mucilage types can have varying effects on rhizosheath formation depending on their composition and properties. With the specific outcomes influenced by the plant, species, soil type and environmental conditions. The main objective of this study is to compare the effect of chia seed and flax seed mucilage (FSM) in rhizosheath formation. Therefore, I investigate the rhizosheath formation under CSM and FSM.

3. **How is rhizosheath formation affected by varying soil volumetric contents?**

   I systematically examine the rhizosheath formation under various soil volumetric water contents. The main aim is to unravel the complex dynamics that govern rhizosheath development in response to fluctuating soil moisture levels and mucilage availability. This knowledge can provide valuable insights into optimizing soil-plant interactions, nutrient acquisition, and soil stability, contributing to sustainable agriculture and improved crop performance under diverse environmental conditions.

4. **Do alternative drying and wetting cycles have implications on rhizosheath formation?**

Alternating cycles of drying and wetting influence the function of mucilage on rhizosheath development and the related soil aggregation within the rhizosphere. To test this, I used jute cord as an artificial model for root systems. These model roots were exposed to soils with varying clay contents (22% and 32% clay) being both sterilized and unsterilized. To mimic natural conditions, I moistened the model roots by adding 0.12 g dry CSM per g of water and incubated them. The incubation period lasted for 20 days at 25 ^{o}C while maintaining a water holding capacity (WHC) of 75% (used as a reference). I also included a DW treatment with five DW cycles to 75% WHC in regular intervals. Soils without added mucilage was used as a control.

5. **How does particle-size distribution and stability of soil aggregates in rhizosphere and rhizosheath behave under dry and wet conditions in the presence of mucilage?**

Mucilage influences the particle size distribution and soil stability of both rhizosphere and rhizosheath soils in the context of DW cycles. By investigating these interactions, the aim is to gain a comprehensive understanding of how mucilage presence modulates soil properties and stability under varying moisture conditions. This knowledge can provide critical insights into the mechanisms by which mucilage enhances soil structure, nutrient availability, and plant resilience, contributing to sustainable agriculture and environmental conservation

6. **How can the contribution of mucilage to rhizosheath formation be modelled?**

In order to qualitatively assess our hypotheses, specifically focusing on the concentration-dependent distribution of mucilage, I employed a basic model for mucilage dispersion through

radial diffusion. This model operates under the assumption that at low mucilage concentrations, there are insufficient molecules to establish polymer-like networks capable of binding soil particles together. Conversely, in highly concentrated gels, the mucilage is excessively adhesive, impeding its diffusion deep into the soil.

CHAPTER II

RHIZOSHEATH FORMATION DEPENDS ON MUCILAGE CONCENTRATION AND WATER CONTENT

Modified on the basis of manuscript

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1 INTRODUCTION

Soil is compacted by root growth, which in turn reduces spatial rhizosphere extension. Exudates are produced in the rhizosphere by plant roots and microorganisms, thus affecting the soil structure along with alternating drying and wetting cycles (Czarnes et al., 2000). Some of these changes affect the subsequent uptake of water and nutrients as well as the associated root penetration resistance and microbial population dynamics (Hinsinger et al., 2009; Oleghe et al., 2017; York et al., 2016). Plant exudates that trigger these processes comprise molecules of various molecular weights (Cortez & Billes, 1982; Mench et al., 1988). A dominant high molecular weight exudate from the root tips is mucilage (Morrél et al., 1967).

Mucilage is a polymeric gel, mostly made up of polysaccharides from plants and microorganisms, which also contains a trace amount of lipids (Vermeer & McCully, 1982: Read et al., 2003). McCully & Boyer (1997) reported that mucilage has a large water holding capacity. Mucilage that is fully hydrated can contain up to 1000 times its own dry weight. The authors discovered that mucilage loses a considerable portion of its water at water potentials lower than - 0.01 MPa, concluding that the water content of mucilage does not play a major role in drought mitigation through water storage. However, it was suggested that mucilage can enhance the drought tolerance of plants by sustaining a better liquid connectivity via mucilage bridges between soil particles and thus a better hydraulic conductivity in the rhizosphere under rather dry soil conditions (Carminati et al., 2011; Ahmed et al., 2014; Carminati et al., 2017).

Mucilage consists of long polymers that build up crosslinks that form a network-like structure in soil, which can attach to soil surfaces (Fig. II-1). The applicability of the typical diffusion equation that was originally developed for spreading low molecular solutes in soils is limited. When freshly

exuded, mucilage – a mixture of polymers, water, and other components – has a certain concentration. At typical concentrations of exudation, mucilage is a fluid of high viscosity. When exuded into dry or wet soil, this exuded liquid can become more concentrated or diluted, transitioning to a more solid-like behavior at high mucilage concentrations or towards a more Newtonian liquid with a low viscosity at lower concentrations (Carminati et al., 2017; Schnepf et al., 2022). A proper simulation of this spreading would require both (a) the simulation of the hydrological process, i.e. the dynamics of the spreading of exuded water into the soil, and (b) the simulation of the distribution of mucilage components, i.e. diffusion, convection, adsorption, and the formation and rupture (due to friction) of crosslinks, and subsequently also the degradation of mucilage.

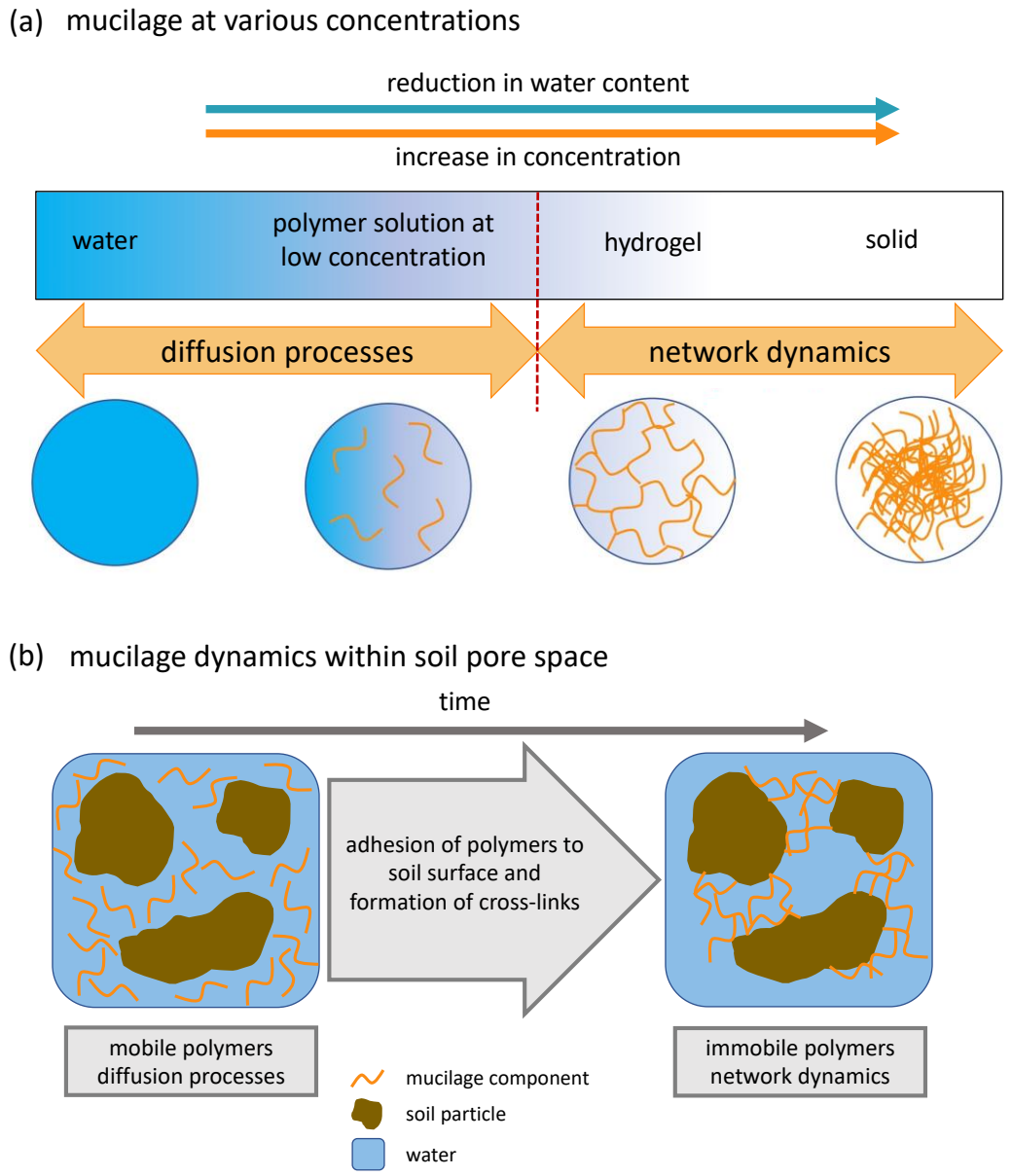


Fig. II- 1 (a) During drying, cross-links are formed between polymers and mucilage undergoes a phase transition from a Newtonian fluid at high water content to a polymeric solution of increased viscosity, a hydrogel and finally to a solid. At low concentrations, diffusion is important for dynamics of polymers, while at higher concentrations cross-links are created and network dynamics become dominating. **(b)** Over time, polymer adhesion to soil particle surface and formation of crosslinks reduce mobility of polymers and form a spider web like structure in the pore space

Hydrological processes and the spreading of mucilage are dynamics that affect each other: the mobility of water molecules is strongly reduced when mucilage polymers at high concentration increase the viscosity of soil solution, build up crosslinks, and become attached to the soil surface, thus forming a spider-web-like structure within the pore space (Brax et al., 2017; Kroener et al., 2018). However, diffusion, convection, adsorption, and the rupture and formation of crosslinks are strongly affected by water content and hydraulic dynamics (Bittelli et al., 2015).

Many of these processes are highly non-linear and we assume that these processes depend on (a) physical and chemical soil properties, and (b) on the chemical composition of the root exudates, i.e. plant species, root age, and growth conditions. For most of these processes, there is still a lack of not only parameters but also a model to describe these mucilage-specific individual physical processes. Although urgently needed, the development of such models and the systematic experimental determination of all required parameters goes far beyond a single research study. In this study, to provide a qualitative description of the effect of mucilage concentration under dry soil conditions, all previously mentioned coupled processes were considered in the model and the simplified diffusion equation was applied.

We assume that these liquid bridges between the soil particles and the mucilage have a strong influence on the formation of rhizosheaths, especially under dry soil conditions. The rhizosheath is formed by mucilage and root hairs that assist the soil clinging to the roots. Rhizosheath was observed in the 19th century as soil particles adhering to grass roots in the desert (Volkens 1887), although its name was established much later (Wullstein et al., 1979). Today, it is sometimes described as the weight of soil that adheres to roots when excavated from the pot or field (George et al., 2014a; McCully, 1999). The rhizosheath should also not be confused with the rhizosphere. Rhizosheath refers to soil that physically adheres to the root system, whereas rhizosphere refers to

soil influenced by roots (Hassan & Mathesius, 2015), which means that the rhizosphere spreads beyond the boundaries of the rhizosheath (York et al., 2016). Under dry conditions, rhizosheaths are very common, especially in cereals and wild grasses (Price, 1911; Watt et al., 1994; Young, 1995: Wullstein et al., 1979).

The rhizosheath is thus only a part of the rhizosphere, and is commonly discovered when the soils are dry (Watt et al., 1994), but is hardly found when they are wet. There was no rhizosheath present when plant species lacked root hairs (Lawrie K Brown et al., 2017), which suggests that mucilage exudation alone is insufficient for the formation of rhizosheaths (Margaret E McCully, 1999). However, the presence of root hair is not an absolute requirement for rhizosheath formation, instead depending on numerous factors such as root type, root system, root length, or the amount of composition of root mucilage (Fan et al., 2001; Muszyński et al., 2015; Peña et al., 2012; Vančura & Hanzlíková, 1972). However, in the root-hairless mutants, the adhesiveness of the exudate became more prominent. This was due to variations in the chemical composition of the root mucilage, which partially compensated for the lack of root hairs that would normally physically entangle soil particles (Burak et al., 2021). Overall, the rhizosheath includes soil particles, an intricate structure of root hairs, a local bacterial community, and mucilage, even in the case of maize (Gochnauer et al., 1989). The surface tension of mucilage increases with the dehydration of the mucilage in dry soil, meaning that the elevated viscosity additionally contributes to the stabilization of both aggregates and its surrounding rhizosheath (Read and Gregory, 1997).

The main aim of this study was to disentangle the complex interactions between mucilage concentration and moisture content and their effect on rhizosheath formation. We hypothesized that the effects were not simply additive. We tested the following hypotheses in depth: (a) at low

concentrations of mucilage, and thus low viscosity, mucilage can easily spread into distant parts of the soil, with the result that the concentration and binding properties of mucilage next to the root are not sufficient to establish a stable, large rhizosheath; (b) at a higher mucilage concentration, the substance can no longer diffuse far into the soil, meaning that the extension of the rhizosheath is small. As a consequence, c) the largest extension of the rhizosheath can be expected at an intermediate concentration of mucilage. Finally, we assume that d) the optimum value of rhizosheath formation shifts with variations in the soil moisture content and the associated mucilage concentrations. To qualitatively test our hypotheses, we used a simple model for spreading mucilage by radial diffusion.

2 MATERIALS AND METHODS

2.1Extraction of mucilage from chia seeds

Physico-chemical conditions of root mucilage can vary significantly depending on plant species, plant age, and soil conditions. Sufficient quantities of real root mucilage are difficult to extract from roots in soil. Therefore, mucilage, both from chia seeds (*Salvia hispanica L.*) and from flax seeds (*Linum usitatissimum L.*), were used in this study as plant model mucilage. Both chia and flax seed mucilage have been used to resemble root mucilage in artificial root–soil systems (Hayat et al., 2021; Naveed et al., 2017, 2019; Oleghe et al., 2017; Paporisch et al., 2021)

The chemical composition of chia seed mucilage is similar to maize (*Zea mays L.*) root mucilage. They are both composed of xylose, glucose, and uronic acids, with the latter making up around 25% of the composition (Carminati & Vetterlein, 2013; Lin et al., 1994). Moreover, chia seed mucilage has similar physical characteristics to maize and lupin (*Lupinus albus L.*) mucilage, as it forms a gel when hydrated and becomes hydrophobic after drying. Hydrophobicity is lower in flax

seed mucilage compared to chia seed mucilage. Moreover, flax seed mucilage is less attached to seeds than chia seed mucilage.

Mucilage was extracted by the method described by Ahmed et al. (2014). In this method, 5 g of seeds were mixed in 50 g of water. The mixture was stirred with a magnetic stirrer for 2 min and kept for 2 h at room temperature. We pushed this mixture through a sieve using a syringe that was cut at the end, thus separating the seeds from their mucilage. It is worth mentioning that the stickiest and gel-like part of the mucilage remained attached to the seeds. The extracted wet mucilage was freeze-dried to obtain a powder of dry mucilage, which can be easily mixed with a certain amount of water to obtain mucilage at a desired concentration.

2.2 Preparation of soil

We used two different soil textures for this study: a sandy loam and quartz sand. The soils were air-dried and sieved to pass through the particle size of 2 mm. The particle size distributions of both soils are presented in table II-1. The soil was homogeneously packed in PVC cylinders with a height of 1.4 cm and an internal diameter of 4.5 cm, corresponding to a soil volume of 22 cm^3. For the soil packing, soil was poured into the cylinder lying horizontally in order to minimize soil layering. This process resulted in a bulk density of 1.45 g cm^{-3} and 1.7 g cm^{-3} for the sandy loam and quartz sand soils, respectively. First, soil was poured into the PVC cylinders. We then inserted the artificial root at the top of the soil layer. The artificial root should resemble a plant root with root hairs and was made up of jute material with a diameter of 3 mm. A wet mucilage solution was prepared at five concentrations: 0.0g (control), 0.02g, 0.04g, 0.12g, and 0.2g dry mucilage g^{-1} water. To prepare the desired mucilage concentrations, freeze-dried mucilage was diluted with deionized water and kept in a sealed container for 15 min to swell. The wet mucilage (2g) was then

uniformly injected by syringe onto the artificial root model to resemble the exudation of mucilage into soil. As a control, we simply used 2 g of water instead of mucilage. Finally, additional soil was uniformly poured over the sample and soil samples were kept for 48 h at 25^{o}C ± 1^{o}C room temperature. For the application of wet mucilage, we calculated its total area [cm^2] assuming that the root had a cylindrical shape with a radius of 3 mm and a root length of 70 mm. Finally, additional soil was uniformly poured over the sample and soil samples were kept for 48 h at 25^{o}C ± 1^{o}C room temperature.

At the end of this experiment, the artificial root was removed from the soil and weighed. This experiment consisted of four replicates for each treatment. We performed two studies using the same method to investigate rhizosheath formation in two soils – sandy loam and quartz – under the influence of CSM concentration. In the other study, we simply monitored the effect of CSM and FSM in only quartz sand. The purpose of this experiment was to compare the influence of the gravimetric concentration of CSM and FSM on rhizosheath formation. We did not observe a visible swelling of the jute material. We repeated these two experiments in four replicates.

In another separate study to investigate the effect of volumetric moisture contents on rhizosheath formation, 40g of oven-dried soil samples were gently packed into a sample holder using a similar method as mentioned above. The soil water content was maintained at five different volumetric moisture contents: control (without moisture), 0.5cm^3 cm^{-3}, 0.15cm^3 cm^{-3}, 0.30cm^3 cm^{-3}, and 0.35 cm^3 cm^{-3} by adding an appropriate amount of distilled water to an oven-dried soil. The soil was prepared by putting some soil into the PVC cylinders. The artificial root was subsequently placed on the soil surface and then (2g) mucilage was spread on the root in similar concentrations (0.0g, 0.02g, 0.4g, and 0.12g dry mucilage g^{-1} water), as outlined above. Soil water content was maintained by weighing soil samples with the addition of an appropriate amount of distilled water.

Once the required moisture content was ensured, the samples were kept for 48 h at 25^{o}C ± 1^{o}C room temperature. The samples were covered with a plastic sheet to avoid any evaporation. After 48 h, the artificial roots were removed from the soil. They were then gently shaken to remove extra soil from the roots. The roots were then weighed on an electric balance to obtain the mass of the fresh rhizosheaths. The rhizosheaths were subsequently oven-dried at 405^{o}C for 48 h to obtain the dried mass of the rhizosheaths. We repeated this experiment with three replicates.

Tab.II.1 Particle size distribution of the sandy loam and quartz sand

Particle size range	**Sandy loam (%)**	**Quartz sand (%)**
> 2 mm	0.28	-
1-2 mm	41.35	-
630 µm	23.76	13
200 µm	25.46	84
63 µm	8.52	2.8
<63 µm	0.63	0.2

2.2.1 Model of mucilage spreading and rhizosheath formation using the radial diffusion equation

Root systems are often described by complex three-dimensional root architecture models that require large computational resources. Since the artificial root–soil system used in our experiment exhibits translational symmetry along the root, it is sufficient to consider the 2D cross section. For this model, we also assume rotational symmetry. This is a simplification of the experimental geometry, which is not exactly rotational symmetric due to gravity and due to the application of

mucilage from the top on the horizontally lying root. The radial rotational symmetric diffusion–adsorption equation for solutes in soil (Bittelli et al., 2015; Landl et al., 2021) is:

$$\rho_b \frac{\partial \mathrm{c}}{\partial \mathrm{t}} = \frac{1}{r}\frac{\partial}{\partial r} \cdot \left(r \rho_l \mathrm{D}(\theta) \frac{\partial \mathrm{c}}{\partial r} \right) + \theta \mathrm{kc} \quad (2)$$

where ρ_b is the soil bulk density (g cm^{-1}), C (g g^{-1} dry soil) is the mucilage concentration per dry soil, θ (cm^3 cm^{-3}) is the volumetric water content, t (s) is time, ρ_l is the water density (g cm^{-1}), c (g g^{-1} water) is the mucilage concentration in soil solution, r (m) is the radial coordinate, D (m^2 s) is the diffusion coefficient, and k (d^{-1}) is the adsorption coefficient. Assuming the simplification of a constant soil water content and using $\rho_b \mathrm{C} = \theta \rho_l c$, this becomes:

$$\theta \frac{\partial \mathrm{c}}{\partial \mathrm{t}} = \frac{1}{r}\frac{\partial}{\partial r} \cdot \left(r \mathrm{D}(\theta) \frac{\partial \mathrm{c}}{\partial r} \right) + \theta \mathrm{kc} \quad (3)$$

Numerous diffusion studies have shown that the diffusion equation can provide reasonable results for solutes of low molecular weights. As discussed in the previous paragraph for the diffusion of mucilage polymers, this is a simplified model. Various mobilities of the polymers of mucilage exuded at high, medium, and low concentrations are represented in the model by choosing a low, medium, and high diffusion coefficient in liquid **(**Tab. 2**)**. The relation between mucilage concentration, polymer mobility, and the diffusion coefficient is highly non-linear. The diffusion coefficient in soil is obtained from the liquid diffusion coefficient by considering soil tortuosity using the equation $D(\theta) = D_0 \cdot \left(\frac{\theta^{10/3}}{\varphi^2} \right)$ (Landl et al., 2021; Millington & Quirk, 1961).

After a certain time t_{end}, we assume that – due to the formation of crosslinks between polymers and due to the attachment of polymers to soil particle surfaces – the spreading of mucilage comes to an end and mucilage polymers can then be considered to be immobile. This assumption of a

t_{end} when mucilage becomes immobile is in agreement with experimental observations by neutron radiography ((Moradi et al., 2012) showing the region of soil around the roots where the presence of mucilage alters the soil hydraulic dynamics. Indeed, the region affected by mucilage has a similar extension around one-day-old roots as around two-week-old roots, which suggests that mucilage diffusion occurs to a large extent during the first few days before coming to an end. In this simplified model, the transition of mucilage from a mobile phase to an immobile mucilage phase is represented by choosing certain values of t_{end} where the diffusion process stops. Technically, the mucilage turning immobile is not an adsorption process where solutes are fully adsorbed to soil particle surfaces. Instead, it is largely a formation of polymer crosslinks, which strengthens the polymeric network, with only partial binding to surfaces, but which may still occupy the entire pore space. We therefore set the adsorption parameter $k = 0$ and the solution of Eqn. (3) is:

$$\mathrm{C}(r,t) = \frac{C_0\theta}{\sqrt{4\pi}Dt}\,\mathrm{e}^{-\frac{r^2\theta}{4Dt}} \quad \text{for} \quad t > 0 \tag{3}$$

C_0 accounts for the initial mucilage distribution, representing the radial exudation from the point source: $\mathrm{C}(r, t = 0) = C_0\delta(0)$, where $\delta(0)$ is the delta function in space.

From a simulated mucilage distribution $\mathrm{C}(r, t_{end})$, the rhizosheath extension can roughly be obtained by assuming that it may occur when the mucilage concentration is high enough to glue soil particles to each other, i.e., in the regions where $\mathrm{C}(r, t_{end}) > \mathrm{C}_{rhiz}$ with C_{rhiz} as a threshold value that may depend on the physico-chemical properties of mucilage and soil.

Tab.II. 2 Parameters of the model of mucilage distribution and rhizosheath formation

Name of parameter	**Parameter**	**Value**	**Unit**	**Source**
Time of polymer immobility	t_{End}	48	h	rough estimation from experiments (Moradi et al., 2012)
Porosity	φ	0.4	m^3m^{-3}	representative for sandy soils
Volumetric soil water content	θ	0.05	m^3m^{-3}	dry case scenario
Liquid diffusion coefficient				
at high concentration	D_0	$4 \cdot 10^{-12}$	m^2s^{-1}	Landl et al. (2021)
at medium concentration	D_0	$4 \cdot 10^{-10}$	m^2s^{-1}	case scenario
at low concentration	D_0	$4 \cdot 10^{-9}$	m^2s^{-1}	case scenario
Threshold for rhizosheath formation	C_{rhiz}/C_0	$2 \cdot 10^6$	-	case scenario of this simulation (depends on various physico-chemical properties of soil, plant, and soil solution)

3 RESULTS

3.1 Rhizosheath formation in a sandy loam soil

The key results of this experimental study are presented in Fig II- 2. The rhizosheaths measured [g cm^{-1}] in a sandy loam soil were studied under various concentrations of applied CSM [g dry mucilage per g water]. At intermediate mucilage concentrations (0.12g dry mucilage g^{-1} water), the average dry mass of consolidated and coherent rhizosheaths per dry mass of root peaked at 3.63 g cm^{-1}. In contrast, the formation and development of rhizosheaths at low (0.02 g dry mucilage g^{-1} water) and high (0.2g dry mucilage g^{-1} water) mucilage concentrations resulted in an average mass of only 0.13 g cm^{-1} and 0.36 g cm^{-1} respectively.

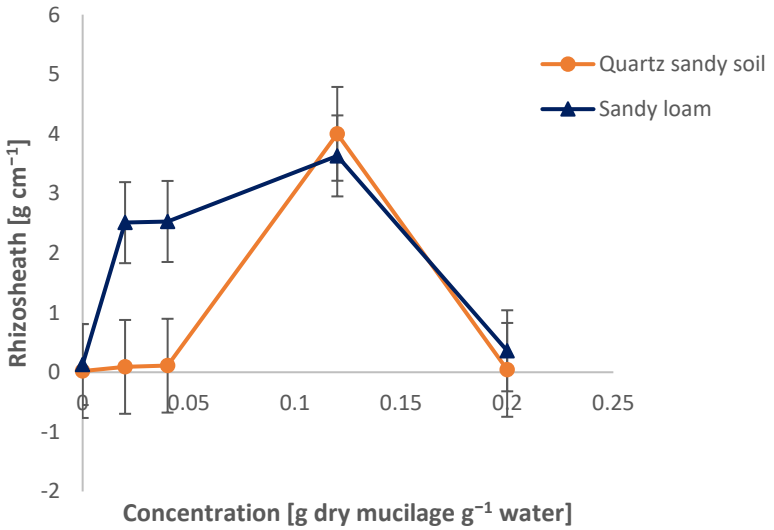


Fig. II- 2 Formation of rhizosheaths under the influence of chia seed mucilage concentration. The color orange represents the formation of rhizosheaths in a quartz sandy soil by a mean value of ± SD, n=4. The color blue represents the rhizosheath in a sandy loam soil by a mean value of ± SD, n=4

3.2 Rhizosheaths formation in a quartz sand soil

There was no significant difference in rhizosheath formation in a quartz sandy soil (Fig. II-2) compared to sandy loam soil under the same concentrations of CSM. Overall, it followed the same trend in terms of stable rhizosheath formation, i.e. the peak in rhizosheath formation was replicated, which was recorded at 4 g cm^{-1} and again at intermediate mucilage concentrations (0.12 g dry mucilage g^{-1} water). In addition, similar to the sandy loam soil, rhizosheath formation was smaller at higher (0.2 g dry mucilage g^{-1} water) and lower mucilage concentrations (0.02g dry mucilage g^{-1} water) of applied CSM.

3.3 Comparison of chiaseed and flaxseed mucilage with respect to rhizosheath formation

Fig. II-3 shows rhizosheath formation under numerous concentrations of FSM. The average mass of rhizosheaths was negligibly small in relation to that of CSM. This is in agreement with our own observations during mucilage extraction from seeds, i.e., CSM was much stickier than FSM.

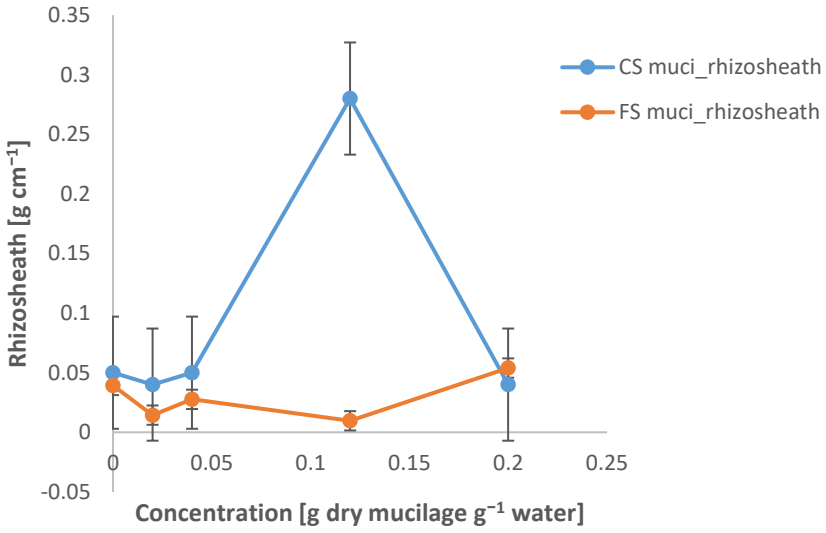


Fig. II- 3 Comparison of the effect of CSM and FSM on rhizosheath formation in a quartz sandy soil under various CSM concentrations. Rhizosheath development under both CSM and FSM were analyzed by a mean value of ± SD, n=4

3.4 Rhizosheath formation as a function of water content

In the quartz sandy soil, rhizosheath formation was also studied as a function of volumetric content Vol. WC ($cm^3\ cm^{-3}$), which is depicted in Fig II-4. In general, the soil water content (dry: soil not very sticky; medium: most sticky; wet: hardly sticky) also had a major influence on the volume of the rhizosheath. At the highest water content (0.35 $cm^3\ cm^{-3}$), the concentration of mucilage did not have a strong impact on rhizosheath formation, and the former peak disappeared (Fig. II-4). Similarly, at a low water content, the formation of rhizosheaths hardly required the presence of mucilage. However, this was different under drought conditions, which are typically critical for plants to take up water and mineral nutrients. Under such conditions, intense root–soil contact is crucial for plant nutrition. In line with these ecological requirements, at 0 (no water) Vol. WC ($cm^3\ cm^{-3}$), the mucilage concentration had a significant influence on rhizosheath extension, with the amount of rhizosheaths peaking at an intermediate content, as indicated above. Mucilage is therefore crucial in facilitating nutrient acquisition by increasing the root surface area, for example

by enhancing nutrient availability through chelating agents and by enhancing plant resilience to various abiotic stresses, such as drought, salinity, and heavy metal toxicity.

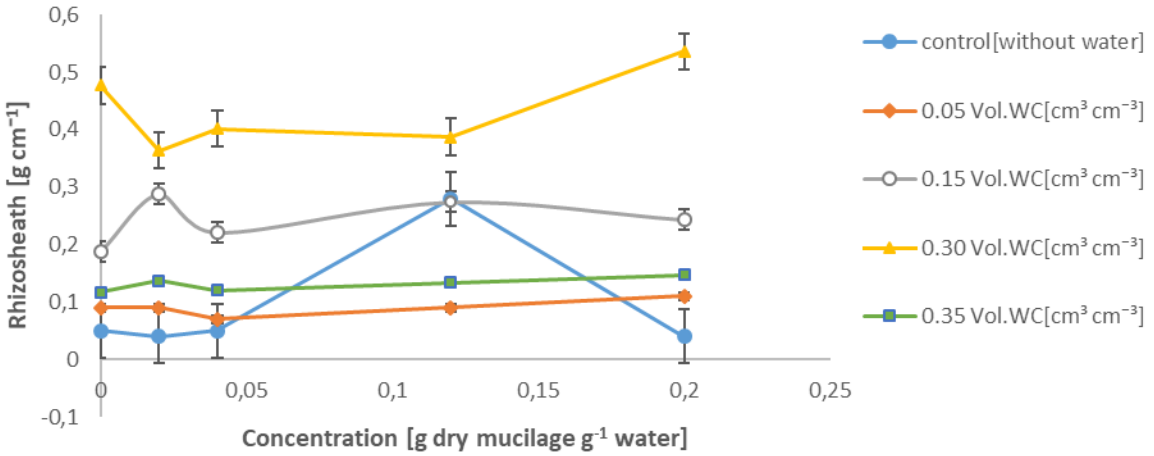


Fig. II- 4 Formation of rhizosheaths as a function of Vol. WC [$cm^3\ cm^{-3}$] in a quartz sandy soil under various CSM concentrations. Control means no water was added in an oven-dried quartz sandy soil by a mean value of ± SD, n=3, control Vol. WC [$cm^3\ cm^{-3}$], while the rest of the soils were irrigated by 0.05, 0.15, 0.30, and 0.35[$cm^3\ cm^{-3}$] Vol. WC, and analyzed for rhizosheath development by a mean value of ± SD, n=3

3.5 Model of mucilage spreading and rhizosheath formation

The simulated spreading of mucilage in soil (Fig. II-5a) shows that when mucilage was applied at high (0.2g dry mucilage g^{-1} water) concentrations, it could only spread a short distance with a high concentration near the initial mucilage pulse. In contrast, when mucilage was applied at intermediate concentrations (0.12g dry mucilage g^{-1} water), it could spread a few millimeters, occupying a considerable soil volume with a relevant amount of mucilage. At a low concentration of applied mucilage (0.02-0.04g dry mucilage g^{-1} water), the polymers were likely to have easily diffused into more distant areas, making it impossible to form a polymeric network between soil

particles that could glue them together to form a rhizosheath. As a result, the formation of rhizosheaths was largest at an intermediate concentration of applied mucilage. At lower and higher concentrations, rhizosheath extension was much shorter, i.e. the conceptual model was able to mimic the observations of the laboratory experiments.

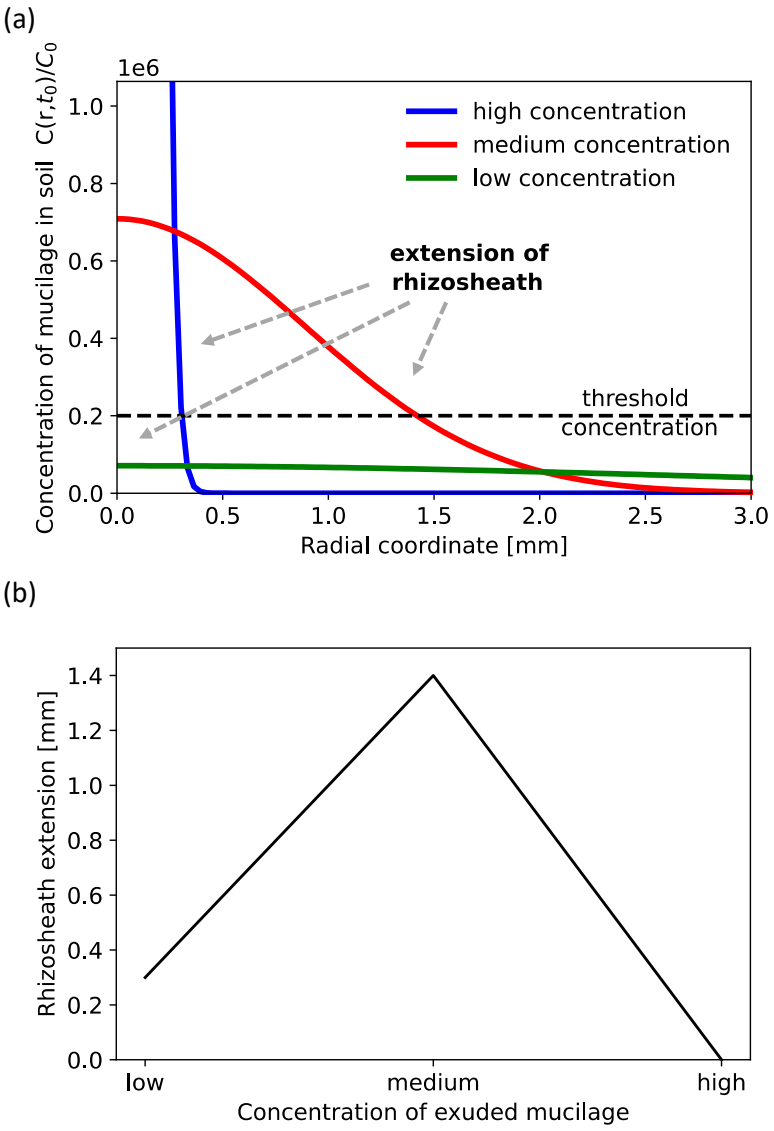


Fig. II- 5 (a) Simulated diffusion of mucilage in soil based on the parameters of Tab. II-2. (b) The extension of rhizosheaths that is expected to form where mucilage concentration in soil is larger than a certain threshold value.

4 DISCUSSIONS

Rhizosheath formation measurements under varied mucilage concentrations as well as the conceptual model of mucilage spreading and rhizosheath extension in dry soil (Fig II-1 and II-2) were able to confirm our hypothesis. We hypothesized that mucilage has a strong influence on rhizosheath formation under dry soil conditions. In dry soils, the intermediate mucilage concentrations of the polymeric network is sufficient to glue and stick soil particles around the root for amplified rhizosheath formation in both soil types. This can be better explained in terms of mucilage diffusion, which allowed a greater volume of rhizosheath extension around the roots. We expected to observe a negligent and weak formation of rhizosheaths at lower concentrations of applied mucilage due to the high diffusion of polymers and, therefore, the wide spreading of mucilage distribution and thus a low concentration in soil. Similarly, higher mucilage concentrations exhibit a lower mobility of polymers. This limits the distribution of mucilage in soil, which in turn resulted in a reduced region of soil agglutination for the development of rhizosheath volume. At higher concentrations, the viscosity of mucilage is expected to be 1000 times higher than pure water, as shown for chia mucilage by Ahmed et al. (2016). Our experiments show that, similar to viscosity, the mobility of mucilage polymers in soil solution also varies strongly with mucilage concentration. We found the same behavior of rhizosheath development in both soils: sandy loam and quartz sand. Moreover, at a low mucilage concentration, the formation of rhizosheaths in sandy loam is higher than in quartz sand. We assume this could be attributed to the presence of large amount of fine particles in sandy loam as compared to quartz sand.

We also compared the effect of CSM and FSM under the same mucilage concentrations on the development of rhizosheaths (Fig II-3). Our findings indicate no significant effect on rhizosheath formation by FSM. We assume that this effect is due to the difference in the viscosity of CSM and

FSM. This is in agreement with the results reported by Brax et al. (2020) and Mazza & Biliaderis (1989), in which they compared the viscosity of CSM and FSM. The findings of both studies confirmed the higher viscosity of CSM compared to FSM at all concentrations studied. Bemiller et al. (1993) also recorded a high viscosity of CSM compared to FSM. Similarly, Naveed et al. (2019) and Brütsch et al. (2019) reported a higher viscosity of CSM than barley.

In a parallel study, rhizosheath formation as a function of soil Vol. WC (cm^3 cm^{-3}) showed a smaller formation of rhizosheaths at low soil Vol. WC (cm^3 cm^{-3}) due to the reduced stickiness of the soil. With high soil WC (cm^3 cm^{-3}), the formation of rhizosheaths was again reduced due to the wet conditions as well as the minor contact between the soil particles and the root to develop rhizosheaths. These observations are also in line with a previous study of Liu et al. (2019), who measured rhizosheath development in response to moisture content. They reported the highest formation of rhizosheaths at 10-14% (W/W) compared to other soil moisture levels. Similarly, we can also assume that at higher moisture contents, the actual influence of mucilage is not significant, particularly because mucilage can dissolve under wet conditions, thus diffusing along with water into the surrounding soil (Watt et al., 1994a), but not concentrating near the roots to form a network of polymers.

Here, we used artificial roots. In real soil, the rhizosphere may dry out again following the transpiration of the plant. Unlike conditions in the model system, there is therefore the chance for mucilage to dry in a system with living plants and to form a polymeric network again, unless it is decomposed by soil microorganisms.

Rhizosheath formation under dry conditions was closely related to mucilage concentration. The maximum rhizosheath formation was found at intermediate concentrations and was about 10 times

higher than rhizosheath formation at other concentrations. These findings were in line with the outcomes of Watt et al. (1993, 1994), who reported that under dry conditions, larger and coherent rhizosheaths were bound to the roots than under wetter conditions. Moreover, in dry soils, the rhizosheaths of certain grasses were approximately three times larger than in wet soils. At low mucilage concentration, facilitated diffusion seemingly resulted in a spreading of the compounds to a degree that soil mucilage concentration was likely not particularly significant with respect to gluing soil particles together, i.e., it only formed a thin, transient rhizosheath layer, whereas rhizosheath formation declined again towards even higher mucilage concentrations (Fig. II-4).

When the water content increased for the given optimum "intermediate" mucilage concentration in the spike solution, the peak of rhizosheath formation shifted towards higher concentrations of the added water (Fig. II-4), which likely reflects the respective dilution of the polymers to optimum concentrations again. In any case, mucilage and water content did not have a purely additively effect, and neither one nor the other appeared to be solely responsible for the degree and amount of rhizosheaths formed. Instead, it was the concentration of the compounds in the gel, i.e. in the available soil water volume.

5 CONCLUSIONS

Overall, the current study showed that rhizosheaths are formed at various mucilage concentrations. This was demonstrated experimentally and based on a radial model using a diffusion equation. The degree of rhizosheath formation, however, is dependent on concentration and moisture, thus requiring a calculation of an effective mucilage concentration in the pore water of the rhizosphere. In the experiment and using the modelling approach, we have seen that under dry soil conditions, i.e. when the soil volumetric moisture content is more or less than zero in the

oven-dried quartz sandy soil, that an intermediate (0.12g dry mucilage g^{-1} water) mucilage concentration resulted in the largest volume of rhizosheaths compared with low (0.02g dry mucilage g^{-1} water) and high (0.2g dry mucilage g^{-1} water) mucilage concentrations. This physical behavior of mucilage in rhizosheaths might be crucial to supporting plant nutrient uptake and water availability under drought and stress conditions. The presence of mucilage in rhizosheath formation provides promising conditions for plants to become more tolerant to abiotic stresses and to improve agricultural yield in drought-prone areas.

CHAPTER III

INTERACTIVE EFFECTS OF MUCILAGE AND DRYING AND WETTING CYCLES ON RHIZOSHEATH DEVELOPMENT

Modified on the basis of manuscript

Rahim, R., Amelung, W., & Siebers, N. (2023). Interactive effects of mucilage and drying and wetting cycles on rhizosheath development. Preprint (Version 1) available at Research Square. https://doi.org/10.21203/rs.3.rs-3432519/v1.

1 INTRODUCTION

Soil aggregates and their stability play a crucial role in soil health and fertility, as they are key components of soil structure, influencing water infiltration, water retention, nutrient cycling, and C sequestration. Soil aggregates form largely through the associations between like clay minerals, oxides as well as microbial substances and plant-derived organic matter (Totsche et al., 2018). Elevated soil microbial activity may both enhance the generation of binding substances like microbial exudates and hyphae (Rahman et al., 2017), but also potentially degrading them, particularly in the absence of additional C sources from plants (Amelung et al., 2023). When soils sterilized, new bacterial populations establish, that may affect aggregation but, potentially alter root growth as well (K. Li et al., 2019; Wertz et al., 2007). The presence of plant roots significantly promotes aggregate formation through their physical enmeshment, alterations of water tension during growth, and excretion exudates from root tips. This leads to better developed soil aggregation in the rhizosphere compared to the adjacent bulk soil environment (J. Li et al., 2020; Amelung et al., 2023). Mucilage, a one of the most efficient plant exudate therewith supports soil aggregation (Monnier, 1965). Mucilage is a polymeric gel composed of high molecular weight polysaccharides and trace amounts of lipids. The specific chemical compounds of root mucilage readily attaches to clay minerals, assisting in aggregate formation and stabilization (Mench et al., 1988; Morel et al., 1991). Particularly, freshly released mucilage from maize roots aids in aggregating the rhizosheath and preventing its disintegration (Morel et al., 1991).

Beyond biological effects, aggregates also form during DW cycles, essential for promoting aggregation, triggering nutrient cycling and organic matter decomposition (Kemper et al., 1985; Krause et al., 2018; Mikha et al., 2005). Under dry conditions, soil particles are tightly bound due to stronger water menisci and final coagulation of particles at low distance, as suggested by the

DLVO theory (Osipov, 2015; Pashley & Karaman, 2021). In contrast, wetting the soil not only leads to the expansion of the electric double layer and related particle dispersion but also results in a rapid incorporation of free water, trapping air in pores, causing swelling and finally also causing inflation of soil aggregates (Kemper et al., 1985; Stewart & Hartge, 1995). Microbial activity can be boosted by DW cycles due to cell death during drought and microbial reconsumption after rewetting (Bell et al., 2014; Clark et al., 2009; Placella et al., 2012). Based on this, it seemed reasonable to hypothesize that aggregate formation, sizes, and stability in the rhizosphere are influenced by DW cycles, therewith potentially modulating the role of mucilage as key bonding partner.

The study's objectives were threefold: first, to quantify the role of mucilage in soil aggregate formation and size distribution in the rhizosheath in the presence of mucilage and under constant moisture conditions for two soils with varying clay content. Second, to evaluate how soil sterilization and resultant inhibition of microbial activity affect soil aggregate formation and size distribution. Third, to assess to what degree the above-mentioned interactions were modulated by DW cycles. Flax cord was used as model-root and chia mucilage as model root-mucilage. To characterize related aggregate formation and individual rhizosheath-aggregate interactions, laser diffraction and scanning electron microscopy (SEM) was used.

2 MATERIALS AND METHODS

2.1 Soil sampling, characteristics, and treatment

The soils used in the study was collected from the Scheyern research station (48° 29' 36'' N, 11° 26' 15'' E) situated in the rural area outside Munich (Germany). The selected soils were sandy to loamy textured Luvisols with mean annual temperature of 7.4 °C and a mean annual precipitation

of 803 mm. Detailed information of the selected soils, i.e., soil properties, sampling plots, and soil sampling is given in Krause et al. (2018). Soil samples with clay contents of 22% and 32% were selected from field triplicates for subsequent experiments. Since all soils originated from the same experimental site, there were no management differences among the different replicates. Illitic clay minerals dominated all soil samples, which were also free of carbonates. Additional clay minerals were Fe chlorite, kaolinite, and smectite. The selected soils were further treated for sterilization. For this purpose, 0.5 kg soil was divided into three portions and placed in an aluminum container covered with aluminum foil. The soil was then autoclaved at 121 ^{o}C under 103 kPa for 30 min and kept left in a laminar flow at room temperature in between for three consecutive days. The purpose of autoclaving the soils was to partially eliminate microorganisms and identify any microbial-induced effects. After those soils were oven dried at 60 ^{o}C and sieved to pass through 2-mm sieve.

2.2 Extraction of chia seed mucilage

We used CSM (*Salvia hispanica L.*) as a model for plant exudates. The choice of CSM was based on two primary reasons: first, its ease of harvest in larger quantities, and second, its similar physical properties to plant root exudates. The extraction of CSM followed the method outlined by Ahmed et al., (2014). In short, 5 g of chia seeds were mixed in 50 g of deionized water and left to swell for 2 min under stirring using a magnetic stirrer. The mixture was then kept at room temperature for 2 h. Subsequently, the chia seed-water mixture was sieved using a syringe that was cut at the one end, while vacuum pressure was applied through a vacuum pump and the chia seed gel was obtained. The harvested chia seed gel was freeze-dried and was subsequently pulverized using a ball mill. The freeze-dried powder was then mixed in a deionized water to get the desired concentrations of 0.12 g of CSM per g of water.

2.3 Aggregation experiment

We used jute cord as a model plant root. Aluminum sample holders measuring 16.6 cm in length, 6.5 cm in height, and 3 cm in width were used. Each sample holder contained 60 g of dry soil, into which jute roots were inserted into the middle. Two g of CSM (0.12 g dry CSM per g water) was injected onto each jute cord. For the control treatment, 2 g of deionized water was used instead of mucilage. The remaining soil was added to cover the jute cord. After treatment preparation, the soil was saturated with deionized water to reach 75% of the soil's WHC (reference treatment). To maintain this level, the soil capillary rise method was applied using perforated aluminum sample holders, with a filter paper placed at the bottom. The sample holders were soaked in tap water until saturation, then the blotting paper was removed and sampled holders were checked for water leakage and the weight was recorded. Five DW cycles were conducted over a 20-day period. Each cycle encompassed 4 days of soil drying to 15% of WHC, followed by rewetting on the 4^{th} day afternoon at 25 ^{o}C. Following four consecutive DW cycles, jute cords were removed, and the mass of rhizosheaths was recorded. To uphold a WHC of 75%, an appropriate amount of deionized water was added. The control treatment was kept at 75% WHC without DW cycles. To monitor soil moisture, samples were weighed daily, and the difference in weight due to evaporation were compensated for by carefully spraying deionized water onto the soil with a syringe. At the end of the DW cycles, rhizosheath formation was determined by weight.

2.4 Particle-size distribution and stability

Due to limited rhizosheath mass, PSD and stability analyses were not possible. Hence, we characterized individual aggregates of the rhizosheath using SEM, and applied general techniques for assessing PSD and stability to the remaining rhizosphere soil, which had also received the mucilage and the DW cycles, but which did not stick the model roots. For PSD and stability tests,

the remaining air-dried bulk soil samples were analyzed using a laser diffraction particle analyzer (Horiba LA960, Kyoto, Japan) as described in Krause et al. (2018) and Tang et al. (2022). This approach involved analyzing the volume-based PSD in an aqueous suspension. Software algorithm based on the Mie theory converts scattered light data into volume-based size distribution. Particle-size distribution was also qualitatively analyzed using SEM. Dried-bulk soil and rhizosheath was prepared for SEM analysis by gently spreading them onto a double-sided C-tape and mounting onto a sample holder. The microstructural analysis was obtained using SEM (Hitachi SU-8000) at 2 KeV.

The determination of soil stability was performed with the same particle analyzer following the method described by others (Kowalenko & Babuin, 2013; Mason et al., 2011; Siebers et al., 2023). For this, while being agitated and circulated aggregates were exposed to a steady and continuous mechanical force, the stability of aggregates was determined by conducting repeated measurements of the particle size distribution over a period of 40 min. We took measurements every 40 s for the first 10 min, then every 3 min for the remaining 30 min. The particle sizes collected were used to calculate the shift in median volume-based diameter (Dv50).

3 STATISTICAL TESTS

For data analysis and plotting we used Microsoft excel (Excel 2016, Microsoft Corporation, Washington, USA) and Origin (OriginPro 2018b, Originlab). To test the rhizosheath formation under both wet conditions and DW cycle we performed paired sample t-test ($p < 0.05$). To compare the stability of the soil aggregates between treatments, we performed one way ANOVA ($p < 0.05$). If significant differences occurred, we used the Least Significant Differences (LSD) to perform a post-hoc separation of means ($\alpha = 0.05$), as proposed by Webster (2007) for soil experiments.

4 RESULTS

4.1 Rhizosheaths mass

Mucilage approximately doubled the amount of rhizosheath compared to the control. In general, it can be seen from the SEM images that larger aggregates were formed in the rhizosheath (Fig. III-1c, d) compared to the control without mucilage (Fig. III-1 a, b). However, the amount of rhizosheath remained relatively low in soils exposed to DW cycles, with no significant effects attributed of soil type (Fig.III-2). Sterilization had no impact on rhizosheath formation in soils under DW cycles. On the other hand, significantly larger rhizosheath formation were visible when soils were maintained under wet conditions. Maximum rhizosheath development was 1.4 g in unsterilized soil with 22% clay (Fig. III-1. c, d). Sterilization consistently yielded lower rhizosheath formation, with statistically significant effects observed solely for the soil with 32% clay (Fig. III-2).

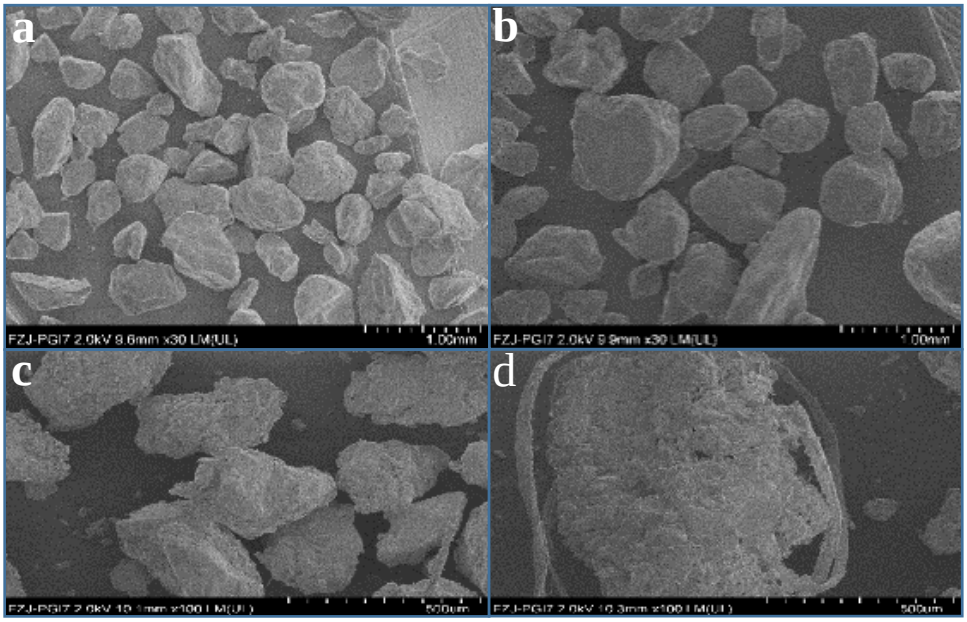


Fig. III- 1 Images of aggregates from rhizosheath soil by scanning electron microscope, **a**) aggregates from rhizosheath having no mucilage under DW-cycle**, b**) aggregates from rhizosheath having no mucilage under wet conditions, **c**) aggregates from composite samples of rhizosheath of unsteri soil_ 22% clay in wet conditions, **d**) aggregates from composite samples of rhizosheath of steri soil_ 32% clay in wet conditions**.**

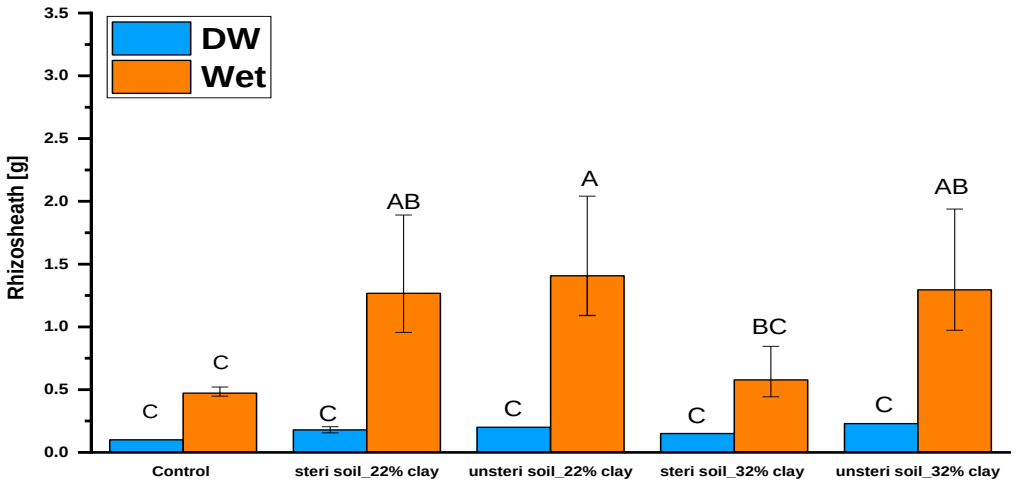


Fig. III- 2 Rhizosheath formation in sterilized and unsterilized soils with 22 and 32% clay under dry-wet (DW) cycles. Mean values ± SD, n=4. Different uppercase letters indicate significant differences between treatments and clay contents ($p < 0.05$)

4.2 Particle size distribution

The PSD was determined in aqueous suspension by laser diffraction analysis. The DW cycles tended to shift the median diameter (Dv50) to larger sizes (50.3 µm) compared to the wet treatments (37.9 µm), but the difference was not significant ($p = 0.05$) (Fig. III-2). The only significant difference was found between sterilized soil with 22% clay and the unsterilized soil with 32% clay. Conversely, a consistent PSD was observed for microaggregates with a median diameter (Dv50) in soils subjected to the wet treatment, regardless of the clay content or the

imposition of sterile conditions. Hence, and in line with our hypothesis, DW cycles increased aggregation but without increase of rhizosheath quantities (Fig. III-2).

The DW cycles and continuous wet conditions had minimal impact on the size distribution of the aggregated particles, which ranged between 0.1 to 35 µm. The average size of isolated soil particles peaked at 6.7 µm (Fig. III-4), constituting between 64% and 68% of soil microaggregates in the sterilized and unsterilized soil with 22% clay, respectively. In the finer textured soil with 32% clay, this peak was shifted to 5.8 µm, with a slightly narrower overall diameter range of 0.1- 26 µm. Evidently, higher clay content appeared to shift particle size distribution towards smaller sizes under DW cycles, with larger amounts of aggregates in the size range > 1 µm being visible in a small shoulder in the particle size distribution (Fig. III-4a). The pattern of PSD under wet conditions was similar to that under DW cycles; yet, with even smaller differences among treatments. The size range of particles was between 0.2-26 µm, peaking at 5.8 µm for all treated soils, with highest percentage 68% for unsteri soil_clay 32% and 64% distribution was noticed for both steri and unsteri soil_clay 22% contents. Noteworthy, in the sterilized treatments, we also noticed a re-aggregation to larger particles ranging from 16-26 µm (Fig. III-4b).

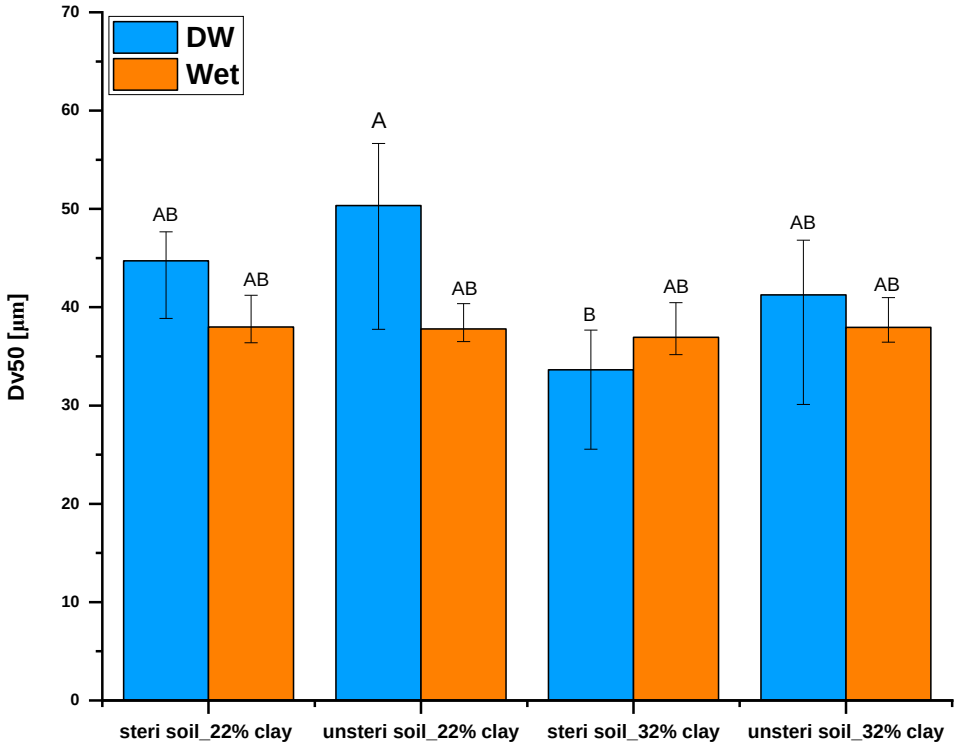
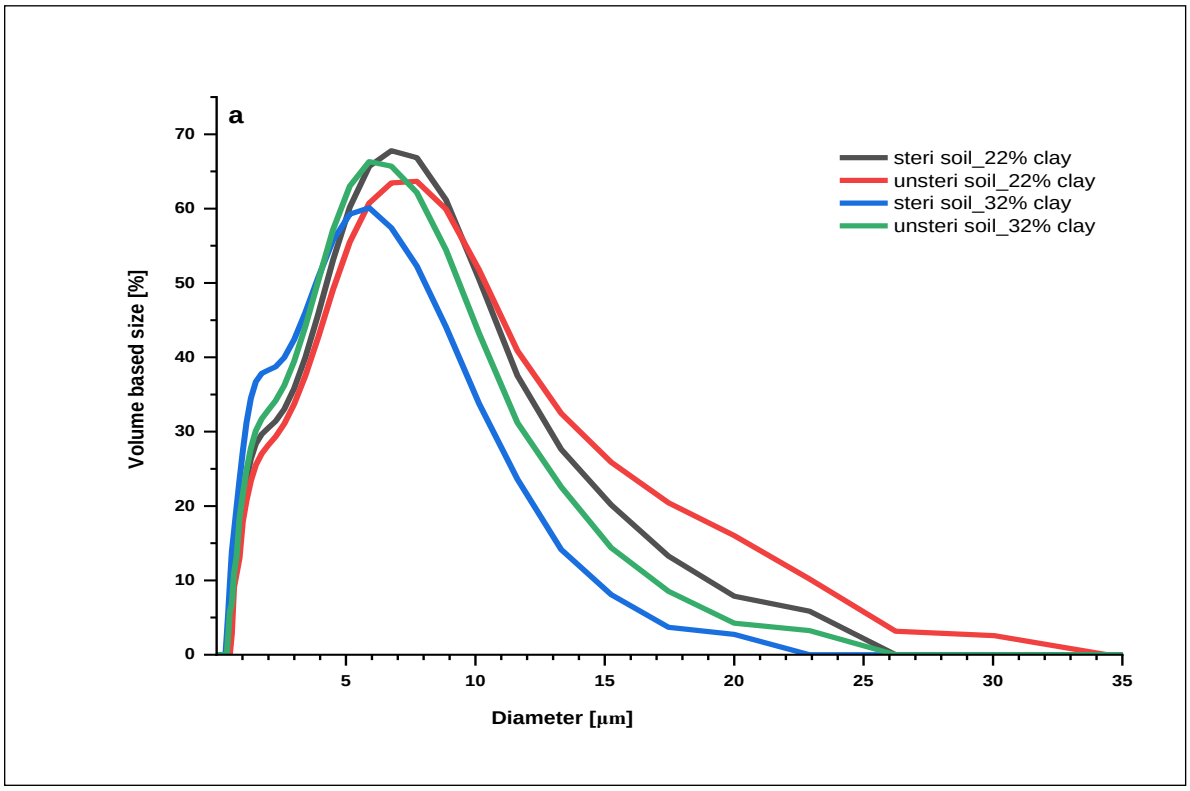


Fig. III- 3 Average median diameter (Dv50) of aggregated particles in the soils exposed to dry-wet (DW) cycles or constant wet conditions. Mean values ± SD, n=4. Different capital letters indicate significant differences between treatments and clay content ($p < 0.05$).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unsteri soil_32% clay
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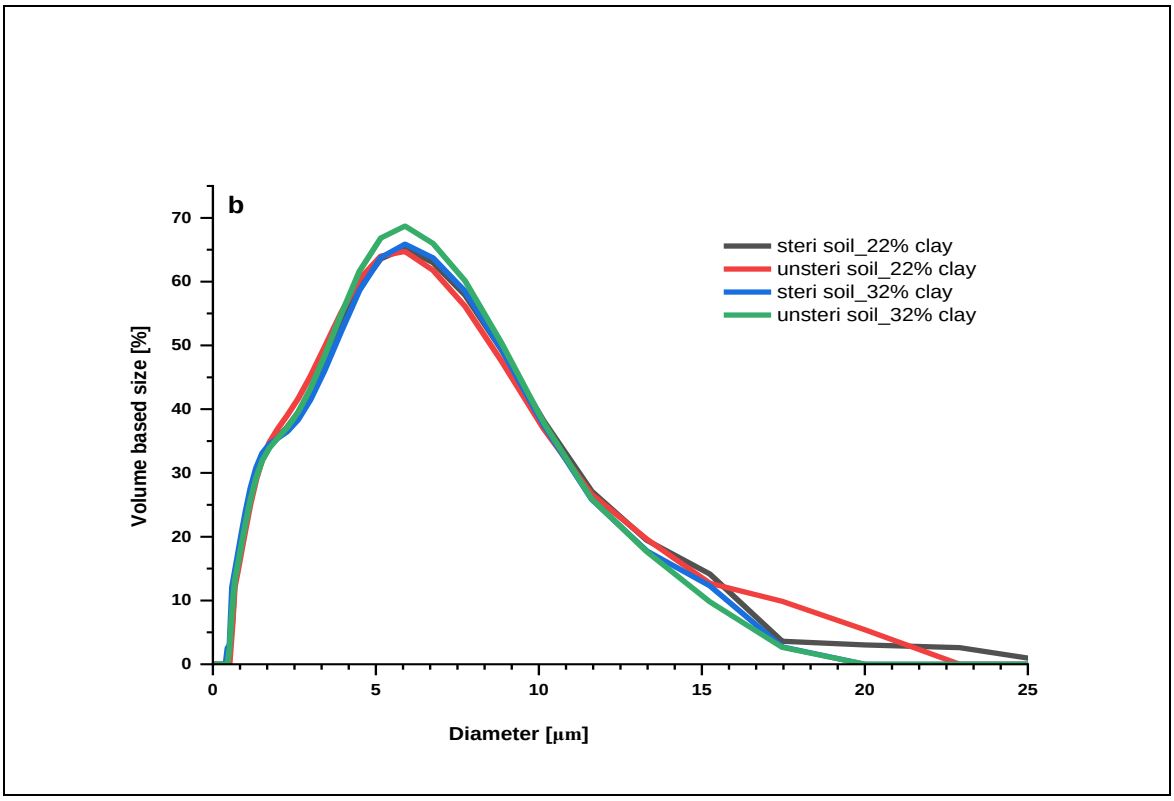
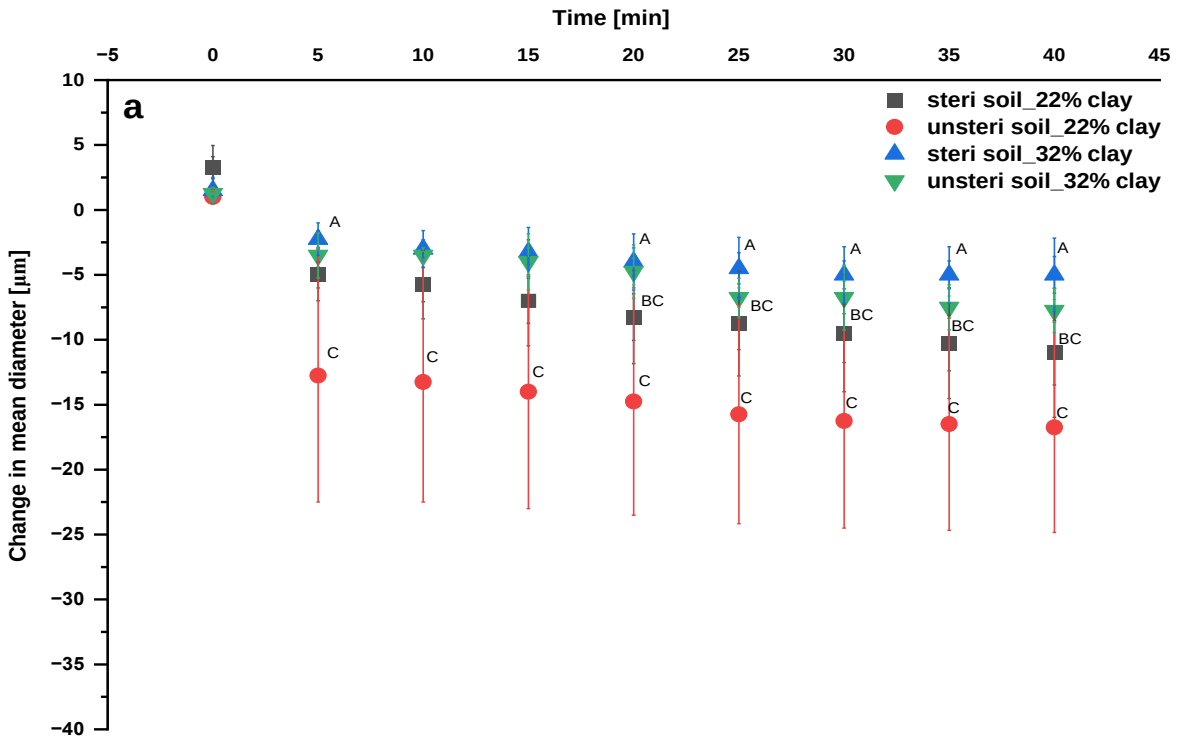


Fig. III- 4 Particle size distribution of sterile and unsterile soils under a) dry-wet (DW) cycles and b) when exposed to constant wet conditions at 75% WHC.

4.3 Soil stability

Under DW cycles the median diameter of the aggregates stronger decreased for samples having 22% clay compared to 32%. Also, this decrease was stronger for unsterilized samples (Fig. III-5a). Under constant wet conditions, the median diameter decreased with time for all soils in a similar manner (Fig. III-5b), with non-significant differences also between the soils with different clay content. Total losses in median diameter averaged -5 µm but did not reach the -15µm as in the soils with DW cycles. In summary, constant wet conditions promoted, thus, the formation of rhizosheath, but it did not promote aggregation, neither in size nor stability. It was rather the other way round that the better aggregated bulk soil in the DW cycles co-incided with less rhizosheath,

as if the formation of aggregates in the surrounding bulk soil interfered with rhizosheath formation by mucilage near the root tips.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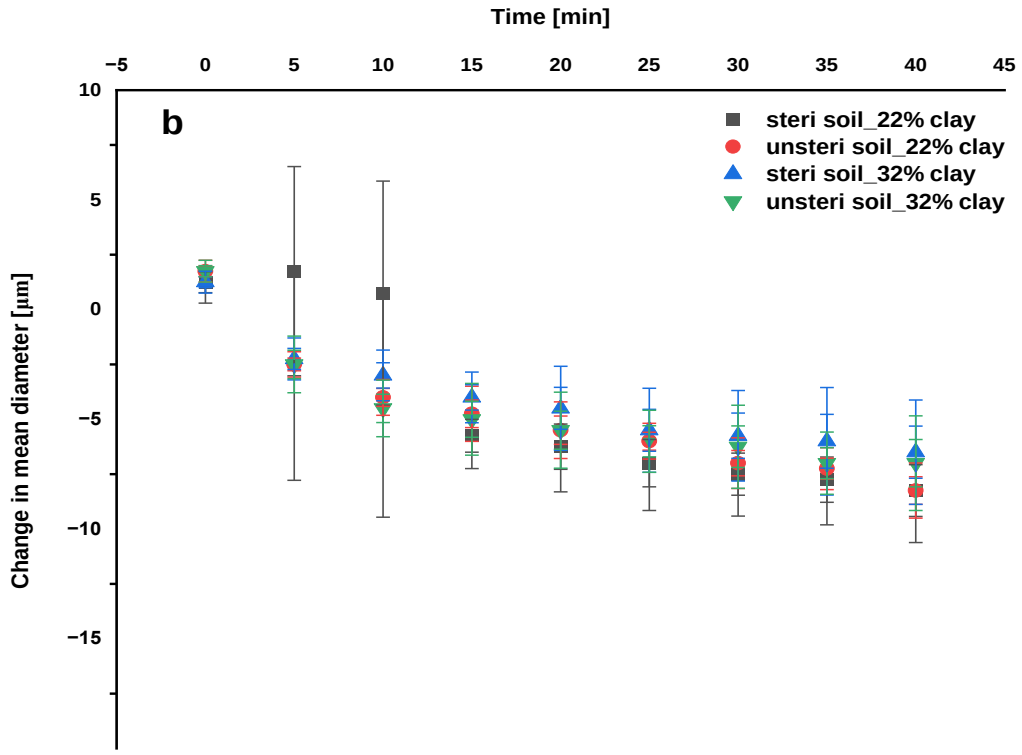

Fig. III- 5 Stability of soil particles as indicated by changes in median diameter with time under **a)** dry-wet (DW) cycles and **b)** constant wet conditions. The capital letters indicate significant differences between treatments but within time point ($p < 0.05$).

5 DISCUSSION

Rhizosheath formation occurs when soil particles, OM, and root exudates accumulate around plant roots, forming a protective sheath. This phenomenon plays a central role in plant-root interactions, nutrient uptake, and soil stabilization. In this study, the development of rhizosheath was significantly enhanced under wetter conditions as compared to DW cycles. In natural environment, several reasons can account for this: firstly, ample moisture promotes enhanced root growth, leading to more root surface area and increased release of exudates (Abdul-Jabbar et al., 1982; Mackay & Barber, 1985). These exudates act as a binding agent, causing soil particles to adhere

to the roots and facilitating rhizosheath formation (Aslam et al., 2022). However, in this study, this reason does not apply, as we exclusively used model roots for our experiments.

The structure of the rhizosheath also creates an ecological niche with favorable microclimatic conditions, which in turn promote the growth and development of microbial populations, including N-fixing bacteria (Bergmann et al., 2009; Othman et al., 2003), as well as the production of related microbial gluing agents (Davinic et al., 2012; Six et al., 2004). Furthermore, excessive moisture reduces the cohesion of soil particles, making them more likely to detach and adhere to the roots (Bates & Lynch, 2001), thereby further enhancing rhizosheath formation. Additionally, constant moisture conditions may lead to an enhanced diffusion of mucilage into the surrounding soil, thus increasing the soil volume participating in mucilage-induced rhizosheath formation (Rahim et al., 2023).

The constant moisture minimizes soil drying and subsequent cracking, ensuring the continuous accumulation of the rhizosheath around the roots. In contrast, alternate DW cycles of drying and wetting, characterized by intermittent moisture fluctuations, negatively affect rhizosheath formation because soil cracking and shrinkage occur during drying, disrupting the continuity of the rhizosheath, and resulting in reduced accumulation around the roots. Furthermore, the movement of soil particles during drying and subsequent rewetting events can wash away or displace the accumulated rhizosheath. Finally, mucilage might coagulate during the drying event, thus not diffusing into the surrounding soil and reducing the amount of mucilage-attached particles for rhizosheath formation (Rahim et al., 2023). Noteworthy, similar effects can also be expected in natural soil environment: during drying cycles, when soil moisture decreases, roots may experience water stress and partial desiccation. This will additionally hinder root growth and reduce exudate release (Hinsinger et al., 2009), thus contributing to the reduced formation of

rhizosheath. The fluctuating moisture conditions likely also impact soil microbial communities involved in rhizosheath formation. Yet, different processes interact, namely the microbial production of additional glues for rhizosheath formation, as well as the decomposition of mucilage and other gluing agents needed for aggregation and rhizosheath formation. The role of DW cycles is hard to predict in this context. It has been shown that DW cycles mighty restrict the availability of available C in the soil, thus limiting microbial decomposition processes (Fierer & Schimel, 2002; Mikha et al., 2005; Sommers et al., 1981); however, it is also well known that rewetting of dried soil usually leads to an increased CO_2 flush (Birch effect), due to enhanced microbial activity (Birch, 1958; Franzluebbers et al., 1994; S. Zhang et al., 2020). Yet, we can decipher the underlying processes as we studied the rhizosheath development in soils in both sterilized and unsterilized soil. In line with Zhang et al., (2020), who reported higher rhizosheath formation and root biomass in rice plants in unsterilized soils, we also found that rhizosheath formation was enhanced when sterilization was lacking. Apparently, the production of microbial gluing agents outcompeted their potential role on the decomposition of mucilage. As similar effects were not recorded for the soils that underwent DW cycles, it seems at least reasonable to assume that microbial effects were less prominent in these samples than in those with constant moisture supply.

We also found significant higher rhizosheath formation in unsterilized soil that had lower clay contents (22%). Rhizosheath formation constitutes a complex mechanism influenced by a multitude of biotic and abiotic factors. One pivotal factor contributing significantly to this process is soil texture. The SEM images revealed smaller aggregates, potentially enveloped by organic matter, in rhizosheath under wet conditions, particularly in unsterilized soil (22% clay content), characterized by a higher rhizosheath mass (see Fig III-1c). Conversely, in soils with a higher clay content (sterilized, 32%), we observe a diminished rhizosheath formation, corroborating our

findings from SEM (see Fig III-1d). In the context of more clayey soils with reduced rhizosheath development, a single prominent aggregate predominates the image, underscoring the cohesive nature inherent to clayey soils and the substantial impact of abiotic factors on aggregate formation.

It is noteworthy that historical data have consistently indicated statistically significant instances of rhizosheath formation in sandy soils. Furthermore, in contrast to loamy and clayey soils, sandy soils, which have lower porosity and limited root-soil contact, typically exhibit the formation of larger rhizosheaths (Hallett et al., 2022). Similar results were reported by multiple studies where sandy soil depicted large and consolidated rhizosheaths (Bailey & Scholes, 1997; Leistner, 1967; Oppenheimer, 1960). These studies have indicated that roots grown in sandy soil tend to develop a greater number of epidermal hairs compared to roots of the same plant species grown in less sandy soil (Duell & Peacock, 1985). Here, however, only model roots were analyzed that did not develop additional root hairs. Nevertheless, our data confirm these findings that rhizosheath formation was facilitated at higher sand content. Two reasons might account for this: a) a better diffusion of mucilage into the surrounding soil when clay content was smaller, and b) less aggregation of the coarser surrounding soil facilitating attachment of particles to the (model) plant root.

To analyze the soil of the aggregate formed, PSD we used laser diffraction. The median diameter of soil microaggregates was < 10 µm, in line with Krause et al. (2018). Yet, also some aggregates > 10 µm were found, possibly reflecting the presence of organic C in > 6.7 µm size fraction and inorganic size fractions like clay minerals and Fe/Al (oxyhydr)oxides in the form of silicate-oxide interaction (Churchman, 2018). The aggregates formed had higher stability when the soil contained more clay (Fig III-5, 1d). Clay particles have a high surface area and possess electrostatic

charges that attract and bind soil particles together to aggregates, through cation bridging, and by interactions with organic gluing agents (Tisdall and Oades, 1982; Bronick and Lal, 2005; Totsche et al., 2018, Krause et al., 2019). Intriguingly, aggregate stability was also enhanced when the soils were sterilized (Fig III-4a). Less clayey soils typically have a coarser texture with larger sand and silt particles. These larger particles tend to form a variety of aggregates with more open structures (Tisdall & Oades, 1982). In contrast, clayey soils, with their fine-textured particles, are more prone to forming larger, compact aggregates due to their higher surface area and greater adhesion. This is further supported by the SEM images, where rhizosheath mass was higher in soils with lower clay content compared to soils with a higher clay content (see Fig III-1c and 1d). Indeed, the formation and stability of soil aggregates is influenced by an interplay between biotic factors (e.g., EPS and microorganisms) and abiotic factors (e.g., clay minerals, pedogenic oxides). As emphasized by Clarholm & Skyllberg (2013), the presence of EPS and microorganisms plays a pivotal role in the initial formation of aggregates owing to their adhesive properties. Nevertheless, when OM becomes depleted or in conditions of sterilization, aggregates may disintegrate due to the loss of the organic "glue" that binds them together (Siebers et al., 2023). This disintegration can lead to the emergence of smaller particles or aggregates that exhibit higher stability, primarily driven by the increased prevalence of abiotic components such as clay minerals and oxides. These abiotic factors, especially electrostatic interactions and cation-bridging, engender robust forces of attraction between soil particles, resulting in enhanced aggregate cohesion and overall stability (Brady et al., 2008; Rengasamy, 2006). The biotic and abiotic processes is crucial for maintaining soil structure and its capacity to provide vital ecosystem services. This phenomenon aligns with our findings that demonstrate a shift towards smaller particle sizes and concurrently increased stability under sterilization conditions (Fig III-5a).

6 CONCLUSIONS

In summary, our study sheds light on the complex dynamics involved in rhizosheath formation and highlights the critical influence of biotic and abiotic factors, particularly soil texture, moisture conditions, and sterilization of the soil. We found that rhizosheath development was hampered by DW cycles but enhanced at constant wet conditions, likely because it interfered less with mucilage diffusion, microbial production of gluing agents, and physical disruption of rhizosheath by desiccation cracks and particle interactions outside the influence area of the roots. Sterilization, surprisingly, promotes rhizosheath formation, possibly by favoring microbial adhesives over mucilage decomposition. Soil texture, particularly clay content, plays a significant role, with lower clay content soils favoring larger rhizosheaths and smaller aggregates. These findings highlight the importance of these factors in plant-root interactions and have broader implications for soil health and ecosystem services. These results enhance our understanding about the role of different plant mucilage traits in soil aggregation and rhizosheath formation against various physiological conditions and shed more light on mucilage behavior in rhizosheath formation under various agro climatic regimes.

CHAPTER IV

PORE-SCALE SIMULATION OF MUCILAGE DRAINAGE

Modified on the basis of manuscript

Esmaeelipoor Jahromi, O., Knott, M., Mysore Janakiram, R. K., **Rahim, R**., & Kroener, E. (2022). Pore-scale simulation of mucilage drainage. *Vadose Zone Journal*, *21*(5), e20218. https://doi.org/10.1002/vzj2.20218.

Contribution of RR to this chapter:
Riffat Rahim (I, 2.1, 4)

1 INTRODUCTION

Mucilage is one of the secretions of plant roots and has very different properties compared to conventional Newtonian fluids. For example, mucilage is able to hold a large quantity of water which can be up to 1000 times of its dry weight (McCully & Boyer, 1997), and is very hydrophilic in these conditions. Dry mucilage, however, remains hydrophobic for a while after being exposed to water. This can delay the rewetting of dry rhizosphere for hours or even days (Brax et al., 2017; Carminati et al., 2010; Naveed et al., 2019; Read et al., 1997). Furthermore, at low concentration, mixture of water and mucilage behaves like a Newtonian liquid, but as its water content is reduced and concentration is increased, its viscosity is increased (Read et al., 1997). Further reduction in water content leads to the formation of a spider web-like polymeric network which solidifies and forms a hydrogel at very low water contents (Brax et al., 2017). Eventually, once the mucilage is dried, it become a brittle solid. The mixture of water and mucilage is a single-phase fluid and it is not possible to distinguish between mucilage and water especially during the drying process with a transition from a Newtonian fluid to a brittle solid. Therefore, in the rest of the paper, whenever we talk about mucilage or liquid phase, we refer to the mixture of water and mucilage unless it is clarified that authors mean dry mucilage or pure water.

Mucilage has a strong impact on soil mechanics: at low concentrations, hydrated mucilage may reduce friction between soil particles and thus reduce root penetration resistance; on the other hand, once the water content decreases and concentration increases, it may act like glue and increase soil strength (Naveed et al., 2017; Oleghe et al., 2017). Due to this reason, mucilage can affect root growth and rhizosheath formation significantly. By altering hydrodynamic and mechanical properties, the presence of mucilage also affects many physical, chemical and biological processes that usually depend on water content. For example, mucilage increases the water holding capacity

of the rhizosphere or, by blocking the large pores within the soil, disconnects the gas phase and therefore affects the gas diffusion (Haupenthal et al., 2021). Moreover, mucilage can contain protons or hydroxyl ions, which the roots release to neutralize the pH of the soil. In addition, due to the presence of organic compounds, the concentration of nutrients in the rhizosphere is affected by mucilage (Fageria & Stone, 2007). These organic compounds provide a food source for microbial activity, which can either be beneficial or harmful to the plant (Benard et al., 2019; Pathan et al., 2020).

The presence of mucilage increases the water holding capacity of the rhizosphere. The water retention curve will be affected and for a specific matrix potential, a higher water content will be observed. As a result, the reduction of hydraulic conductivity will be limited. During soil drying, this phenomenon maintains hydraulic contact between roots and soil, and water will be available to the roots (Carminati, 2012; Schwartz et al., 2016). Many root water uptake models still consider rhizosphere as bulk soil (Doussan et al., 2006; Gardner, 2003; Roose & Fowler, 2004); however, more models have been recently introduced, which consider the differences induced by mucilage in the rhizosphere (Carminati, 2012; Kroener et al., 2014; Schwartz et al., 2016) and show the relevance of rhizosphere hydraulic properties especially at dry conditions and during irrigation.

Kroener et al., (2018) have proposed a qualitative pore-scale concept of how mucilage alters water retention and saturated hydraulic conductivity and demonstrated that the mechanism how mucilage alters liquid phase distribution strongly depends on pore sizes. However, a proper pore-scale model is still needed to study the impact of mucilage on the distribution and especially on the connectivity of liquid phase in the rhizosphere. To develop such a model, the complex physical properties of mucilage must be incorporated. It needs to be capable of describing the spider web like structure

of the polymers in the hydrogel and its transition towards a rather solid state of mucilage at higher concentrations. Therefore, we examine available models to simulate such materials.

The Lattice Spring Method, also known as LSM, is a method to model network and solids. In this method, an elastic solid is modeled using a set of interconnected springs. System nodes are considered at those points where these springs collide. Despite its simplicity, this model is well able to simulate the deformation as well as fracturing of solids (Arbabi & Sahimi, 1990; Chung et al., 2002). Micromechanics of materials is another field in which this method can be applied (Ostoja-Starzewski, 2002). Along with the above, the LSM is well coupled to discrete particle-based methods, such as the Lattice Boltzmann Method (LBM), and can be applied to systems where fluids collide with elastic solids (Afra et al., 2018; GA et al., 2005; Wu & Qi, 2017).

For visco-elastic materials, the Spring-Damper or Spring-Dashpot method is used where a damper is incorporated alongside a spring to imply both viscosity and elasticity (Roscoe, 1950; Roylance, 2001). Numerous studies have been carried out using this method, such as those in the food industry (Jena & Bhattacharya, 2003; Wang, 2003) or biology for instance, by measuring the visco-elastic properties of living cells (Bu et al., 2019) or simulating the viscoelastic flow of blood (Rojas, 2007; Wu & Qi, 2017). Also, in the polymer industry this method is frequently used (Alcoutlabia & Martinez-Vega, 2003; Haario et al., 2014; Men cík et al., 2011). Beside this wide range of applications, to our knowledge, these models have not yet been applied to describe polymer dynamics within a porous medium as it is the case in the rhizosphere. Here, the presence of the porous medium fundamentally alters mucilage drying dynamics: free mucilage shrinks to a tiny volume of a small extension. However, when present in a porous medium and bound to soil particle surfaces, mucilage during drying creates a spider-web like structure spanning across the pore space with a tension on the web that increases during drying.

In this study, we simulate the drying of mucilage under quasi-steady-state conditions and show that indeed the geometry of drying of mucilage structures between soil particles strongly depends on certain properties, such as strength of springs and the distance between the soil particles. For this purpose, we have developed a new model using the LS method, which will be discussed in more detail below. The emergence of specific spatial mucilage structures in the pore space are qualitatively validated via ESEM measurements of mucilage bridges between glass beads.

2 MATERIALS AND METHODS

2.1 Environmental scanning Electron Microscopy (ESEM)

Brown flax seeds (*L. usitatissimum*) were washed and swollen in distilled water (1:6, w/w) for six hours at room temperature. Mucilage was extracted under vacuum filtration through a 500 μm sieve and seed cells were removed by filtration through a 100 μm sieve. Maize seeds were washed with hydrogen peroxide (10%), rinsed with distilled water and spread on a stainless-steel mesh mounted in PE boxes partly filled up with water, in which two air diffusers cared for high humidity. Mucilage was collected from aerial young maize roots after 3 days under vacuum suction once a day over 3 days and sieved through a 100 μm sieve. Further details from the procedure and the set-up figure in Brax et al. (Brax et al., 2020). Both mucilage was freeze-dried and dissolved at 0.2 mg/mL for maize root and 0.5 mg/mL for FSM. Mucilage was pipetted on beforehand weighted glass beads (150 μm and 350 μm diameter) in a watch glass until saturation. Concentrations of mg dry mucilage per g glass beads were 0.065 for maize root and 3.02 for FSM. The moist samples were dried in the ESEM under vacuum and ESEM images were directly taken with the FEI Quanta 250 ESEM (FEI Company Hillsboro, United States) under low vacuum with chamber pressures

between 60 and 80 Pa. A large field detector was used with an acceleration voltage between 12.5 and 15 kV.

2.2 Model of mucilage network

Size exclusion chromatography revealed mucilage to be composed of several populations of polymers, whose molecular weight can reach up to 107 Da (Roulard et al., 2016). The mixture of mucilage and water behaves differently depending on its concentration (Fig. IV-1). At very low concentrations, the diluted mixture of mucilage and water can be considered as a classical Newtonian liquid (Mezger, 2019). If the water content decreases, this mixture will become more concentrated and turn into a shear thinning non-Newtonian liquid (Naveed et al., 2019). By further decrease in water content, the polymeric strands will link together and form a network which traps water due to capillary forces. The mixture will then turn into a hydrogel, which is expected to have viscoelastic properties (Brax et al., 2019; Read et al., 1999). Eventually, mucilage will form a wide variety of solid brittle structures such as thin filament, hollow cylinders or connected foil shape plates (Benard et al., 2018; Haupenthal et al., 2021).

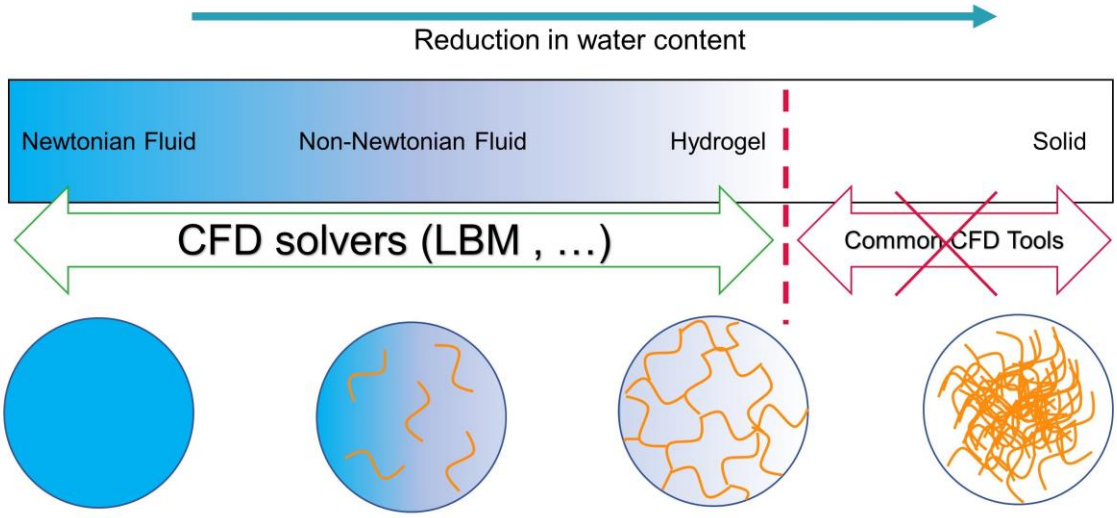

Fig. IV- 1 During drying, mucilage undergoes phase transitions from an almost Newtonian fluid at high water content to a hydrogel and finally to a solid. Conventional computational fluid dynamic (CFD) methods such as Lattice Boltzmann Method (LBM) cannot be applied on dry states of mucilage

It is worth mentioning that based on viscosity measurements (Benard et al., 2021; Brax et al., 2020), a smooth transition is expected for the change in mucilage states (Fig. IV-1). Besides that, because of heterogeneity of mucilage during drying, different states of mucilage exist simultaneously in a small volume. Therefore, defining threshold concentrations for the transition from Newtonian fluid to a brittle solid is very difficult. The following model in this study will be applied to rather dryer states of mucilage when it behaves like a rather solid gel with a spider-web like structure.

For modeling viscoelastic materials, the spring-damper model is the most well-known one. In this model, springs and dampers are manipulated in series, parallel, or in a combination of both models (Funk et al., 2009). The nodes will be located at the ends of these combined components. The spring provides elastic properties while the damper, which contains a viscous fluid, is used to incorporate the viscosity of the viscoelastic material.

Drying processes in the rhizosphere are usually induced by external changes of water potential and water fluxes, e.g. due to root water uptake, drainage or evaporation. These drying processes typically happen on the time scale of hours or days. When a certain water potential is applied locally and creates tension on the mucilage network at the µm-pore-scale, we assume that the network adjusts within minutes to the applied tension. Therefore, we assume that during rhizosphere drying processes, at the µm-pore-scale, the mucilage network can locally be considered in a quasi-steady state, defined by the locally applied tension on the network.

Elongation of linear springs are described via Hooke's equations where time is not involved and the force is proportional to the displacement and stiffness of the spring. In the Spring-Damper Model, time parameter is provided via the damper intervention that accounts for viscosity.

As long as mucilage drying happens slowly under quasi-steady-state conditions, the time parameter will not affect the distribution of mucilage at a given tension. Therefore, in this study we considered it unnecessary to account for the dampers and simplified the bonds between nodes as single springs. However, for more advanced models that also consider fluid flow coupled to polymer network dynamics especially for fast rhizosphere rewetting, it may be necessary to keep dampers in the model.

In summary, mucilage will here be characterized using a simple LSM, the process is considered quasi-steady-state and there is no dynamic fluid flow simulated. Now it is not relevant how long it will take for the nodes to displace and we only care about their final location as a result to an applied tension on the network. In more advanced studies and by coupling with a two-phase fluid flow model, this tension on the springs may be locally related to the water potential present at the specific spring. Here, however, we focus mainly on the spatial network arrangements at certain tensions and network and pore parameters.

In each quasi-steady state equilibrium, the network will adjust in such a way that for each network node, the forces of all n springs attached to this node sum up to zero ($\Sigma_{i=0}^{n} F_s^i = 0$). As a first approximation, the force, F_s^i(N), on a single spring is calculated according to Hooke's law:

$$F_s^i = -K^i \Delta x^i \qquad [1]$$

where Δx^i(m) represents the spring displacement $\Delta x^i = L_a^i - L_e^i$ with actual length of the spring L_a^i(m) and equilibrium length L_e^i(m), K^i(N/m) is the stiffness of spring which is inversely proportional to its equilibrium length $K^i = k \cdot \frac{1}{L_e^i}$

While we are applying the simplified linear model as a first approximation for the relation between displacement and restoring force, we are aware that materials typically have a specific stress strain relation, with a region of elastic deformation, reversible deformation, permanent deformation and a fracture point (Fig. IV-2). In the region between O and A, there is a linear relation between stress and strain and Hooke's law is obeyed. When the applied force is removed, the body returns to its original dimensions. This region is known as the elastic region.

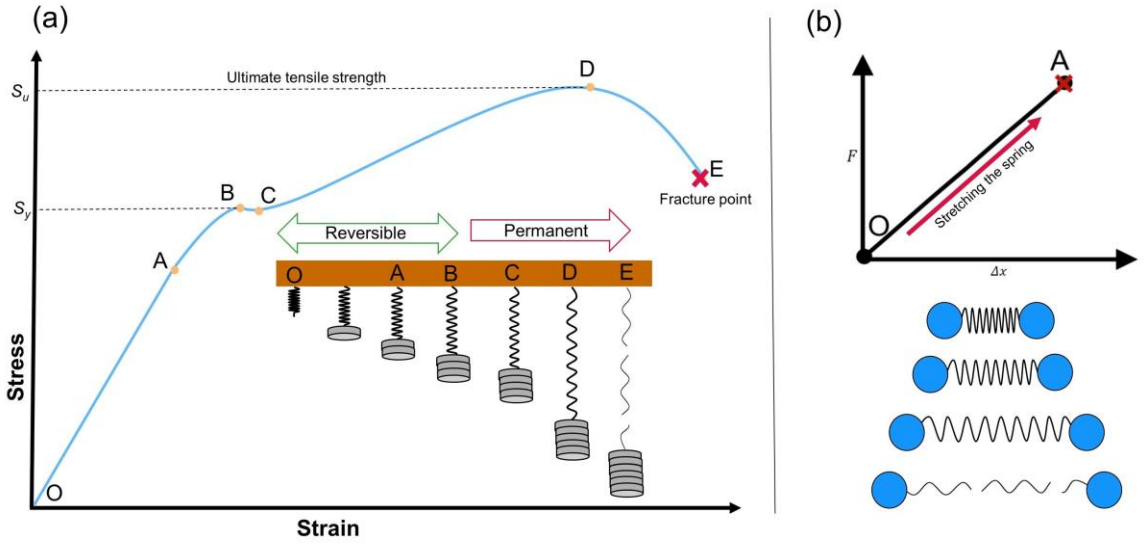

Fig. IV- 2 a) Typical stress-strain relation of a solid body. **b**) Evolution of a system of two node and single spring based on the simplified stress-strain relation.

The polymer strands of mucilage are responsible for the stretching described in Fig. IV-2. Usually such a material specific stress strain relation can be determined experimentally, but in the case of the mucilage polymeric network, such data is not available for individual polymeric strings and is very challenging to measure at the nano-micro scale. One approach could be via a Micro-Wilhelmy method using constant-diameter Nano-needle-tipped atomic force microscope probes as has been presented by Yazdanpanah et al., (2008) applied for withdrawing tens of microns in diameter cylindrical wire from a liquid. However, it is expectable that in the case of mucilage such a curve will strongly depend on various parameters, such as mucilage type, age, concentration and soil solution that may vary from species to species and change over time. Therefore, in this study a simple stress-strain relationship is used as a first approximation: we will consider the elastic region and skip the rest of the curve: Hooke's law will be followed and point A will correspond to the

breaking point. Now, just two parameters are sufficient to define the stress strain relation: the slope which corresponds to the stiffness K of the spring and the breaking point A (Fig. IV-2b).

In our model, it is assumed that initially, when mucilage is wet, all of the polymer strings can be considered at equilibrium within in the liquid phase and their actual length L_a, is equal to the equilibrium length $L_e = L_a$. When water potential decreases and mucilage become dryer, a tension will act on the mucilage network which decreases the equilibrium length of each spring. To represent this tension in our model, we introduced a correction factor T that represents tension via reducing the apparent equilibrium length of each spring. Displacement of each spring can then be calculated as $\Delta x^i = L_a^i - \frac{L_e^i}{T}$ and the corresponding force on a spring at certain T value is:

$$F_s^i = -K^i \cdot (L_a^i - \frac{L_e^i}{T}) = -k \cdot \frac{L_a^i - \frac{L_e^i}{T}}{L_e^i} \qquad [2]$$

The initial value of T, when mucilage is quite saturated, is equal to unity. It will increase as the water content decreases. As a result, it is expectable that when T value increases, a force is induced in each spring that pulls nodes closer towards each other (Fig. IV-3). Here we have assumed an inverse relation between water content and T value. Finding experimentally a physical quantitative relation between them is very difficult.

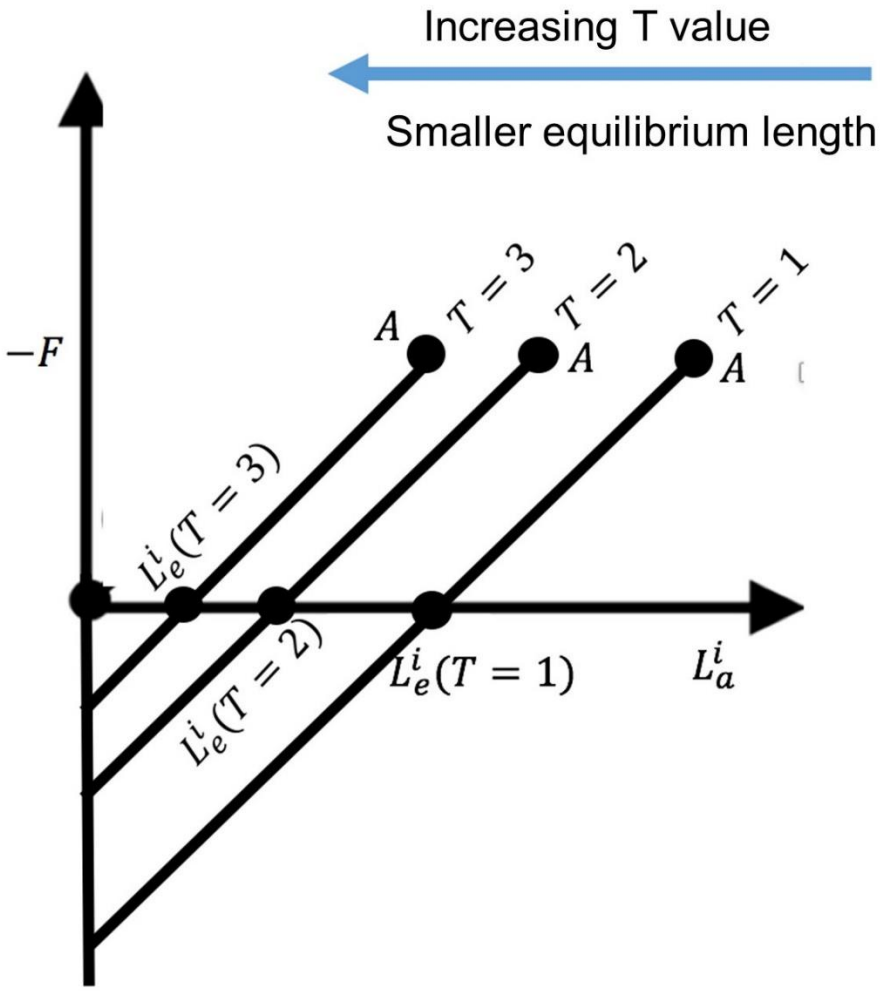

Fig. IV- 3 Effect of increasing T value on apparent equilibrium length of a single spring, its breaking point and relation between force and actual length.

The goal of the simulation is to calculate the network configurations during drying, i.e. equilibrium configurations at various T values. For each T value step, equilibrium is achieved when at each node the sum of the forces on all connected springs equals zero (in three dimensions: $\Sigma_{i=0}^{n} F_s^i = 0$). Note that in such an equilibrium configuration, individual springs can still be under strong tension, especially when the network is bound to soil surfaces at opposite sides of a pore.

The movement of the nodes in this study, is motivated based on physics via Stokes' law: Stokes' law predicts that a spherical object in a viscous fluid moves at a constant velocity v(m/s), i.e. constant displacement per time step, that is proportional to the force acting on it $F_D = 6\pi\mu Rv$ where μ(pa.s) is the dynamic viscosity, R(m) is the radius of the object (Laidler, 1982; Bird et al., 2002). Moving at a constant velocity means summation of applied force should be zero. Then F_s (Eq. 2) would be equal to the drag force $F_s = F_D = F$ and then we will have:

$$F = 6\pi\mu Rv = 6\pi\mu R\frac{dx}{dt} \qquad [3]$$

Then by rearranging the equation and choosing a very small time-step:

$$\overrightarrow{\Delta x} = C\vec{F}\ ;\ C = \frac{\Delta t}{6\pi\mu R} \qquad [4]$$

For each T value, the specific equilibrium configuration is calculated iteratively. In each iteration, at first, for each node, all forces of springs attached to the node are summed up $\vec{F}^{Tot} = \Sigma_{i=0}^{n} F_s^i = 0$. Secondly, each node will be displaced by distance $\overrightarrow{\Delta x} = C\vec{F}^{Tot}$ (Eq. IV-4) that is proportional to the forces acting on it. C is chosen sufficiently small, to ensure convergence and to avoid numerical oscillations and overshoots. The simulations here are conducted in a dimensionless space, and the physical units in Stoke's law (Eq. IV-3) are not considered because the simulations will be continued over a sufficiently large number of time-steps until the steady-state condition is reached. This way, the simulation will not be affected by time and other time-dependent parameters such as viscosity.

These iterations will be applied until steady-state condition for a given T value is achieved and then T in Eq. (IV-2) will be increased and the new configuration will be calculated. With respect

to the main directions, each node can have a maximum of six neighbors within the network. (Fig. IV-4).

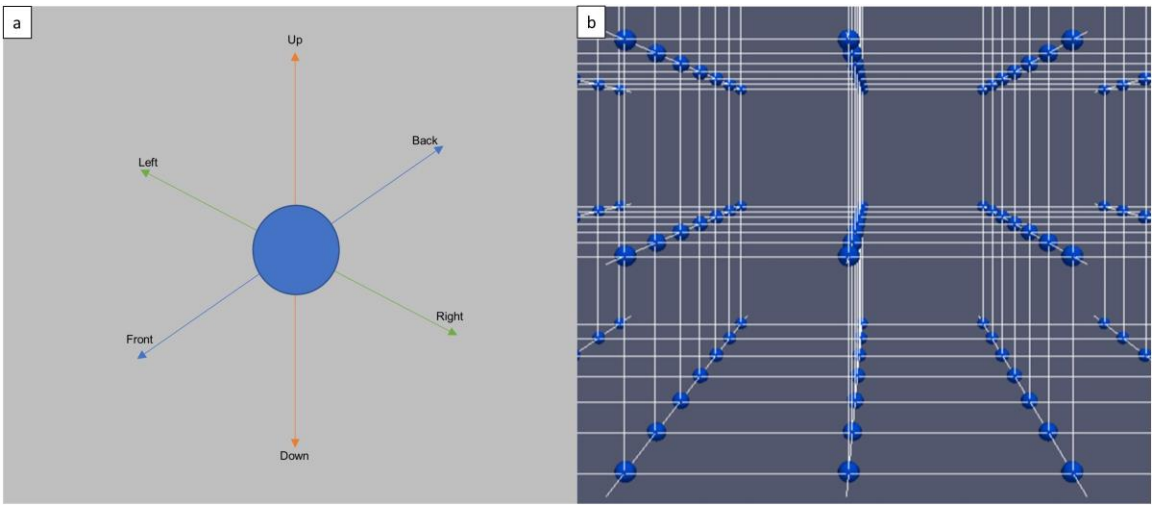

Fig. IV- 4 The maximum number of neighbors a single node can have and b) mucilage nodes within the network.

To avoid isolation of single nodes, we defined a minimum value of connections that each node should always have. Current model can have a minimum value of 4 or 5. Obviously, we do not expect the remaining springs to survive forever, then, regardless of their numbers, they will follow Hooke's law as before but a higher breaking point will be defined for them. We can explain this assumption, from a physical point of view. The water content of a broken spring is divided among remaining springs, increasing their flexibility, and so a higher Δx_{max} can be expected for the remaining springs. This new maximum value of Δx is called the ultimate value. In the current model it is chosen 5 times greater than Δx_{max}.

The initial distribution corresponds to a mucilage bridge in the pore space between two spherical solid particles (Fig. IV-5) and the evolution of spatial configuration of mucilage will be simulated several times based on a set of parameters (Tab. IV-1) with 12 different breaking points. Initially, all of the spring bonds are individually at equilibrium and distributed following a regular cubic

grid. The bridge has a cylindrical shape (Fig. IV-5). Although real soil pore spaces are normally between more than two particles, here we have chosen two soil particles as calculation domain to focus mainly on the evolution of polymeric network regardless of pore size and shape.

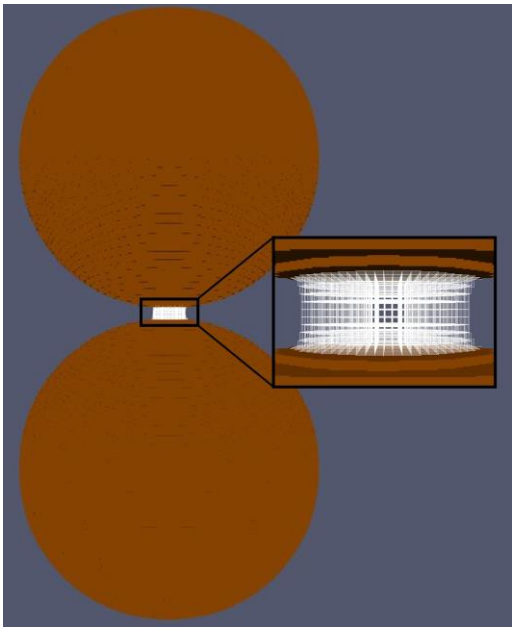

Fig. IV- 5 The pore geometry and initial distribution of the network.

Table IV- 1: Parameters used for 12 sets of simulations. Length is reported here in lattice unit, "lu", which is equal to the initial distance between two nodes.

12 different Breaking Points (Δx_{max})	**1.3, 1.5, 1.7, …, 3.5**
Minimum of neighbours	5
Ultimate Breaking Point	5*Δx_{max}
Distance between Soils(lu)	10
Soil Radius(lu)	100
T-value growth rate	0.01
C (Transforms force to displacement)	0.001
Force Soil-Node	1000*Force Node-Node
Soil Force range(lu)	1.2

3 RESULTS

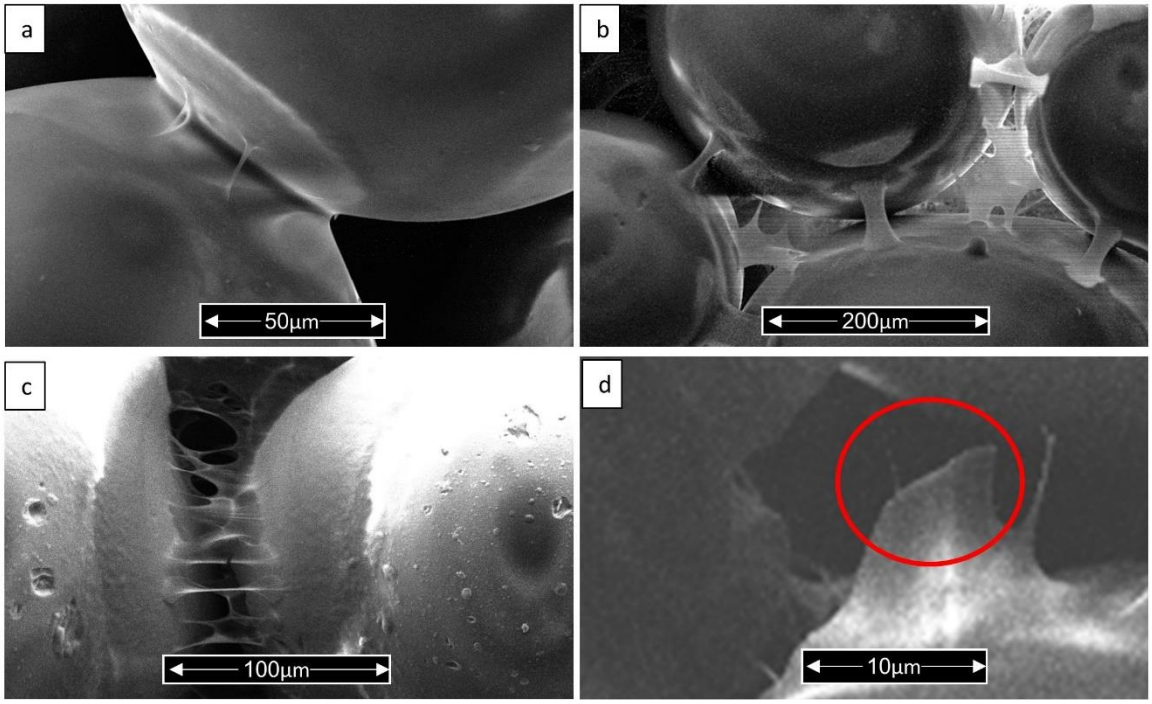

Fig. IV- 6 (a) Maize mucilage (0.065 mg/gGB)) and glass beads (150 microns, (**b-d**) Flax mucilage (3,025 mg/g GB) and glass beads (350 microns). Different types of connected structures are shown from (**a to c)**, while the contact between mucilage and top solid surface is broken in **d** (red circle).

ESEM images (Fig. IV-6) of mucilage drying between glass beads reveal the formation of specific structures, such as filaments (Fig. IV-6a), hollow cylinders (Fig. IV-6b), combinations of filaments and hollow layers (Fig. IV-6c) and broken structures (Fig. IV6d). Fig. IV-7 compares the results of the simulation with the ESEM images and indeed the simulations can generate these mucilage specific structures.

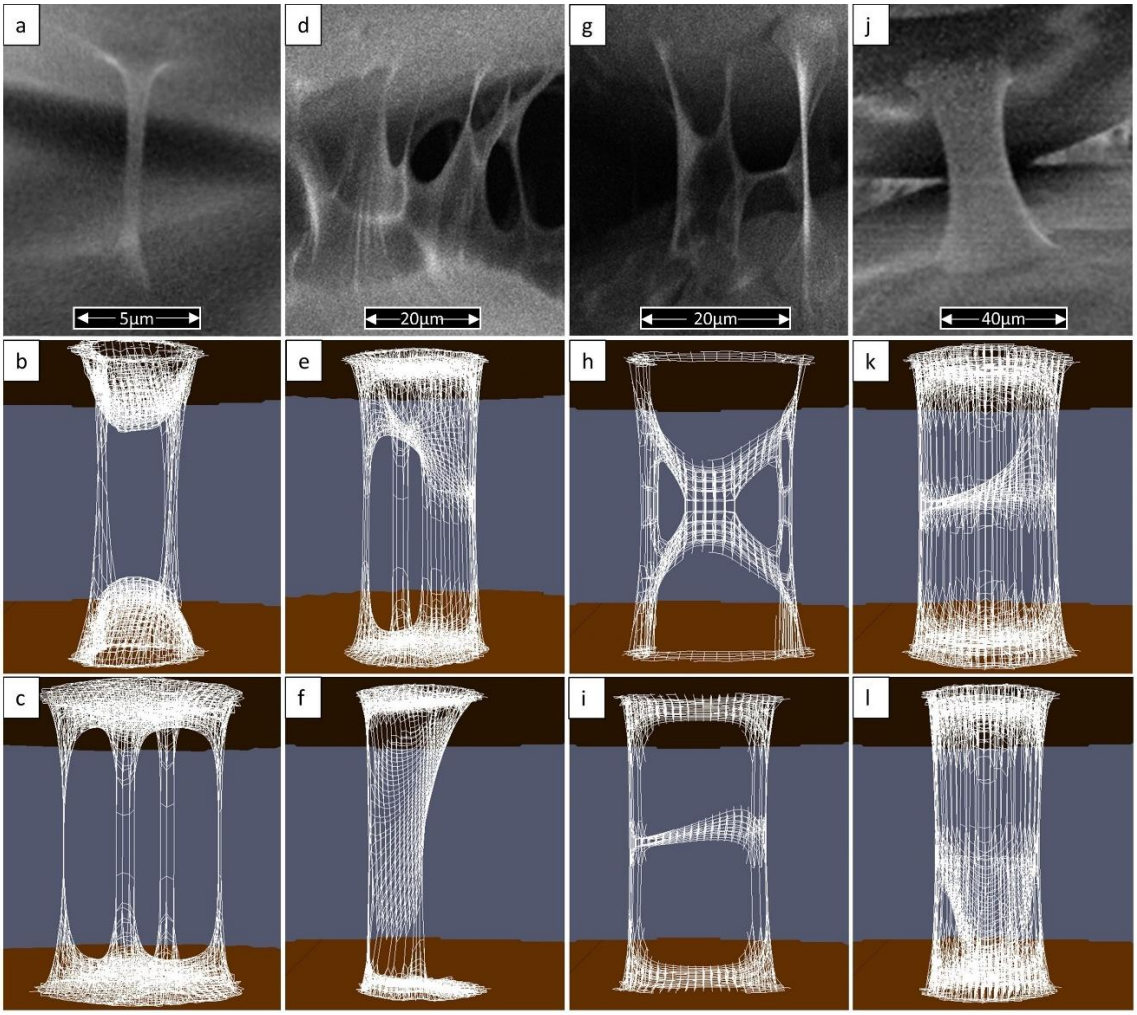

Fig. IV- 7 Qualitative validation of simulations by comparing them with ESEM images of each column. Columns show thin filament (a-c), combination of layer and filament (d-f), forming of H-shape structure (g-i) and hollow cylinders (j-l).

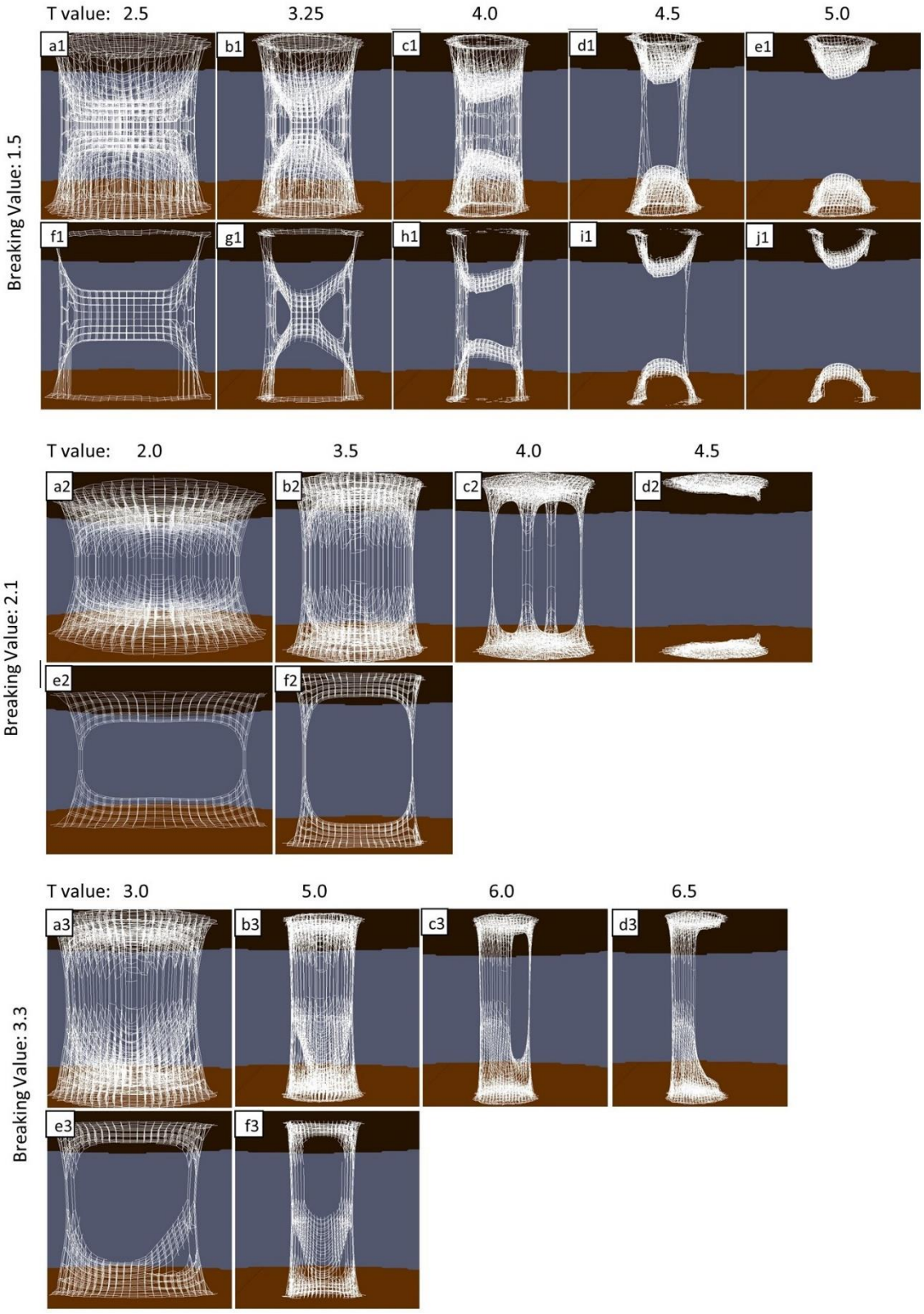

Fig. IV- 8 Dynamics of mucilage network based on the proposed model for three different breaking values of (a1 – j1) 1.5, (a2 – f2) 2.1 and (a3 – f3) 3.3 respectively. Top row shows the 3D structure and bottom row is a 2D slice of the inside.

Fig. IV-8 illustrates the network evolution for the breaking lengths of 1.5, 2.1, and 3.3, respectively. The top row shows the 3D network and the bottom row shows a 2D slice through the network. Smaller breaking value leads the network to break faster and break from regions near to the soil surface (Fig. IV-8 – a1). Higher breaking values will shift the breaking region to the center (Fig. IV-8 – a2). The reason is that, initially, the springs near soil surface will be under higher tensions compared to central springs but further shrinkage of the whole network and applying the equilibrium condition will cause the central springs experience higher tensions compared to those near soil surface and it will shift the breaking region. Increasing the breaking value to a higher number, will cause the springs to survive longer while the whole network is shrinking (Fig. IV-8 – a3). The structure of the network at the time it is breaking, will significantly affect the consequent structures and patterns it will have at the remaining drying process.

For a breaking value of 1.3, it will end up with two filaments (Fig. IV-8) and later with two hollow caps sticking to the soil surface while the next simulations of the breaking value 2.1 and 3.3 are drying with different patterns. It should be mentioned that although a single spring with the breaking value of 2.1 can sustain stronger forces than a spring of 1.5, the network of breaking value 1.5 is still connected at T value of 4.5 while the network of 2.1 is already broken.

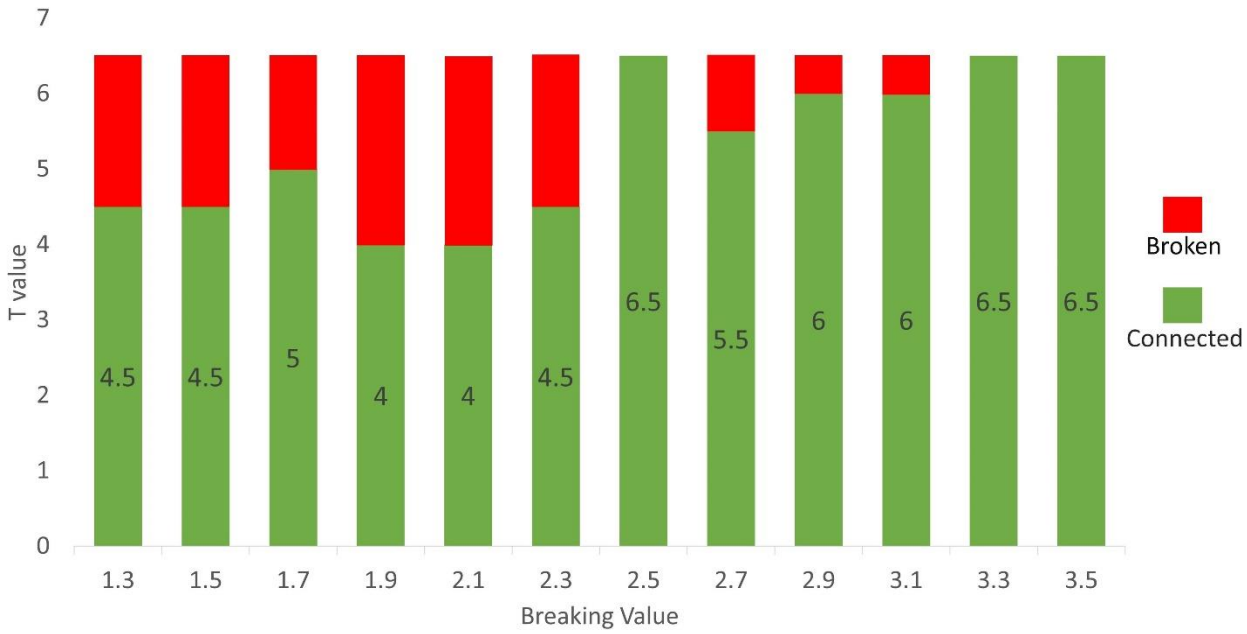

Fig. IV- 9 Connectivity of network for 12 sets of simulations in different T values (Eq. IV-2).

Fig. IV-9 compares the connectivity of networks under different T values for all 12 simulation sets. We can roughly say that networks of springs with a greater breaking length will remain connected at higher T values. It seems that the breaking value is not the only parameter affecting the connectivity of the network and more precise studies should evaluate the effect of other variables. For instance, in reality, mucilage strands can diffuse within the liquid phase and link to another strand and change the network structure. Incorporating this phenomenon to the current model may help to achieve a better insight.

Fig. IV-10 shows the outer diameter of the network at different T values. This diameter is measured at the center of the pore space as the maximum distance between two points of the network. The same as Fig. IV-10, it roughly shows that a network of springs with lower breaking value shrinks faster and it can be observed very well at lower T values. However, it is not an absolute trend and

in some cases a network of lower breaking value survives longer. As we mentioned earlier, we think the location of first breaking in the network which can be located in the center of the bridge or near soil surfaces (Fig. IV-8) is the main reason of this observation.

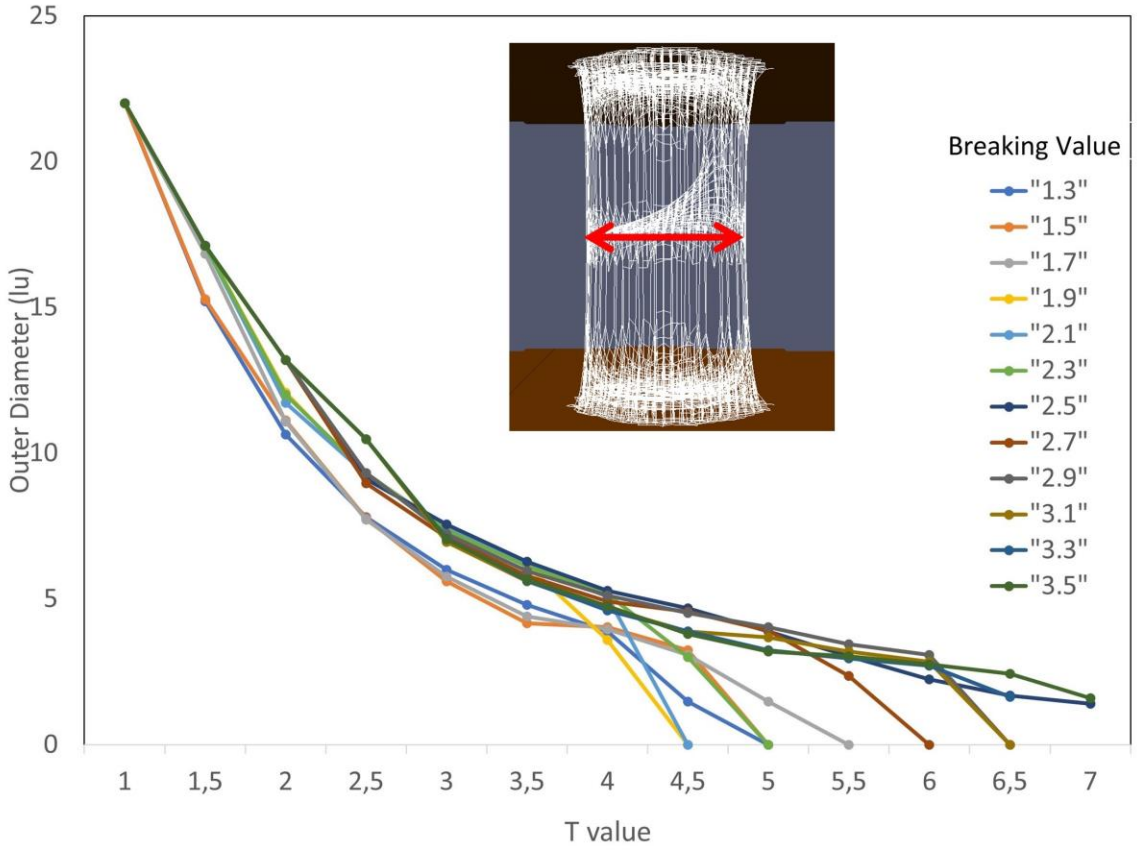

Fig. IV- 10 The outer diameter of the network under different T values.

4 DISCUSSION

In this study, a new pore-scale model is developed, to simulate the drying of mucilage within the pore space in the rhizosphere. During the drying process, the mixture of water and mucilage forms a single phase and it is not possible to separate them into two different phases. Therefore, whenever we refer to liquid phase or mucilage, the mixture of them is meant. By losing water during drying,

mucilage will deform to a brittle solid. The proposed Lattice Spring (LS) Method is well suited for modeling this gradual behavior since it exploits virtual springs corresponding to this evolution.

Previous studies revealed that mucilage forms various shapes between two soil particles during the drying process, such as thin filament, hollow structures or combination of them, which is consistent with the results of the proposed model. This way, the pore distribution of liquid phase which is a mixture of water and mucilage will be achieved and consequently, the exact connectivity of liquid phase in the pore space can be observed and studied.

It had been studied before that the presence of mucilage increases the water holding capacity of the rhizosphere and therefore affects the water retention curve. It also prevents the severe reduction in hydraulic conductivity during the drying of soil and provides water for the roots. This first application of the LSM for mucilage dynamics could well reproduce the distribution of the mucilage phase in the pore space and, for future research, it offers the possibility of better and more accurate models of the mentioned hydraulic rhizosphere processes.

Nutrients and micro-pollutants are transported to the plant roots through the same liquid connections. Therefore, with the help of the introduced model, the exact pathway of the solute transport can be studied. In addition, having information about liquid (mucilage) distribution also provides information about gas phase connections in the pores because we will see the presence of gas wherever there is no soil or liquid (mucilage). On the other hand, drying mucilage, in some pore, clogged the pore, thus affecting the gas phase connectivity and gas diffusion.

Previous studies have shown that plant root exudates affect soil stability. For example, the presence of mucilage eases soil compression, increases its mechanical flexibility, and improves the conditions for plant root growth (Naveed et al., 2018; Oleghe et al., 2017). The origin of this

observation is in the soil-soil particle interactions because mucilage directly affects and facilitates it. Root growth requires breaking the connections between soil particles in the root growth path and compacting the soil around the roots. The model presented in this study presents the connections between soil particles in the form of a set of springs. According to the properties of these springs, the force required to break or compress them can be calculated and by upscaling and calculating the forces on a larger domain, the plant root growth conditions can be predicted. In addition to the role of lubricant for root growth, mucilage also has a sticky nature that enmeshes soil particles around the plant roots and forms rhizosheath (L. K. Brown et al., 2012; Delhaize et al., 2015; I. P. Moreno-Espíndola et al., 2007; Watt et al., 1994). This phenomenon is also directly related to the soil-soil interaction and requires strong enough connections to overcome the weight of soil particles so that the particles stick to each other and to the plant roots and do not separate. As in the previous case, the calculation of the breaking force for the network of springs between the soil particles can help to predict the conditions for the formation of rhizosheath.

Despite already being capable of producing realistic spatial configurations, this mucilage network model bears the potential of further modifications and improvements. For instance, a relation between T value and water content and also advancing the lattice structure by adding diagonal bonds (Fig. IV-4) may be useful because in reality particles interact with each other from any direction and more bonds will consider this phenomenon better. Besides that, coupling the current model with a reliable CFD tool like Lattice Boltzmann, enables us to simulate a wide range of mucilage concentrations from early stages onwards where mucilage can be considered as a liquid.

At lower mucilage concentrations, physical properties like viscosity and surface tension become relevant. Coupling current model with two phase flow models, enables us to consider phase entrapment in small pores. Diffusion of polymers and reconfiguration of the network is another

relevant phenomenon at low concentrations of mucilage. It may be considered by incorporating polymer diffusion via spring reconfigurations in the model.

The ability of the polymers to unfold under stress, varies depending on the size of the polymers and the physico-chemical interactions between them and may lead to different stress-strain relations. The viscosity of mucilage varies depending on the amount and size of the polymers and the physico-chemical interactions between them (Brax et al., 2019, 2020). While flax seed mucilage is composed of 25% of acidic polymer (Qian et al., 2012) able to interact with Ca ions to build ionic crosslinks, which increase gel strength and viscosity, maize root mucilage contains only around 5 wt% galacturonic acid equivalents (Brax et al., 2020). The chemical environment can also affect strongly the ability of the polymers to elongate and displace the breaking point to different values. Brax et al., (2019) showed that in root and seed mucilage, the viscosity drops strongly due to an addition of calcium leading to a collapse of the polymeric network due to the coiling of polymers around the calcium ions. To tune the parameters used in the model, it may be beneficial – although very challenging - to conduct nano-micro-scale experiments on the mechanical properties of mucilage and to find out the values of physical parameters

CHAPTER V
FINAL DISCUSSION

1 SUMMARY OF THE RESEARCH OBJECTIVES

Root exudates, including mucilage, are vital for establishing soil adhesion and forming the protective rhizosheath around plant roots (Carminati et al., 2017; Read et al., 2003). However, specific influence of CSM concentrations on this process remains an unexplored area. The aim of this study is to fill the current knowledge gap by investigating the impact of different concentrations of CSM on the formation of rhizosheaths.

In this thesis, I for the first time conducted a systematic assessment, prioritizing the investigation of the specific impact of different levels of CSM and its impact on the development of rhizosheaths. I hypothesized that the most favorable conditions for rhizosheath formation occur when there is a moderate level of mucilage concentration and water content, yet it faces limitations in both high mucilage concentration under arid conditions and low mucilage concentration in wet conditions. Moreover, I also hypothesized that alternating cycles of drying and wetting influence the function of mucilage on rhizosheath development and related soil aggregation within the rhizosphere. Therefore, the primary objectives of this research encompass comprehending the intricate interplay between mucilage, water content, soil characteristics, and environmental dynamics concerning rhizosheath formation. This investigation entails assessing the impact of varying concentrations of CSM on rhizosheath development across diverse soil types, elucidating the role of mucilage quality derived from chia and flax seeds, and investigating the interactions influenced by drying and wetting cycles. Moreover, the study aims to decipher alterations in aggregate size and soil stability within the surrounding milieu and ultimately construct a predictive model that integrates these critical factors. Collectively, these goals seek to deepen our understanding of plant-soil interactions, potentially offering valuable insights for the advancement of sustainable agricultural practices and efficient soil management strategies.

To achieve the aims of this thesis, I adapted the method followed by Ahmed et al. (2014) for the extraction of CSM. In my study, I conducted a simple experiment involving the use of jute cords as an artificial model for root systems. Jute cords were positioned in both sandy loam soil and quartz sand. Furthermore, in a subsequent investigation, I introduced variations in their moisture levels by amending them with five different concentrations of mucilage (ranging from 0 to 0.2 2g dry mucilage g^{-1} water). Afterward, these cords were isolated from the mucilage obtained from chia and flax seeds following their swelling in distilled water for 15 minutes. In another study, I again used jute cord as artificial model for root systems and exposed them to sterilized and unsterilized soils with varying clay contents (22% and 32%). To mimic natural conditions, we moistened the model roots by adding 0.12 g dry CSM g^{-1} water and incubated them for 20 days at 25 ^{o}C and a moisture content that was either set to 75% of water holding capacity (used as a reference), or which was included DW cycles to 75% water holding capacity in regular intervals. Soils without added mucilage was used as a control. Subsequently, we isolated the rhizosheath by gently shaking (Brown et al., 2017a). For PSD and stability tests, I analyzed the remaining air-dried bulk soil samples using a laser diffraction particle analyzer. Particle-size distribution was also qualitatively analyzed using SEM. Dried-bulk soil and rhizosheath was prepared for SEM analysis by gently spreading them onto a double-sided C-tape and mounting onto a sample holder. The microstructural analysis was obtained using SEM (Hitachi SU-8000) at 2 keV.

The determination of soil stability I performed with the same particle analyzer as described by previously (Kowalenko & Babuin, 2013; Mason et al., 2011; Siebers et al., 2023). For this, while being agitated and circulated aggregates were exposed to a steady and continuous mechanical force, the stability of aggregates was determined by conducting repeated measurements of the PSD over a period of 40 min. I recorded data at 40-second intervals during the initial 10 minutes, and

thereafter, I extended the measurement intervals to every 3 minutes for the remaining 30 minutes. The particle sizes collected were used to calculate the shift in median volume-based diameter (Dv50).

Regarding the research inquiries outlined in chapter I-IV, I may now provide a summary of the ensuing results:

1. **Does mucilage concentration influence rhizosheath formation under different soils?**

I proposed that the best rhizosheath development occurs with an intermediate CSM concentration. To test this, I experimented with rhizosheath formation in quartz sand and sandy loam soils, using varying CSM concentrations: 0.0g (control), 0.02g, 0.04g, 0.12g, and 0.2g of dry mucilage per g^{-1} water. I applied wet mucilage (2g) evenly on jute cord root models to simulate mucilage release into the soil. Results showed peak rhizosheath formation at 3.63 g cm^{-1} in sandy loam soil with 0.12g dry mucilage g^{-1} water. Quartz sand also peaked at 4 g cm^{-1} with a moderate CSM concentration. Lower (0.02 g dry mucilage per gram of water) and higher (0.2 g dry mucilage g^{-1} water) concentrations resulted in lower rhizosheath formation, measuring 0.13 g cm^{-1} and 0.36 g cm^{-1}, respectively. In conclusion, mucilage concentration drives rhizosheath formation, with optimal levels at 0.12g per gram of rhizosphere water. The study's peak rhizosheath formation was observed at this concentration, emphasizing its crucial role.

2. **How is rhizosheath formation and stability influenced by mucilage from different plants?**

This research aimed to explore the impact of different mucilage types on the formation of rhizosheaths. The study specifically focused on comparing the effects of two mucilage types—CSM and FSM—at various concentrations (0.0g, 0.02g, 0.04g, 0.12g, and 0.2g dry mucilage g^{-1}

water) in a quartz sandy soil environment. To simulate plant roots, jute cord models were employed in this experiment. Both CSM and FSM were administered in dry conditions to observe their influence on rhizosheath formation. The findings consistently demonstrated a noteworthy increase in rhizosheath development in the presence of CSM across all concentrations tested. In contrast, FSM consistently led to negligible masses of rhizosheaths. This observation emphasizes the critical role of mucilage composition and viscosity in driving rhizosheath formation, showcasing the significant impact that different mucilage types can have on soil-root interactions and their implications for plant growth and soil structure. I concluded that this formation of rhizosheath depends largely on the composition and viscosity of mucilage.

3. How is rhizosheath formation affected by varying soil moisture volumetric contents?

This study investigates the combined effects of soil volumetric water content and CSM concentrations on rhizosheath formation in quartz sandy soil. Soil samples were packed into holders and subjected to varied moisture levels: control, 0.5 cm^3 cm^{-3}, 0.15 cm^3 cm^{-3}, 0.30 cm^3 cm^{-3}, and 0.35 cm^3 cm^{-3}. CSM concentrations of 0.0g, 0.02g, 0.4g, and 0.12g dry mucilage g^{-1} water were applied to artificial roots placed on the soil surface. Results revealed that high water content reduced the impact of mucilage on rhizosheath formation. At 0.35 cm^3 cm^{-3}, mucilage concentration had minimal effect. Conversely, low water content made rhizosheath formation less reliant on mucilage presence. Under drought conditions, intermediate mucilage concentrations significantly extended rhizosheaths. This suggests that mucilage enhances root surface area, aiding nutrient uptake and fortifying plant resilience against abiotic stresses such as drought and salinity.

4. **Do alternative drying and wetting cycles have implications on rhizosheath formation?**

To understand how alternative drying and wetting cycles effects aggregation in rhizosheath soil, I measured rhizosheath in soils with various clay contents under the presence of CSM. The current hypothesis suggests that the presence of alternating dry and wet cycles in the rhizosphere may impact the development of the rhizosheath, thereby altering the significance of mucilage as a crucial bonding partner. I analyzed the rhizosheath formation under five DW cycles and assessed the impact on aggregate formation and stability in rhizosheath soil. The procedure involved drying the soil to 15% WHC for four days, followed by rewetting and monitoring of rhizosheath mass. I also use SEM to see rhizosheath under DW cycles at the end. I found Mucilage doubled rhizosheath amount compared to control, forming larger aggregates. DW cycles had minimal impact on rhizosheath formation, while sterilization reduced it. Wet conditions resulted in significant rhizosheath development, with maximum observed in unsterilized soil with 22% clay.

5. **How does PSD and stability of soil aggregates in rhizosphere and rhizosheath behave under dry and wet conditions in the presence of mucilage?**

The study aimed to assess the impact of soil sterilization and DW cycles on soil aggregates' size distribution using jute cord as model roots and CSM as model root-mucilage. Due to limited rhizosheath mass, PSD and stability analysis weren't feasible. SEM was used to examine individual rhizosheath aggregates, while standard techniques evaluated the remaining rhizosphere soil's PSD and stability, unaffected by model roots. Laser diffraction analysis on air-dried bulk soil samples assessed volume-based size distribution, showing a slight shift in median diameter (Dv50) from DW cycles (50.3 μm) to wet treatments (37.9 μm), albeit not statistically significant ($p = 0.05$). The notable distinction was between sterilized (22% clay) and unsterilized soil (32% clay). Wet treatments showed consistent microaggregates PSD, irrespective of clay content or sterilization.

Under DW cycles, particle sizes ranged from very small to 35 µm, with 22% clay soils exhibiting particles around 6.7 µm (64%-68%) and 32% clay soils slightly smaller at 5.8 µm (0.1 to 26 µm). More clay led to smaller particles, especially under DW cycles, with increased aggregates larger than 1 µm. Wet conditions displayed similar effects but with smaller differences, ranging from 0.2 to 26 µm, peaking at 5.8 µm (68% for unsterilized 32% clay, and 64% for both sterilized and unsterilized 22% clay). Sterilized soils showed some re-aggregation into larger particles (16 to 26 µm). Soil stability analysis revealed more significant median diameter reduction under DW cycles in 22% clay samples compared to 32%, especially in unsterilized soil. Constant wetness caused a smaller average decrease in median diameter (-5 µm) compared to DW cycles (-15 µm). Wet conditions promoted rhizosheath formation without significantly impacting aggregation, suggesting a possible interference with rhizosheath formation near root tips in bulk soil aggregate formation.

6. How can the contribution of mucilage to rhizosheath formation be modelled?

In this study, I employed a simplified diffusion equation to offer a qualitative description of how the presence of mucilage influences arid soil conditions. I applied a fundamental model that considers the displacement of polymers within the gel-like substance at different levels, covering minimal, intermediate, and substantial diffusion coefficients in the fluid stage. The association among the concentration of the gel-like substance, mobility of the polymers, and the diffusion coefficient follows a nonlinear pattern. Soil tortuosity was utilized to deduce the soil diffusion coefficient using the liquid diffusion coefficient as a reference. My findings indicate that at high mucilage concentrations (0.2g of dry mucilage per gram of water), the spread of mucilage is limited to a spitting distance, resulting in a high concentration near the initial mucilage application point. In contrast, at intermediate concentrations (0.12g of dry mucilage per gram of water),

mucilage spreads a few millimeters, occupying a substantial volume of soil. At low concentrations (0.02-0.04g of dry mucilage per gram of water), the polymers likely diffuse over greater distances, preventing the formation of a polymeric network capable of binding soil particles into a rhizosheath. In summary, the conceptual model effectively reproduces the results of my laboratory experiments.

2 SYNTHESIS AND OUT LOOK

2.1 Rhizosheath development

Mucilage adheres to soil, facilitating the creation of rhizosheaths, pertaining to soil that remains adhered to the roots despite mild agitation (Brown et al., 2017). I postulated that the optimal conditions for rhizosheath formation occur at an intermediate mucilage concentration. However, both high mucilage concentration and dry conditions were found to limit its formation. To investigate this, I employed an artificial root soil system that allowed for independent variation of mucilage concentrations and their respective impact on rhizosheath formation. In this thesis **(Chap II)**, I observed that the formation of rhizosheaths in dry soil reached its peak at an intermediate level of mucilage concentration (0.12 g dry mucilage g^{-1} water). This phenomenon aligns with the conceptual model, which utilizes a radial diffusion equation **(**Fig. II-1**)**. This phenomenon can be elucidated through the concept of mucilage diffusion, which is crucial in the expansion of the rhizosheath around plant roots in dry conditions. The initial hypothesis was that at lower concentrations of applied mucilage, rhizosheath formation would be minimal and feeble due to the rapid diffusion of mucilage polymers. This rapid diffusion results in a widespread distribution of mucilage in the soil, leading to lower local concentrations. Conversely, at elevated mucilage levels (0.2 grams of dry mucilage per gram of water), the mobility of polymers diminishes, restricting

the dispersion of mucilage in the soil and subsequently reducing the area of soil cohesion essential for rhizosheath expansion. For instance, Ahmed et al. (2016) demonstrated that chia mucilage, like other mucilage, can exhibit viscosities up to 1000 times greater than that of pure water at elevated concentrations. **The empirical results validate that the concentration of mucilage significantly influences the movement of mucilage polymers within the soil solution.** While fresh mucilage initially displays limited water retention capacity (Guinel and McCully, 1986), exposure to desiccation significantly enhances its ability to retain water, as evidenced in ESEM studies (Albalasmeh & Ghezzehei, 2014). This response is attributed to the polysaccharides' ability to contract and adapt their morphology under drying conditions (Sutherland, 2001). Hence, the model posits at minimal mucilage concentrations (0.02g of dry mucilage per gram of water), there aren't enough molecules to create polymer-like networks that bind soil particles. Conversely, **when mucilage is highly concentrated, it becomes too adhesive to penetrate deep into the soil.** This suggests that plants with similar mucilage concentrations in their root exudates might exhibit more substantial rhizosheath development. **The findings are transferable to real soil-plant systems in which mucilage plays a role in root-soil interactions.** Researchers and agricultural practitioners can consider optimizing mucilage concentration to enhance nutrient uptake and plant resilience.

2.2 Viscosity influence on rhizosheath development

The viscosity of mucilage varies based on factors such as polymer quantity, size, and chemical interactions. FSM, with 25% acidic polymer, interacts with calcium ions, forming ionic crosslinks that boost gel strength and viscosity. Conversely, maize root mucilage, containing about 5% galacturonic acid equivalents, resulted in negligible rhizosheath formation compared to the substantial and stable formation observed with CSM in quartz soil **(**Fig. II-3**)**. **This occurrence was explained by the difference in thickness or consistency between the mucilage derived**

from chia seeds and that from flax seeds. This observation aligns with the findings of the research of Brax et al. (2020) and Mazza & Biliaderis (1989), both of whom conducted viscosity comparisons between CSM and FSM. The results from these studies consistently validate the higher viscosity of CSM in comparison to FSM across all examined concentrations. Further corroboration for this trend can be found in the study by Bemiller et al. (1993), which also reported elevated viscosity levels for CSM when contrasted with FSM. Likewise, Naveed et al. (2019) and Brütsch et al. (2019) recorded elevated viscosity measurements for CSM when compared to barley. Likewise, Naveed et al. (2019) and Brütsch et al. (2019) documented higher viscosity values for CSM in comparison to barley. **In real soil-plant systems, understanding the specific properties of mucilage derived from different plant species can inform plant selection and breeding strategies**. For example, choosing plant varieties with higher-quality mucilage could enhance rhizosheath formation and its associated benefits.

Notably, my observations regarding rhizosheath development were consistent across two different soil types: sandy loam and quartz sand. Intriguingly, **we noticed that at low mucilage concentrations, rhizosheath formation in sandy loam exceeded that in quartz sand**. This deviation might be ascribed to the existence of a higher amount of fine particles in sandy loam compared to quartz sand, which may influence the interactions between mucilage and soil particles, thereby affecting rhizosheath development.

2.3 Moisture regime

Numerous research investigations have explored the effects of varying moisture conditions and the adsorption of mucilage on the formation of rhizosheaths (Czarnes et al., 2000; Reid and Goss, 1981, 1982; Watt et al., 1993, 1994), there is a significant knowledge gap regarding the influence of CSM concentration on rhizosheath formation in quartz sandy soil. In the context of this thesis,

I observed that the augmentation of soil moisture promoted rhizosheath formation, encompassing both low and high mucilage concentration ranges, albeit up to an intermediate volumetric water content of 0.15 cm^3 cm^{-3}. **Under conditions of high soil water content (expressed in cm^3 cm^{-3}), the formation of rhizosheaths was once again diminished, primarily due to excessive soil moisture hindering substantial contact between soil particles and the roots, thereby impeding rhizosheath development**. These findings align with a prior investigation by Liu et al. (2019), which explored the formation of rhizosheaths under different moisture conditions and identified that the most significant rhizosheath development occurred within the moisture range of 10-14% (by weight), surpassing the performance of other soil moisture levels. Likewise, one can deduce that under elevated moisture levels, the immediate impact of mucilage diminishes. This is partly because mucilage has a propensity to dissolve in wet conditions, dispersing along with water into the adjacent soil, as reported by Watt et al. (1994a). Consequently, mucilage fails to accumulate in sufficient quantities near the roots to facilitate the formation of a polymer network essential for rhizosheath development.

Mucilage, distinguished by features like water absorption, elevated viscosity, and diminished surface tension, modifies the spatial arrangement of the liquid component in drying soil, enhancing its connectivity. (Carminati et al., 2017; Benard et al., 2018). In arid conditions, there was a notable connection between mucilage concentration and the development of rhizosheaths. **The most substantial rhizosheath formation was observed at moderate concentrations, which were approximately tenfold greater than those at other concentration levels.** These findings are consistent with the conclusions documented by Watt et al. (1993, 1994), where they observed that in drier conditions, rhizosheaths surrounding the roots were larger and exhibited increased cohesion in comparison to wetter conditions. Furthermore, in arid soils, certain grasses produced

rhizosheaths approximately three times larger than those in moist soils. In drier midsummer soils, rhizosheaths encompassing maize and other mesophytic grasses exhibited increased thickness and stronger attachment to the roots (Watt et al., 1994b). In contrast, they were less sturdy and flourishing in the more humid soils of the early spring from roots growing in the moister soils of early spring. This scrutiny corresponds to a scenario where a segment of the root length experienced a matric potential of <−1.5 MPa, while the entire plant was subjected to a fully saturated moisture regime. They exhibited decreased vigor and were more easily separated from roots thriving in the more saturated soils of early spring (Nambiar 1976). Conversely, at low mucilage concentrations, facilitated diffusion appeared to disperse the compounds to an extent where soil mucilage concentration may not significantly contribute to binding soil particles together. This resulted in the formation of a thin and transient rhizosheath layer, with rhizosheath development declining again as mucilage concentrations increased. While the study focused on quartz sandy soil, similar principles can be applied to various soil types. Agriculture practices often involve managing soil moisture, **and the findings emphasize the need to consider both mucilage and soil moisture in soil management strategies to optimize plant growth.**

2.4 Simulations of mucilage in pore space

At reduced mucilage concentrations, elements like viscosity and surface tension assume substantial importance. Viscosity characterizes a liquid's resistance to flow. In general, pure liquids and weak solutions of low-molecular-weight substances exhibit Newtonian behavior: they deform in proportion to the applied stress and do not revert when the stress is relieved. Newtonian fluids possess a viscosity that remains constant regardless of the applied shear rate or shear stress. Conversely, solutions with higher concentrations of high-molecular-weight substances like polysaccharides display non-Newtonian behavior and often demonstrate viscoelastic properties, as

demonstrated in the study by Read & Gregory (1997) on root mucilage. When applying stress to a viscoelastic material, a portion of the energy dissipates as heat during deformation, while the remaining energy is stored elastically. The viscosity of non-Newtonian fluids changes in response to the shear rate. Shear-thinning behavior is evident in non-Newtonian liquids as their viscosity decreases with an escalation in shear rate. The differences in viscosity among various exudates and mucilages may be attributed to their polysaccharide content. Specifically, higher concentrations of polysaccharides in these substances lead to increased viscosities, as reported in studies by Naveed et al., (2017) and Read & Gregory (1997). This relationship offers an indirect assessment of their potential to function as hydrogels. An analysis of the existing literature and previous models reveals a noticeable gap in understanding the intricate relationships among different phases within the pore space of the rhizosphere during wetting and drying processes. A significant challenge arises from the complex behavior of mucilage, which behaves as a liquid at lower concentrations, transforms into a solid state at higher concentrations, and takes on a viscoelastic state, akin to a hydrogel, in the intermediate range. To bridge this knowledge gap, this study introduces an innovative three-dimensional pore-scale model, employing the LSM (**Chapter IV**). This model is employed to simulate the drying process of mucilage positioned between two soil particles. Furthermore, a promising avenue for enhancement involves the incorporation of the current model with a robust CFD tool like the lattice Boltzmann method. This fusion would facilitate the simulation of a broad range of mucilage concentrations, spanning from the initial stages where mucilage exhibits liquid-like behavior. **Remarkably, the model demonstrates the capacity to reproduce the distinct spider-web-like structures that are characteristic of mucilage**. The validity of the three-dimensional mucilage drying model is evaluated through

qualitative validation, which entails comparing the model-generated results with ESEM images depicting dry mucilage located between glass beads.

Integrating the model with two-phase flow models (**Chap IV**) allows us to account for phase entrapment in small pores. Additionally, polymer diffusion and network reconfiguration are important phenomena at low mucilage concentrations, and these can be addressed by incorporating polymer diffusion through spring reconfigurations into the model. The model under consideration holds the potential to offer fresh insights into hydrodynamic phenomena occurring within the rhizosphere's pore space. Furthermore, it has the capacity to enhance our comprehension of various significant processes intricately tied to rhizosphere hydraulic dynamics. **These processes encompass solute transport, the connectivity of the liquid phase, resistance encountered during root penetration, rhizosheath formation, and the activity of microorganisms.**

The model presented in this research employs an arrangement of interconnected springs to symbolize the connections among soil particles. By evaluating the characteristics of these springs, we can calculate the force needed to either break or compress them. Through scaling up and determining the forces acting on a broader spatial domain, we can forecast the conditions conducive to plant root growth. Furthermore, mucilage, apart from serving as a lubricant for root development, exhibits adhesive characteristics that entangle soil particles in proximity to plant roots, leading to the formation of rhizosheaths (Brown et al., 2012; Delhaize et al., 2015; Moreno-Espíndola et al., 2007; Watt et al., 1994).

This phenomenon is inherently linked to the interactions among soil particles, requiring robust connections to counteract the gravitational forces acting on soil particles. These connections ensure the cohesion of soil particles with each other and with plant roots, thereby preventing their separation. Much like the previous case, the calculation of the breaking strength for the network

of springs that connect the soil particles can assist in predicting the circumstances conducive to rhizosheath formation. While the current mucilage network model already demonstrates the capacity to generate realistic spatial configurations, it offers opportunities for further improvements. For example, establishing a correlation among the "T" value and water content, and refining the lattice configuration by introducing diagonal bonds (as illustrated in Figure IV-4) could prove to be beneficial. This modification is warranted because, in real-life scenarios, particles engage in interactions from all directions, and the inclusion of more bonds would better account for this phenomenon. **The development of a model to describe mucilage effects on rhizosheath formation can serve as a valuable tool for predicting outcomes in real soil-plant systems.** Such models can be **integrated into decision support systems for agricultural practices and land management, allowing for more precise and efficient soil management**.

2.5 Rhizosheath assessment under alternative dry and wet cycles

Plant roots play a pivotal role in enhancing aggregate formation within the soil by physically intertwining with soil particles, changing water tension during their growth, and releasing exudates from their root tips. Consequently, this results in the development of more robust soil aggregation in the rhizosphere compared to the surrounding bulk soil environment, as observed in studies by J. Li et al. (2020) and Amelung et al. (2023). Notably, mucilage, considered one of the most effective plant exudates, further contributes to soil aggregation, as documented by Monnier (1965). Apart from their biological influence, aggregates are also formed during DW cycles, serving as a pivotal factor in promoting aggregation and initiating essential processes like nutrient cycling and the decomposition of organic matter, as observed in research conducted by Kemper et al. (1985), Krause et al. (2018), and Mikha et al. (2005). In chapter III, I presented the results of rhizosheath formation after incorporation of dry and wet cycles in the presence of chia seed mucilage. The

presence of mucilage doubled the amount of rhizosheath relative to the soil that did not receive mucilage additions. However, an even stronger enhancement was found with the application of constant wet conditions, which significantly enhanced rhizosheath development, particularly in unsterilized soil with only 22% clay (Fig. III-2). **The application of DW cycles reduced the amount of rhizosheath significantly compared to the wet treatment**. This reduction was likely due to physical interactions affecting rhizosheath formation, as sterilization did not affect rhizosheath development in the treatment with DW cycles. The structure of the rhizosheath also establishes an ecological niche characterized by favorable microclimatic conditions. In this particular habitat, conditions are favorable for the growth and development of microbial communities, including those encompassing nitrogen-fixing bacteria (Bergmann et al., 2009; Othman et al., 2003), along with the synthesis of associated microbial adhesives (Davinic et al., 2012; Six et al., 2004). Moreover, excessive moisture levels diminish the cohesion among soil particles, rendering them more susceptible to dislodging and adhering to the roots (Bates & Lynch, 2001). Consequently, this further augments the development of the rhizosheath. **In addition, a consistent presence of moisture can facilitate the diffusion of mucilage into the adjacent soil. This, in turn, expands the volume of soil participating in the process of mucilage-induced rhizosheath formation** (Rahim et al., 2023).

Fluctuations in moisture conditions are likely to exert an influence on soil microbial communities actively participating in the formation of rhizosheaths. However, it's crucial to recognize the intricate interplay of various processes at work. This encompasses the microbial synthesis of supplementary adhesives crucial for rhizosheath formation, as well as the breakdown of mucilage and other bonding agents essential for aggregation and rhizosheath development. The specific role of DW cycles in this context remains challenging to predict.

On one hand, it has been demonstrated that DW cycles can potentially limit the availability of accessible C in the soil, consequently constraining microbial decomposition processes (Fierer & Schimel, 2002; Mikha et al., 2005; Sommers et al., 1981). However, it is equally well-documented that the rewetting of dried soil typically triggers an increased release of CO_2 (referred to as the Birch effect), owing to heightened microbial activity (Birch, 1958; Franzluebbers et al., 1994; S. Zhang et al., 2020). This knowledge can be applied to real-world situations, such as understanding how natural precipitation patterns or irrigation practices may influence rhizosheath development. **It highlights the need for soil management practices that consider the impact of these cycles.**

2.6 Particle size distribution and soil stability

Based on investigations PSD and aggregate properties of surrounding soil of rhizosheaths carried by laser diffraction, I found a minimal treatment effects on the aggregation of particles outside the rhizosheath, all exhibited an average median diameter of < 10 µm. In Figure III-4, it can be observed that the mean size of individual soil particles reached its maximum at 6.7 µm. This size accounted for approximately 64% and 68% of soil microaggregates in both the sterilized and unsterilized soil samples containing 22% clay, respectively. Our results confirms the outcomes of Krause et al., (2018). However, larger aggregates exceeding 10 µm were also detected, which could be indicative of the existence of organic carbon within the size fraction greater than 6.7 µm, as well as inorganic components such as clay minerals and iron/aluminum (oxyhydr) oxides, likely in the form of silicate-oxide interactions.

Under DW cycles, the median aggregate size exhibited a more significant decrease in the 22% clay samples than the 32% clay samples, with a particularly pronounced reduction in unsterilized samples (Fig. III-5a). Conversely, under continuous wet conditions (Fig. III-5b), the median diameter consistently decreased over time for all soil types, with no significant differences between

clay content. The average reduction in median diameter was approximately -5 µm, contrasting with the -15 µm reduction seen in soils subjected to DW cycles. Greater clay content in the soil resulted in increased stability of the formed aggregates also interestingly, sterilization of the soils also led to improved aggregate stability. Conversely, soils rich in clay, characterized by their fine-textured particles, have a propensity to create larger and denser aggregates owing to their expanded surface area and increased adhesive properties. This observation is reinforced by the SEM images, where rhizosheath mass appeared to be more substantial in soils with lower clay content as opposed to those with higher clay content (refer to Fig. III-1c and III-1d). I assume the formation and stability of soil aggregates is influenced by an interplay between biotic factors (e.g., EPS and microorganisms) and abiotic factors (e.g., clay minerals, pedogenic oxides). The interplay of biotic and abiotic processes is essential in upholding soil structure and its ability to deliver essential ecosystem services. This observation is in line with my results, which reveal a transition towards reduced particle sizes and simultaneously high stability in sterilized conditions (Fig. III-5a). The analysis of PSD and stability in real soil-plant systems can help predict the impact of mucilage on soil aggregation and nutrient availability. **This information is relevant for soil management practices, as it can guide decisions on soil amendments and cultivation techniques**.

2.7 Temporal and spatial distribution of mucilage

The role of mucilage in rhizosheath formation has garnered significant attention in recent scientific research due to its implications for plant health and ecosystem functioning. These structures are not static but rather dynamic, and their formation depends on various factors, including the temporal and spatial distribution of mucilage. Temporal distribution refers to the changes in mucilage secretion over time, influenced by factors such as plant species, root age, and environmental conditions. Mucilage secretion typically follows a diurnal pattern, with higher

exudation during the daytime (George et al., 2014a). This temporal aspect can affect rhizosheath formation, particularly during DW cycles, which have been shown to play a pivotal role in influencing rhizosheath dynamics (Ahmed et al., 2014). DW cycles, characterized by alternating periods of soil drying and wetting, influence rhizosheath formation by promoting the aggregation of soil particles around plant roots (Carminati et al., 2010).

Spatial distribution of mucilage is equally crucial, as it determines the localization and concentration of mucilage in the rhizosphere. Different plant species produce distinct mucilage types and concentrations (Carminati et al., 2010; Holz et al., 2018), affecting rhizosheath formation. Studies have demonstrated that mucilage concentration is a key driver of rhizosheath formation (Ahmed et al., 2014; Van Deynze et al., 2018). The formation of optimal rhizosheaths often occurs at intermediate mucilage concentrations (Rahim et al., 2023: Ahmed et al., 2014). Rhizosheath formation can vary under different scenarios, depending on factors such as plant species, soil type, environmental conditions, and the presence of specific microbes (Haling et al., 2010; Watt et al., 1993). Additionally, the impact of temporal and spatial distribution of mucilage on rhizosheath formation can be complex, influenced by interactions with other rhizosphere components, such as root hairs and exudates (George et al., 2014b; Holz et al., 2018).

3 CONCLUSIONS

My work places a spotlight on the pivotal role of mucilage in rhizosheath formation. Through a comprehensive investigation, I have underscored the significance of mucilage concentration and its intricate interplay with soil moisture, particularly highlighting the importance of an optimal mucilage concentration (0.12g dry mucilage g^{-1} water) under dry soil conditions. This observation holds exceptional promise for enhancing plant resilience to arid conditions and underpins the potential for elevated agricultural productivity in water-scarce regions. The key findings of my

work provide compelling evidence of mucilage's crucial role in sustaining rhizosheath development, cementing its status as a linchpin in mitigating abiotic stress and supporting ecosystem services.

Additionally, my research introduces a novel perspective on hydrodynamic processes within the rhizosphere. I reveal the broader significance of this model, extending beyond rhizosheath formation, encompassing a spectrum of vital processes dependent on rhizosphere hydraulic dynamics. These findings underscore the indispensable nature of understanding mucilage's function and behavior within the rhizosphere, holding the key to unlocking an array of essential ecosystem services, from efficient solute transport to enhanced microbial activity.

Besides, my research discovered that DW cycles posed challenges to rhizosheath development, whereas constant wet conditions fostered it. This phenomenon was likely due to the reduced interference of DW cycles with mucilage diffusion, microbial production of adhesives, and the physical integrity of rhizosheaths, specifically related to desiccation cracks and particle interactions beyond the root's reach. Strikingly, my study revealed that soil sterilization had an unexpected and beneficial effect on rhizosheath formation, possibly by promoting the production of microbial adhesives over mucilage decomposition. Furthermore, I unraveled the significant role played by soil texture, especially clay content, where lower clay content soils supported the formation of more substantial rhizosheaths and smaller aggregates. These findings underscore the paramount importance of these factors in plant-root interactions and have far-reaching implications for soil health and ecosystem services. The result of my work thus enrich the comprehension of the diverse roles played by various plant mucilage traits in the context of soil aggregation and rhizosheath formation, under varying physiological conditions.

CHAPTER VI
REFERENCES

REFERENCES

Abdul-Jabbar, A. S., Sammis, T. W., & Lugg, D. G. (1982). Effect of moisture level on the root pattern of alfalfa. *Irrigation Science, 3,* 197–207.

Ahmed, M. A., Kroener, E., Benard, P., Zarebanadkouki, M., Kaestner, A., & Carminati, A. (2016). Drying of mucilage causes water repellency in the rhizosphere of maize: measurements and modelling. *Plant and Soil*, *407*(1–2), 161–171. https://doi.org/10.1007/s11104-015-2749-1

Ahmed, M. A., Kroener, E., Holz, M., Zarebanadkouki, M., & Carminati, A. (2014). Mucilage exudation facilitates root water uptake in dry soils. *Functional Plant Biology*, *41*(11), 1129–1137.

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 and Soil*, *428*, 57–65.

Albalasmeh, A. A., & Ghezzehei, T. A. (2014). Interplay between soil drying and root exudation in rhizosheath development. *Plant and Soil*, *374*(1–2), 739–751. https://doi.org/10.1007/s11104-013-1910-y

Amelung, W., Meyer, N., Rodionov, A., Knief, C., Aehnelt, M., Bauke, S. L., Biesgen, D., Dultz, S., Guggenberger, G., & Jaber, M. (2023). Process sequence of soil aggregate formation disentangled through multi-isotope labelling. *Geoderma*, *429*, 116226.

Aslam, M. M., Karanja, J. K., Dodd, I. C., Waseem, M., & Weifeng, X. (2022). Rhizosheath: An adaptive root trait to improve plant tolerance to phosphorus and water deficits? *Plant, Cell*

& Environment, *45*(10), 2861–2874.

Badri, D. V, & Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant, Cell & Environment*, *32*(6), 666–681.

Bailey, C., & Scholes, M. (1997). Rhizosheath occurrence in South African grasses. *South African Journal of Botany*, *63*(6), 484–490.

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.*, *57*, 233–266.

Bates, T. R., & Lynch, J. P. (2001). Root hairs confer a competitive advantage under low phosphorus availability. *Plant and Soil*, *236*, 243–250.

Bell, C. W., Tissue, D. T., Loik, M. E., Wallenstein, M. D., Acosta-Martinez, V., Erickson, R. A., & Zak, J. C. (2014). Soil microbial and nutrient responses to 7 years of seasonally altered precipitation in a Chihuahuan Desert grassland. *Global Change Biology*, *20*(5), 1657–1673.

BEMILLER, J. N., WHISTLER, R. O. Y. L., BARKALOW, D. G., & CHEN, C.-C. (1993). Aloe, chia, flaxseed, okra, psyllium seed, quince seed, and tamarind gums. In *Industrial gums* (pp. 227–256). Elsevier.

Bengough, A. G., Loades, K., & McKenzie, B. M. (2016). Root hairs aid soil penetration by anchoring the root surface to pore walls. *Journal of Experimental Botany*, *67*(4), 1071–1078.

Bennett, A. B., Pankievicz, V. C. S., & Ané, J.-M. (2020). A model for nitrogen fixation in

cereal crops. *Trends in Plant Science*, *25*(3), 226–235.

Bergmann, D., Zehfus, M., Zierer, L., Smith, B., & Gabel, M. (2009). Grass rhizosheaths: associated bacterial communities and potential for nitrogen fixation. *Western North American Naturalist*, *69*(1), 105–114.

Birch, H. F. (1958). The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil*, *10*, 9–31.

Bittelli, M., Campbell, G. S., & Tomei, F. (2015). *Soil physics with Python: transport in the soil-plant-atmosphere system*. OUP Oxford.

Brady, N. C., Weil, R. R., & Weil, R. R. (2008). *The nature and properties of soils* (Vol. 13). Prentice Hall Upper Saddle River, NJ.

Brax, M., Buchmann, C., & Schaumann, G. E. (2017). Biohydrogel induced soil–water interactions: how to untangle the gel effect? A review. *Journal of Plant Nutrition and Soil Science*, *180*(2), 121–141.

Brown, L. K., George, T. S., Neugebauer, K., & White, P. J. (2017a). The rhizosheath–a potential trait for future agricultural sustainability occurs in orders throughout the angiosperms. *Plant and Soil*, *418*, 115–128.

Brown, L. K., George, T. S., Neugebauer, K., & White, P. J. (2017b). The rhizosheath–a potential trait for future agricultural sustainability occurs in orders throughout the angiosperms. *Plant and Soil*, *418*(1–2), 115–128.

Brütsch, L., Stringer, F. J., Kuster, S., Windhab, E. J., & Fischer, P. (2019). Chia seed mucilage–a vegan thickener: Isolation, tailoring viscoelasticity and rehydration. *Food & Function*,

10(8), 4854–4860.

Burak, E., Quinton, J. N., & Dodd, I. C. (2021). Root hairs are the most important root trait for rhizosheath formation of barley (Hordeum vulgare), maize (Zea mays) and Lotus japonicus (Gifu). *Annals of Botany*, *128*(1), 45–57.

Canarini, A., Kaiser, C., Merchant, A., Richter, A., & Wanek, W. (2019). Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. *Frontiers in Plant Science*, *10*, 157.

Carminati, A., Benard, P., Ahmed, M. A., & Zarebanadkouki, M. (2017). Liquid bridges at the root-soil interface. *Plant and Soil*, *417*(1–2), 1–15. https://doi.org/10.1007/s11104-017-3227-8

Carminati, A., Moradi, A. B., Vetterlein, D., Vontobel, P., Lehmann, E., Weller, U., Vogel, H.-J., & Oswald, S. E. (2010). Dynamics of soil water content in the rhizosphere. *Plant and Soil*, *332*, 163–176.

Chaboud, A. (1983). Isolation, purification and chemical composition of maize root cap slime. *Plant and Soil*, *73*, 395–402.

Churchman, G. J. (2018). Game Changer in Soil Science. Functional role of clay minerals in soil. *Journal of Plant Nutrition and Soil Science*, *181*(1), 99–103.

Clarholm, M., & Skyllberg, U. (2013). Translocation of metals by trees and fungi regulates pH, soil organic matter turnover and nitrogen availability in acidic forest soils. *Soil Biology and Biochemistry*, *63*, 142–153.

Clark, J. S., Campbell, J. H., Grizzle, H., Acosta-Martìnez, V., & Zak, J. C. (2009). Soil

microbial community response to drought and precipitation variability in the Chihuahuan Desert. *Microbial Ecology*, *57*, 248–260.

Czarnes, S., Hallett, P. D., Bengough, A. G., & Young, I. M. (2000). Root-and microbial-derived mucilages affect soil structure and water transport. *European Journal of Soil Science*, *51*(3), 435–443.

Dakora, F. D., & Phillips, D. A. (2002). *Root exudates as mediators of mineral acquisition in low-nutrient environments*. 35–47.

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 and Biochemistry*, *46*, 63–72.

De León-González, F., Gutiérrez-Castorena, M. C., González-Chávez, M. C. A., & Castillo-Juárez, H. (2007). Root-aggregation in a pumiceous sandy soil. *Geoderma*, *142*(3–4), 308–317.

de Oliveira Nascimento, L., Ferreira, J. B., de Oliveira Nascimento, G., dos Santos, V. B., de Souza, C. S., de Lima, A. F. B., & de Oliveira Nascimento, F. I. (2020). Effect of reduced spacing on relationship of physiological, morphological and productive traits of corn yield. *Australian Journal of Crop Science*, *14*(7), 1202–1208.

Delhaize, E., James, R. A., & Ryan, P. R. (2012). Aluminium tolerance of root hairs underlies genotypic differences in rhizosheath size of wheat (Triticum aestivum) grown on acid soil. *New Phytologist*, *195*(3), 609–619.

Delhaize, E., Rathjen, T. M., & Cavanagh, C. R. (2015). The genetics of rhizosheath size in a multiparent mapping population of wheat. *Journal of Experimental Botany*, *66*(15), 4527–4536.

Dennis, P. G., Miller, A. J., & Hirsch, P. R. (2010). Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiology Ecology*, *72*(3), 313–327.

Duell, R. W., & Peacock, G. R. (1985). Rhizosheaths on mesophytic grasses 1. *Crop Science*, *25*(5), 880–883.

Fan, T. W.-M., Lane, A. N., Shenker, M., Bartley, J. P., Crowley, D., & Higashi, R. M. (2001). Comprehensive chemical profiling of gramineous plant root exudates using high-resolution NMR and MS. *Phytochemistry*, *57*(2), 209–221.

Fierer, N., & Schimel, J. P. (2002). Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry*, *34*(6), 777–787.

Franzluebbers, K., Weaver, R. W., Juo, A. S. R., & Franzluebbers, A. J. (1994). Carbon and nitrogen mineralization from cowpea plants part decomposing in moist and in repeatedly dried and wetted soil. *Soil Biology and Biochemistry*, *26*(10), 1379–1387.

Galloway, A. F., Knox, P., & Krause, K. (2020). Sticky mucilages and exudates of plants: putative microenvironmental design elements with biotechnological value. *New Phytologist*, *225*(4), 1461–1469. https://doi.org/10.1111/nph.16144

George, T. S., Brown, L. K., Ramsay, L., White, P. J., Newton, A. C., Bengough, A. G., Russell, J., & Thomas, W. T. B. (2014a). Understanding the genetic control and physiological traits

associated with rhizosheath production by barley (H ordeum vulgare). *New Phytologist*, *203*(1), 195–205.

George, T. S., Brown, L. K., Ramsay, L., White, P. J., Newton, A. C., Bengough, A. G., Russell, J., & Thomas, W. T. B. (2014b). Understanding the genetic control and physiological traits associated with rhizosheath production by barley (Hordeum vulgare). *New Phytologist*, *203*(1), 195–205. https://doi.org/10.1111/nph.12786

Gohel, V., Vyas, P., & Chhatpar, H. S. (2005). Activity staining method of chitinase on chitin agar plate through polyacrylamide gel electrophoresis. *African Journal of Biotechnology*, *4*(1), 87–90.

Hallett, P. D., Marin, M., Bending, G. D., George, T. S., Collins, C. D., & Otten, W. (2022). Building soil sustainability from root–soil interface traits. *Trends in Plant Science*.

Hassan, S., & Mathesius, U. (2015). Flavonoids Play Multiple Roles in Symbiotic Root–Rhizosphere Interactions. *Biological Nitrogen Fixation*, 499–510.

Hayat, F., Abdalla, M., & Munir, M. U. (2021). Effect of Chia Seed Mucilage on the Rhizosphere Hydraulic Characteristics. *Sustainability*, *13*(6), 3303.

Hinsinger, P., Bengough, A. G., Vetterlein, D., & Young, I. M. (2009). Rhizosphere: biophysics, biogeochemistry and ecological relevance. *Plant and Soil*, *321*(1–2), 117–152.

Holz, M., Zarebanadkouki, M., Kuzyakov, Y., Pausch, J., & Carminati, A. (2018). Root hairs increase rhizosphere extension and carbon input to soil. *Annals of Botany*, *121*(1), 61–69.

Inderjit, & Dakshini, K. M. M. (1994). Algal allelopathy. *The Botanical Review*, *60*, 182–196.

Jones, D. L., Hodge, A., & Kuzyakov, Y. (2004). Plant and mycorrhizal regulation of

rhizodeposition. *New Phytologist*, *163*(3), 459–480.

Jones, D. L., & Oburger, E. (2011). Solubilization of phosphorus by soil microorganisms. *Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling*, 169–198.

Kemper, W. D., Rosenau, R., & Nelson, S. (1985). Gas displacement and aggregate stability of soils. *Soil Science Society of America Journal*, *49*(1), 25–28.

Kowalenko, C. G., & Babuin, D. (2013). Inherent factors limiting the use of laser diffraction for determining particle size distributions of soil and related samples. *Geoderma*, *193*, 22–28.

Krause, L., Rodionov, A., Schweizer, S. A., Siebers, N., Lehndorff, E., Klumpp, E., & Amelung, W. (2018). Microaggregate stability and storage of organic carbon is affected by clay content in arable Luvisols. *Soil and Tillage Research*, *182*, 123–129.

Kroener, E., Holz, M., Zarebanadkouki, M., Ahmed, M., & Carminati, A. (2018). Effects of mucilage on rhizosphere hydraulic functions depend on soil particle size. *Vadose Zone Journal*, *17*(1), 1–11.

Kuzyakov, Y., & Razavi, B. S. (2019). Rhizosphere size and shape: Temporal dynamics and spatial stationarity. *Soil Biology and Biochemistry*, *135*, 343–360.

Lambers, H., Shane, M. W., Cramer, M. D., Pearse, S. J., & Veneklaas, E. J. (2006). Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany*, *98*(4), 693–713.

Landl, M., Haupenthal, A., Leitner, D., Kroener, E., Vetterlein, D., Bol, R., Vereecken, H., Vanderborght, J., & Schnepf, A. (2021). Simulating rhizodeposition patterns around growing and exuding root systems. *In Silico Plants*, *3*(2), diab028.

Leistner, O. A. (1967). The plant ecology of the southern Kalahari. Botanical Survey Memoir No 38. *Dept. of Agriculture, Technical Services, Johannesburg, South Africa*.

Li, J., Yuan, X., Ge, L., Li, Q., Li, Z., Wang, L., & Liu, Y. (2020). Rhizosphere effects promote soil aggregate stability and associated organic carbon sequestration in rocky areas of desertification. *Agriculture, Ecosystems & Environment*, *304*, 107126.

Li, K., DiLegge, M. J., Minas, I. S., Hamm, A., Manter, D., & Vivanco, J. M. (2019). Soil sterilization leads to re-colonization of a healthier rhizosphere microbiome. *Rhizosphere*, *12*, 100176. https://doi.org/https://doi.org/10.1016/j.rhisph.2019.100176

Liu, T., Ye, N., Song, T., Cao, Y., Gao, B., Zhang, D., Zhu, F., Chen, M., Zhang, Y., & Xu, W. (2019). Rhizosheath formation and involvement in foxtail millet (Setaria italica) root growth under drought stress. *Journal of Integrative Plant Biology*, *61*(4), 449–462.

Mackay, A. D., & Barber, S. A. (1985). Soil moisture effects on root growth and phosphorus uptake by corn 1. *Agronomy Journal*, *77*(4), 519–523.

Marasco, R., Fusi, M., Ramond, J.-B., Van Goethem, M. W., Seferji, K., Maggs-Kölling, G., Cowan, D. A., & Daffonchio, D. (2022). The plant rhizosheath–root niche is an edaphic "mini-oasis" in hyperarid deserts with enhanced microbial competition. *ISME Communications*, *2*(1), 47.

Mason, J. A., Greene, R. S. B., & Joeckel, R. M. (2011). Laser diffraction analysis of the disintegration of aeolian sedimentary aggregates in water. *Catena*, *87*(1), 107–118.

Mazzola, M., & Manici, L. M. (2012). Apple replant disease: role of microbial ecology in cause and control. *Annual Review of Phytopathology*, *50*, 45–65.

McCully, M. E. (1999). Roots in soil: unearthing the complexities of roots and their rhizospheres. *Annual Review of Plant Biology*, *50*(1), 695–718.

McCully, M. E., & Boyer, J. S. (1997). The expansion of maize root-cap mucilage during hydration. 3. Changes in water potential and water content. *Physiologia Plantarum*, *99*(1), 169–177.

Mench, M., Morel, J. L., Guckert, A., & Guillet, B. (1988). Metal binding with root exudates of low molecular weight. *Journal of Soil Science*, *39*(4), 521–527.

Mikha, M. M., Rice, C. W., & Milliken, G. A. (2005). Carbon and nitrogen mineralization as affected by drying and wetting cycles. *Soil Biology and Biochemistry*, *37*(2), 339–347.

Millington, R. J., & Quirk, J. P. (1961). Permeability of porous solids. *Transactions of the Faraday Society*, *57*, 1200–1207.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, *7*(9), 405–410.

Mo, X., Wang, M., Zeng, H., & Wang, J. (2023). Rhizosheath: Distinct features and environmental functions. *Geoderma*, *435*, 116500.

Monnier, G. (1965). Action des matieres organiques sur la stabilite structurale du sol. *Sols Africains*, *10*(1), 5–42.

Moradi, A. B., Carminati, A., Lamparter, A., Woche, S. K., Bachmann, J., Vetterlein, D., Vogel, H.-J., & Oswald, S. E. (2012). Is the rhizosphere temporarily water repellent? *Vadose Zone Journal*, *11*(3), vzj2011-0120.

Morel, J. L., Habib, L., Plantureux, S., & Guckert, A. (1991). Influence of maize root mucilage

on soil aggregate stability. *Plant and Soil*, *136*(1), 111–119.

Morré, D. J., Jones, D. D., & Mollenhauer, H. H. (1967). Golgi apparatus mediated polysaccharide secretion by outer root cap cells of Zea mays. *Planta*, *74*(3), 286–301.

Muszyński, A., O'Neill, M. A., Ramasamy, E., Pattathil, S., Avci, U., Peña, M. J., Libault, M., Hossain, M. S., Brechenmacher, L., & York, W. S. (2015). Xyloglucan, galactomannan, glucuronoxylan, and rhamnogalacturonan I do not have identical structures in soybean root and root hair cell walls. *Planta*, *242*, 1123–1138.

Naveed, M., Ahmed, M. A., Benard, P., Brown, L. K., George, T. S., Bengough, A. G., Roose, T., Koebernick, N., & Hallett, P. D. (2019). Surface tension, rheology and hydrophobicity of rhizodeposits and seed mucilage influence soil water retention and hysteresis. *Plant and Soil*, *437*, 65–81.

Naveed, M., Brown, L. K., Raffan, A. C., George, T. S., Bengough, A. G., Roose, T., Sinclair, I., Koebernick, N., Cooper, L., & Hackett, C. A. (2017). Plant exudates may stabilize or weaken soil depending on species, origin and time. *European Journal of Soil Science*, *68*(6), 806–816.

Nazari, M. (2021). Plant mucilage components and their functions in the rhizosphere. *Rhizosphere*, *18*, 100344.

Ndour, P. M. S., Gueye, M., Barakat, M., Ortet, P., Bertrand-Huleux, M., Pablo, A.-L., Dezette, D., Chapuis-Lardy, L., Assigbetsé, K., & Kane, N. A. (2017). Pearl millet genetic traits shape rhizobacterial diversity and modulate rhizosphere aggregation. *Frontiers in Plant Science*, *8*, 1288.

Neumann, G., & Römheld, V. (1999). Root excretion of carboxylic acids and protons in phosphorus-deficient plants. *Plant and Soil*, *211*, 121–130.

Oleghe, E., Naveed, M., Baggs, E. M., & Hallett, P. D. (2017). Plant exudates improve the mechanical conditions for root penetration through compacted soils. *Plant and Soil*, *421*(1–2), 19–30.

Oppenheimer, H. R. (1960). Adaptation to drought: xerophytism. *Plant-Water Relationships in Arid and Semi-Arid Conditions: Reviews of Research*.

Ordentlich, A., Elad, Y., & Chet, I. (1988). The role of chitinase of Serratia marcescens in biocontrol of Sclerotium rolfsii. *Phytopathology*, *78*(1), 84–88.

Osipov, V. I. (2015). *Physicochemical theory of effective stress in soils*. Springer.

Othman, A., Shawky, M., Amer, W., Fayez, M., Monib, M., & Hegazi, N. (2003). Biodiversity of microorganisms in semi-arid soils of north Sinai deserts. *Archives of Agronomy and Soil Science*, *49*(3), 241–260.

Pang, J., Ryan, M. H., Siddique, K. H. M., & Simpson, R. J. (2017a). Unwrapping the rhizosheath. *Plant and Soil*, *418*(1–2), 129–139. https://doi.org/10.1007/s11104-017-3358-y

Pang, J., Ryan, M. H., Siddique, K. H. M., & Simpson, R. J. (2017b). Unwrapping the rhizosheath. *Plant and Soil*, *418*, 129–139.

Paporisch, A., Bavli, H., Strickman, R. J., Neumann, R. B., & Schwartz, N. (2021). Root exudates alters nutrient transport in soil. *Water Resources Research*, *57*(10), e2021WR029976.

Pashley, R. M., & Karaman, M. E. (2021). *Applied colloid and surface chemistry*. John Wiley &

Sons.

Peña, M. J., Kong, Y., York, W. S., & O'Neill, M. A. (2012). A galacturonic acid–containing xyloglucan is involved in Arabidopsis root hair tip growth. *The Plant Cell*, *24*(11), 4511–4524.

Phillips, R. P., Finzi, A. C., & Bernhardt, E. S. (2011). Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO2 fumigation. *Ecology Letters*, *14*(2), 187–194.

Placella, S. A., Brodie, E. L., & Firestone, M. K. (2012). Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. *Proceedings of the National Academy of Sciences*, *109*(27), 10931–10936.

Price, S. R. (1911). The roots of some North African desert-grasses. *New Phytologist*, *10*(9), 328–340.

Rahim, R., Jahromi, O. E., Amelung, W., & Kroener, E. (2023). *Rhizosheath formation in soil depends on mucilage concentration and water content*.

Rahman, M. T., Zhu, Q. H., Zhang, Z. B., Zhou, H., & Peng, X. (2017). The roles of organic amendments and microbial community in the improvement of soil structure of a Vertisol. *Applied Soil Ecology*, *111*, 84–93.

Read, D. B., Bengough, A. G., Gregory, P. J., Crawford, J. W., Robinson, D., Scrimgeour, C. M., Young, I. M., Zhang, K., & Zhang, X. (2003). Plant roots release phospholipid surfactants that modify the physical and chemical properties of soil. *New Phytologist*, *157*(2), 315–326.

Read, D. B., & Gregory, P. J. (1997). Surface tension and viscosity of axenic maize and lupin

root mucilages. *The New Phytologist*, *137*(4), 623–628.

Rengasamy, P. (2006). World salinization with emphasis on Australia. *Journal of Experimental Botany*, *57*(5), 1017–1023.

Rovira, A. D. (1959). Root excretions in relation to the rhizosphere effect: IV. Influence of plant species, age of plant, light, temperature, and calcium nutrition on exudation. *Plant and Soil*, *11*, 53–64.

Sasse, J., Martinoia, E., & Northen, T. (2018). Feed your friends: do plant exudates shape the root microbiome? *Trends in Plant Science*, *23*(1), 25–41.

Schnepf, A., Carminati, A., Ahmed, M. A., Ani, M., Benard, P., Bentz, J., Bonkowski, M., Knott, M., Diehl, D., & Duddek, P. (2022). Linking rhizosphere processes across scales: Opinion. *Plant and Soil*, *478*(1–2), 5–42.

Seneviratne, G., Weerasekara, M., Seneviratne, K., Zavahir, J. S., Kecskés, M. L., & Kennedy, I. R. (2011). Importance of biofilm formation in plant growth promoting rhizobacterial action. *Plant Growth and Health Promoting Bacteria*, 81–95.

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 and Tillage Research*, *79*(1), 7–31.

Smith, S. E., Smith, F. A., & Jakobsen, I. (2004). Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist*, *162*(2), 511–524.

Sommers, L. E., Gilmour, C. M., Wildung, R. E., & Beck, S. M. (1981). The effect of water

potential on decomposition processes in soils. *Water Potential Relations in Soil Microbiology*, *9*, 97–117.

Stewart, B. A., & Hartge, K. H. (1995). *Soil structure: its development and function* (Vol. 7). CRC Press.

Tang, N., Siebers, N., Leinweber, P., Eckhardt, K.-U., Dultz, S., Nischwitz, V., & Klumpp, E. (2022). Implications of Free and Occluded Fine Colloids for Organic Matter Preservation in Arable Soils. *Environmental Science & Technology*, *56*(19), 14133–14145.

Tisdall, J. M. (2020). Formation of soil aggregates and accumulation of soil organic matter. In *Structure and organic matter storage in agricultural soils* (pp. 57–96). CRC Press.

Tisdall, J. M., & OADES, J. M. (1982). Organic matter and water-stable aggregates in soils. *Journal of Soil Science*, *33*(2), 141–163.

Totsche, K. U., Amelung, W., Gerzabek, M. H., Guggenberger, G., Klumpp, E., Knief, C., Lehndorff, E., Mikutta, R., Peth, S., & Prechtel, A. (2018). Microaggregates in soils. *Journal of Plant Nutrition and Soil Science*, *181*(1), 104–136.

Van Deynze, A., Zamora, P., Delaux, P.-M., Heitmann, C., Jayaraman, D., Rajasekar, S., Graham, D., Maeda, J., Gibson, D., & Schwartz, K. D. (2018). Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. *PLoS Biology*, *16*(8), e2006352.

Vančura, V., & Hanzlíková, A. (1972). Root exudates of plants: IV. Differences in chemical composition of seed and seedlings exudates. *Plant and Soil*, *36*, 271–282.

Vermeer, J., & McCully, M. E. (1982). The rhizosphere in Zea: new insight into its structure and

development. *Planta*, *156*(1), 45–61.

Vetterlein, D., Carminati, A., Kögel-Knabner, I., Bienert, G. P., Smalla, K., Oburger, E., Schnepf, A., Banitz, T., Tarkka, M. T., & Schlüter, S. (2020). Rhizosphere spatiotemporal organization–a key to rhizosphere functions. *Frontiers in Agronomy*, *2*, 8.

Volkens, G. (1887). *Die Flora der aegyptisch-arabischen Wüste: auf Grundlage anatomisch-physiologischer Forschungen*. Gerbrüder Borntraeger (Ed. Eggers).

Watt, M., McCully, M. E., & Canny, M. J. (1994a). Formation and stabilization of rhizosheaths of Zea mays L.: Effect of soil water content. *Plant Physiology*, *106*(1), 179–186. https://doi.org/10.1104/pp.106.1.179

Watt, M., McCully, M. E., & Canny, M. J. (1994b). Formation and stabilization of rhizosheaths of Zea mays L.(Effect of soil water content). *Plant Physiology*, *106*(1), 179–186.

Watt, M., McCully, M. E., & Jeffree, C. E. (1993). Plant and bacterial mucilages of the maize rhizosphere: comparison of their soil binding properties and histochemistry in a model system. *Plant and Soil*, *151*(2), 151–165.

Webster, R. (2007). Analysis of variance, inference, multiple comparisons and sampling effects in soil research. *European Journal of Soil Science*, *58*(1), 74–82.

Wertz, S., Czarnes, S., Bartoli, F., Renault, P., Commeaux, C., Guillaumaud, N., & Clays-Josserand, A. (2007). Early-stage bacterial colonization between a sterilized remoulded soil clod and natural soil aggregates of the same soil. *Soil Biology and Biochemistry*, *39*(12), 3127–3137. https://doi.org/https://doi.org/10.1016/j.soilbio.2007.07.005

Williams, K. A., Ruiz, S. A., Petroselli, C., Walker, N., Fletcher, D. M. M., Pileio, G., & Roose,

T. (2021). Physical characterisation of chia mucilage polymeric gel and its implications on rhizosphere science-Integrating imaging, MRI, and modelling to gain insights into plant and microbial amended soils. *Soil Biology and Biochemistry*, *162*, 108404.

Wullstein, L. H., Bruening, M. L., & Bollen, W. B. (1979). Nitrogen fixation associated with sand grain root sheaths (rhizosheaths) of certain xeric grasses. *Physiologia Plantarum*, *46*(1), 1–4.

York, L. M., Carminati, A., Mooney, S. J., Ritz, K., & Bennett, M. J. (2016). The holistic rhizosphere: integrating zones, processes, and semantics in the soil influenced by roots. *Journal of Experimental Botany*, *67*(12), 3629–3643.

Zhang, S., Yu, Z., Lin, J., & Zhu, B. (2020). Responses of soil carbon decomposition to drying-rewetting cycles: a meta-analysis. *Geoderma*, *361*, 114069.

Zhang, X., Kuzyakov, Y., Zang, H., Dippold, M. A., Shi, L., Spielvogel, S., & Razavi, B. S. (2020). Rhizosphere hotspots: root hairs and warming control microbial efficiency, carbon utilization and energy production. *Soil Biology and Biochemistry*, *148*, 107872.

Zhang, Y., Du, H., Xu, F., Ding, Y., Gui, Y., Zhang, J., & Xu, W. (2020). Root-bacteria associations boost rhizosheath formation in moderately dry soil through ethylene responses. *Plant Physiology*, *183*(2), 780–792.

APPENDIX A

Supporting material for chapter II

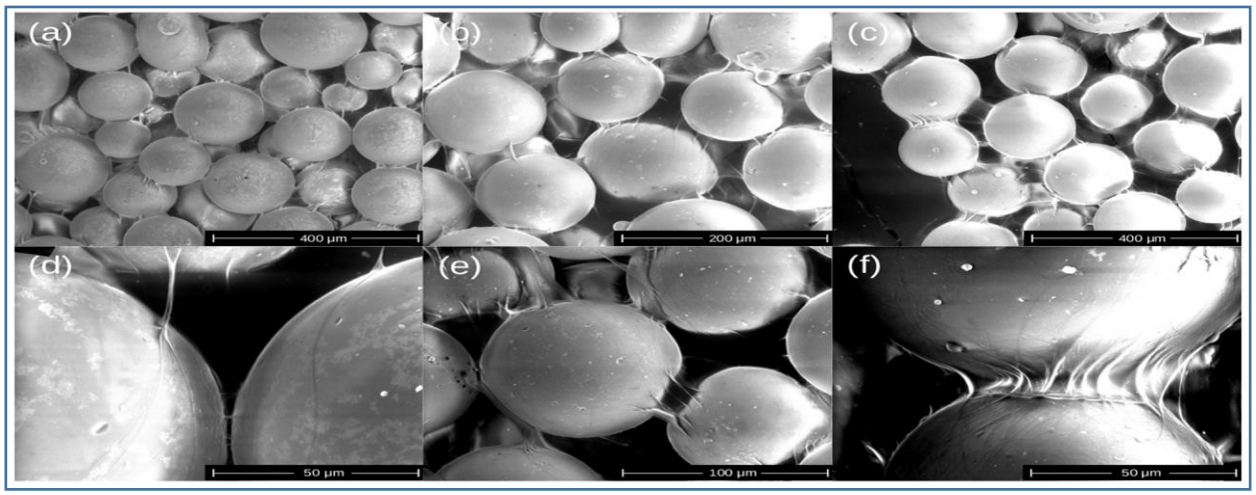

Fig A- 1 Structure of mucilage bridges, ESEM images from Mathilde Brax

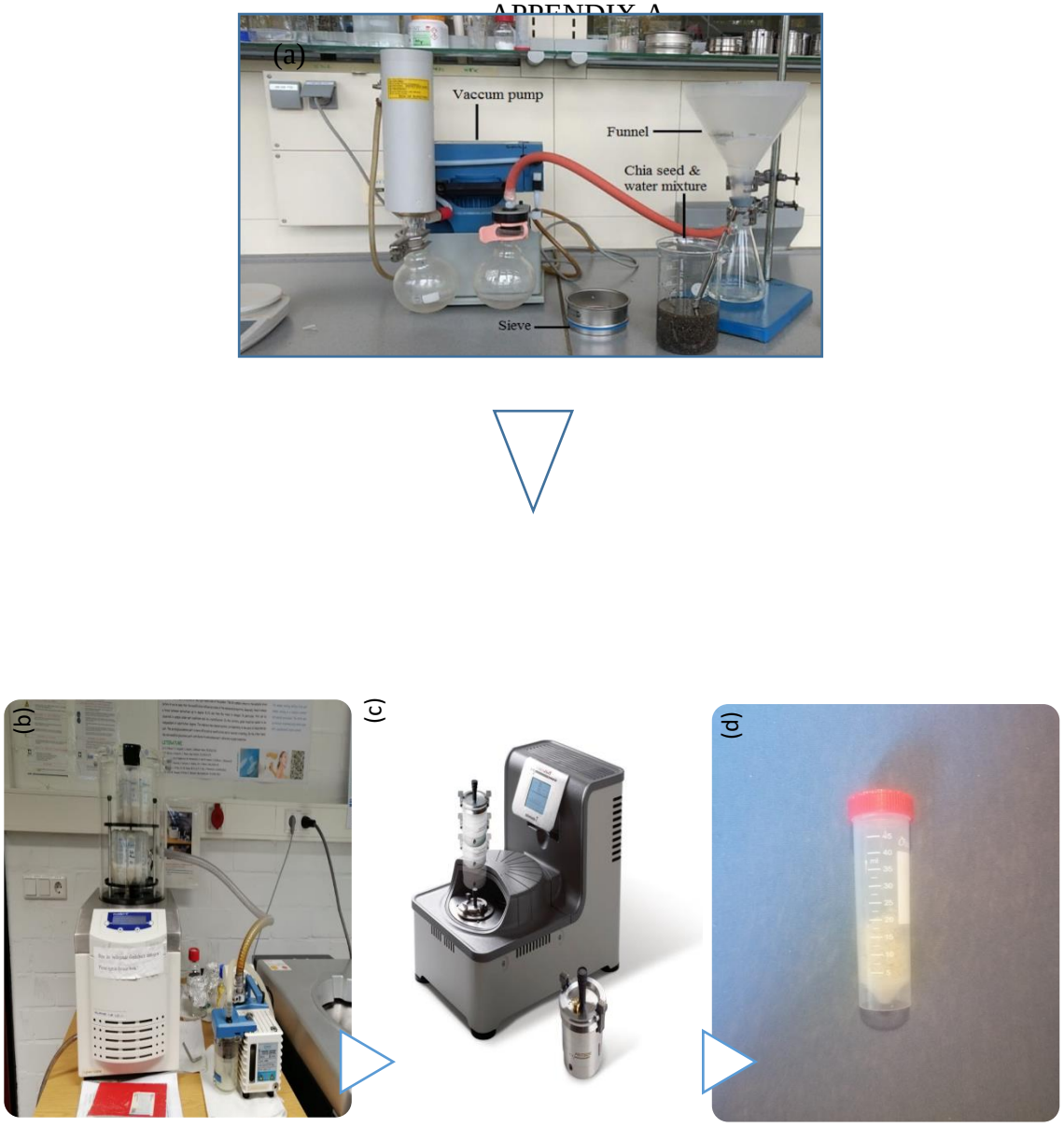

Fig A-2 Process of extraction of chia and flax seed mucilage (a) Vacuum pump (b) freeze dryer (c) Pulverizer (d) Freeze dried chia seed mucilage

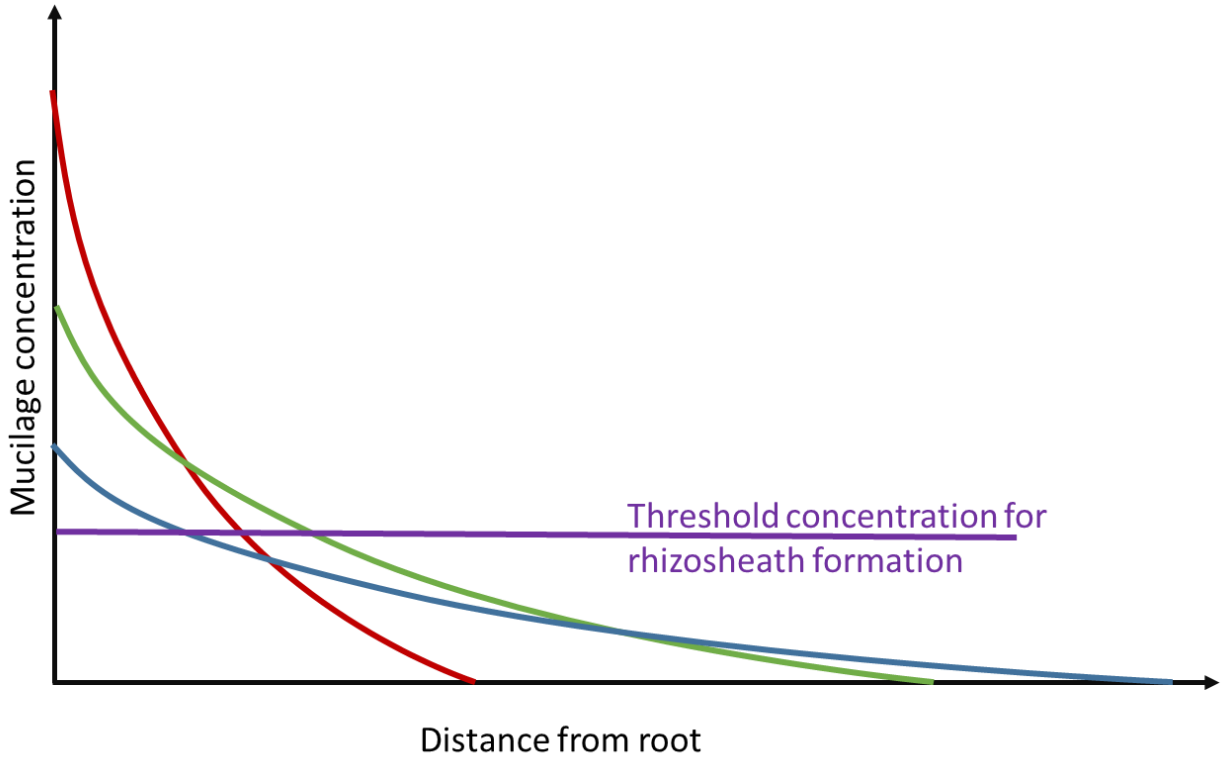

Fig A-3 Mucilage deposition based on single root model: low mucilage concentration -> larger diffusion coefficient D -> it spreads wider -> more dilutes -> less total volume with mucilage concentration above threshold for stable rhizosheath formation- high mucilage concentration -> very small diffusion coefficient D -> it spreads only short distance -> less total volume with mucilage concentration above threshold for stable rhizosheath formation, intermediate concentration: largest rhizosheath volume.

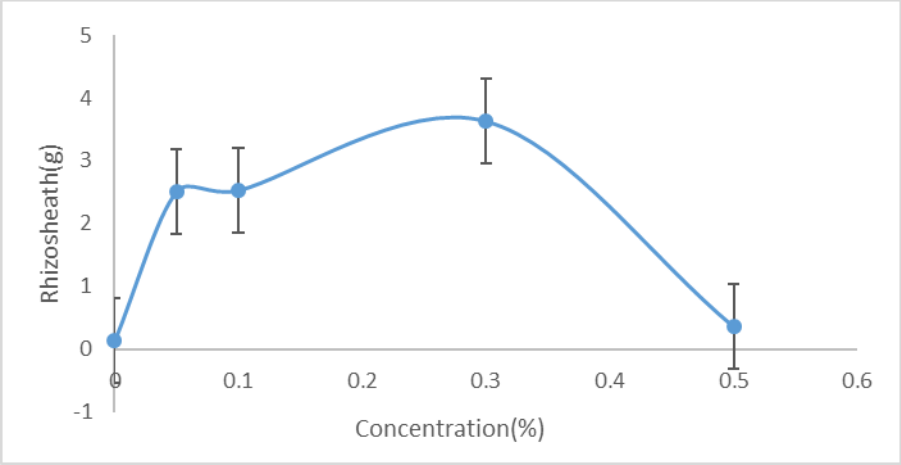

Fig A-4 Rhizosheath formation observed in sandy loam soil under the influence of various concentrations of chia seed mucilage

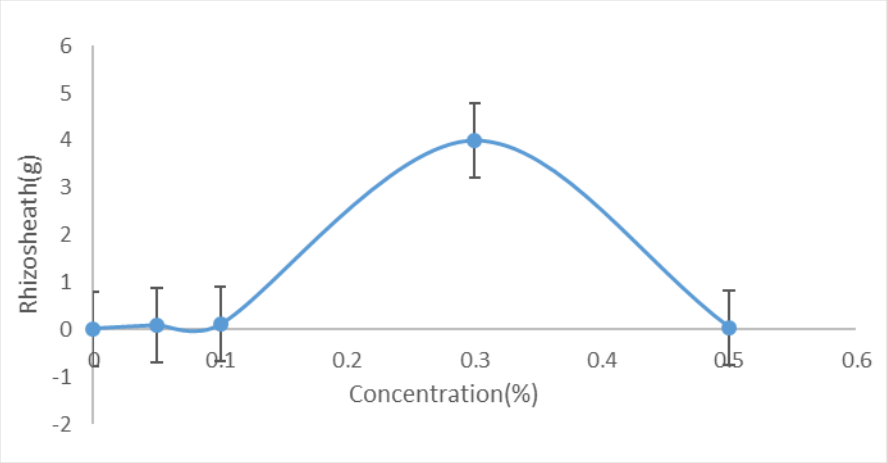

Fig A-4 Rhizosheath formation observed in quartz sandy soil under the influence of various concentrations of chia seed mucilage

APPENDIX B

Supporting material for chapter III

	Clay [%]	Clay [%]
H_2O_2-assessed	**22**	**32**
Bulk Al_D [g kg^{-1}]	1.38 ± 0.03	1.52 ± 0.07
Bulk Fe_D [g kg^{-1}]	9.57 ± 0.03	8.53 ± 0.03
Bulk Mn_D [g kg^{-1}]	0.72 ± 0.03	0.82 ± 0.04
Mass heavy fraction (>2.5 g cm^{-3}) [%]		
8000-250 µm	91.43	96.15
250-20 µm (free)	74.76	76.82
250-20 µm (occluded)	80.68	87.12

Tab B- 1. Characteristics of soil from at the research station Scheyern used in chapter 3 (Krause et al., 2018)

Plot [#]	X	Y	T	U	S
1	4458485	5373014	30.5	47.0	22.5
2	4458535	5373064	24.0	52.3	23.7
3	4458635	5373064	8.5	29.5	62.0
4	4458685	5373064	12.0	22.5	65.5
5	4458735	5373114	18.5	42.0	39.5

Tab B- 2. Soil sampling from DGPRS positions for the defined plots at the research station Scheyern (Krause et al., 2018)

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