Interacting effects of root exudate compounds and δ^{13}C-barley shoot residue on micro-mechanical behaviour of soil measured by rheometry

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Abstract

Laboratory studies have shown that rhizodeposits could lead to either soil structural formation or dispersion depending on plant species, soil conditions, and microbial activity. However, these studies have usually been conducted in dry soils and rarely considered the combined effect of rhizodeposit and organic residues on soil structure. This study hypothesizes that root exudates promote soil dispersion initially, but over time decomposition of root exudates produce binding agents that promote stable soil structure in the rhizosphere. To test this hypothesis, a sandy loam soil sieved to < 500 μm particle size was first amended with root exudate compounds (14.4 mg C g^{-1}), δ^{13}C-barley residue (0.44 mg C g^{-1} soil), or both. Six replicate samples per treatment were packed in cores to a bulk density of 1.27 g cm^{-3} and then equilibrated on a tension table at -2 kPa matric potential. Rheological measurements of flow characteristics (dynamic viscosity) and strength (storage modulus, loss modulus, tan δ, and yield stress) of the control and amended soils were obtained immediately after amendment and after twelve days of incubation at 20 ^{o}C. Only root exudate compounds initially decreased the capacity of soil to retain water at -2 kPa by 21% and by 49% after incubation. Likewise, the yield stress of root exudate amended soil was significantly ($P < 0.05$) lower than that of the unamended soil, reflecting dispersion of soil. However, microbial decomposition/activities significantly ($P < 0.05$) increased yield stress over the corresponding pre-incubation values for these treatments by 200% (root exudate) and 230% (root exudate + δ^{13}C-barley residue). These results confirmed the hypothesized dual effect of root exudates on rhizosphere structure. The initial , whereas stable soil structure is achieved upon decomposition of root exudates.

Keywords: rheology, organic residue, root exudate, soil structure, microbial decomposition and greenhouse gases.

Introduction

Plant roots drive changes in soil structure and stability through localized compaction from growth stresses (Aravena *et al*., 2010; Helliwell *et al*., 2017; Oleghe *et al*., 2017; Dupuy *et al*., 2018) and the release of rhizodeposits that change the mechanical behaviour of the soil in immediate contact with the roots (Iijima *et al*., 2003; Galloway *et al*., 2017). Among the rhizodeposits involved in rhizosphere structure and stability, the production of exudates is of great importance (Morel *et al*., 1991; Czarnes *et al*., 2000; Di Marsico *et al*., 2017). Root exudates serve as a source of energy and drive microbial activities (Sessitsch *et al*., 2001; Bailey *et al*. 2013; Blaud *et al*., 2014) that impact the rate of soil organic matter (SOM) decomposition (Kuzyakov, 2002; Keiluweit *et al*., 2015; Rousk *et al*., 2015). Furthermore, SOM decomposition changes the properties and abundance of microbial-derived mucilage, which has knock-on impacts on soil structural aggregation and stability (Oades, 1993; Rashid *et al*., 2016).

Many factors drive the formation and stability of rhizosphere structure (Denef *et al*., 2002; Six *et al*., 2004), among the most important ones are exudates, micro-organisms, and organic matter, which affect inter-particle bonding and flocculation at the microscale (Hallett *et al*., 2003; Albalasmeh and Ghezzehei 2014; Rashid *et al*., 2016; Buchmann *et al*., 2020). Most studies exploring the impact of exudates and associated microbial activities on rhizosphere mechanical properties have typically focused on aggregation and aggregate stability (Ndour *et al*., 2017; Demenois *et al*., 2018). Increasingly, studies have started exploring how exudate addition affects the underlying mechanisms involved in mechanical stability. For example, Carrizo *et al*. (2018) demonstrated that root exudates increased soil strength and structural stability. Naveed *et al*. (2018) found that seed and root exudates increase hardness and elasticity markedly but postulated that changes in water content caused by these exudates could be driving soil mechanical behaviour. Zhang *et al*. (2008)

quantified interparticle bonding properties in the rhizosphere by demonstrating that polygalaturonic acid increased the fracture toughness and bond energy of clay samples. While Wang *et al*. (2017) showed that secretion of exudate tends to stabilize soil aggregates by simultaneously increasing bonding strength while decreasing the wetting rate. These studies explored relatively dry soils, but much of structure formation in the rhizosphere likely occurs when the soil is wet.

The rhizosphere tends to be temporally wetter than bulk soil (Young 1995; Carminati 2012, Carminati & Vetterlein, 2013) and exudation may enhance the mobility of soil particles, thus promoting the onset of aggregation (Naveed *et al*., 2017). Markgraf *et al*. (2006) suggested that rheometry could provide physically-based measurements of the mechanical properties of wet soils. The impact of mechanical stress on soil rheological properties and stability varies with time, water content (Ghezzehei and Or, 2001; Pértile *et al*., 2016) and the types of organic compounds added to the soil (Tarchitzky and Chen, 2002). Barré and Hallett (2009) quantified the underlying physical mechanisms affecting simulated rhizosphere soil from rheological studies and found evidence that exudate analogues (polygalacturonic acid, PGA, and scleroglucan) increased viscosity and shear resistance in clay soils. Naveed *et al*. (2017) characterised the mechanical stability of rhizosphere soils by demonstrating that rhizodeposits from different plant origin and their microbial decomposition have differing impacts on yield stress and subsequently rhizosphere mechanical stability. Most importantly, they observed that barley rhizodeposits initially caused mechanical dispersion, followed by gelling after microbial decomposition. Maize exudates, on the other hand, gelled the soil. More recent research using nuclear magnetic resonance relaxometry has quantified chemical interactions that drive gelling by mucilages in soils (Buchmann *et al*., 2020). Some of the gelling in soils are driven by improved interparticle bonding (Brax *et al*., 2020), but mucilages may also have non-

Newtonian behaviour affecting rheological response to fast (e.g., rapid aggregate breakdown) or slow (e.g., root growth) mechanical stresses in soil (Haas *et al*., 2018).

Further impacts on soil structural stability could result from the interaction between rhizodeposits and decomposing residues in soil. A rheological study by Markgraf *et al*. (2012) found that the application of farmyard manure increased water content and soil organic carbon, which resulted in increased viscoelasticity, stability and shear behaviour. On the other hand, the interacting effect of root exudates and added organic residues, which is the typical situation in freshly tilled soils, has yet to be explored. We address this gap in understanding by applying rheology-related measurements to wet soils amended with root exudate compounds and/or labelled δ^{13}C-barley shoot residue. The rheological properties of the amended soils (yield stress, dynamic viscosity, loss and storage modulus, and tan δ) were measured with amplitude sweep tests in a parallel plate rheometer (Barré and Hallett, 2009; Mezger, 2014). A model root exudate cocktail previously used in other laboratory studies (Paterson *et al*., 2007; de Graaff *et al*., 2010; Oleghe *et al*., 2019) was used because of the difficulty in extracting and preserving real plant root exudates under sterile conditions. Decomposition of the added compounds was measured from microbial respiration, with the isotopic label used to separate the added barley residue from native soil organic matter. This approach enabled us to assess exudate, microbial activities and biological impacts together, so that interacting effects could be explored.

Materials and methods

Soil sampling and description

Sandy loam topsoil (0-20 cm) was sampled from the Bullion field at the James Hutton Institute, Dundee, UK (56.27N 3.40W). The soil is a Dystric Cambisol (FAO classification) with sand, silt and clay fractions of 60%, 24% and 16%. The respective carbon, C, and nitrogen, N, contents of the soil were 2.25 ± 0.14 mg g^{-1}, and 0.16 ± 0.03 mg

g^{-1} respectively, which resulted in a C: N ratio of 16:1. The pH in $CaCl_2$ was 5.48. The bulk sample was air-dried at 30 ^{o}C to about 1 % water, sieved through 500 µm and then stored at 4 ^{o}C.

Root exudate preparation

An artificial root exudate cocktail was produced after Paterson *et al*. (2007) by combining common sugars, organic acids, and amino acids found in root exudates (Rovira and McDougall, 1967; Jones 1998; Hütsch *et al*., 2002). Labelled δ^{13}C-barley (*Hordeum vulgare*, *spp*. Belgravia) shoot fine powder with a C content of 44.2% and a C: N ratio of 10.8 was used. The bulk plant material was 1.98 atom% excess δ^{13}C using a 20/20 isotope ratio mass spectrometer (Sercon Ltd, Crewe, UK). Details of the production of the labelled barley can be found in Kuntz *et al*. (2016).

Gravimetric moisture content

Six unamended soil samples were saturated before placing them onto a tension table (EcoTech MeBaystem GmbH, Germany) and dried to water content at equilibrium with -2 kPa matric potential while keeping the temperature at 4 ^{o}C to suppress microbial decomposition. The soil gravimetric water content, *w*, was measured as the mass of water per unit mass of soil (*w*, (%) = [mass of moist soil (g) − mass of oven-dried soil (g)/mass of oven-dried soil (g)] × 100). This water content at -2 kPa matric potential (0.40 g g^{-1}) was considered to be the upper limit at field capacity and used as a reference throughout the experiment.

Sample preparation

Sieved air-dried soils <500 µm were treated with or without δ^{13}C-barley residue (0.44 mg C g^{-1} soil) and brought to 0.20 g g^{-1} water content by mixing the soil with either model root exudate (14.4 mg C g^{-1} soil) solution or distilled water. This results in four treatments i.e. unamended or control soil, soil treated with root exudate compounds only, soil treated with δ^{13}C-barley residue only, and soil treated with root exudate compounds and δ^{13}C-barley residue together. In total 48 soil samples with 6 replicates for each treatment were prepared by packing 4.0 g of soil in plastic rings that were 4 mm in height and 40 mm in diameter, which resulted in a bulk density of 1.27 g cm^{-3}. Out of 48 soil samples, 24 were tested immediately after equilibrating at -2 kPa on the tension table at 4 ^{o}C representing fresh or before decomposition treatment. The rest of the 24 soil rings were placed in respiration pots, covered and randomly placed in a plant culture incubator (SANYO Electric Co. Ltd, Japan) at a controlled temperature of 20 ^{o}C for 12 days. The water content was adjusted and maintained at field capacity with deionised water by replacing the mass of water lost to evapotranspiration using a 5 ml syringe. The hourly rates of microbial respiration for days 0, 1, 3, 7 and 12 were determined by feeding the emissions captured from the respiration pots into a Picarro G2201-i isotopic-CO_2 gas analyser (Picarro Inc., Santa Clara, CA, USA), to determine the total and isotopic carbon signature (δ^{13}C-COO).

Rheological measurements

The rheological properties of the soils when freshly packed and after 12 days of incubation were measured on samples that were first saturated and then dried to -2 kPa matric potential using the described tension table and temperature conditions. Amplitude sweep tests at constant frequency and temperature were performed with a Discovery Hybrid Rheometer (DHR, TA instruments, Delaware, USA), equipped with parallel plates of 40 mm in diameter. The lower plate was fixed and the upper plate rotated. The faces of the plates were serrated to improve grip. To ensure minimal disturbance, the samples were placed on the lower plate of the rheometer and gently pushed out of the rings with a spatula, resulting in samples that fit closely to the diameter of the plates.

The experimental program included linear amplitude sweep tests with a single loading profile. The parallel plate was separated by a gap of 4 mm and the temperature of the lower plate was maintained constant at 25 °C (controlled by a Peltier unit). The resting period before the test was 60 s and the variation of the amplitude of deformation ranged from 0.001 to 1000 %. The angular frequency was 10 rad/s and the number of measuring points was 30. The test duration was about 15 min. The normal force on the sample did not exceed 40 kPa at the beginning of the test and tended to 0 kPa at the end of the test (Naveed *et al*., 2018).

Rheological characterization of soil

Soil rheological behaviour was assessed from the curves of storage modulus (G'), and loss modulus (G'') as a function of oscillation strain (Figure 1). The storage modulus, G' measures the elastic component of a soil where the induced energy from applied stress is temporarily stored and is fully recoverable upon withdrawal of the stress. The loss modulus, G'', is the viscous component or dissipated energy, which means that the energy used to initiate the flow is irreversible. The dynamic viscosity (η = stress/strain rate) is the measure of its resistance to flow when an external force is applied (Markgraf *et al*., 2006).

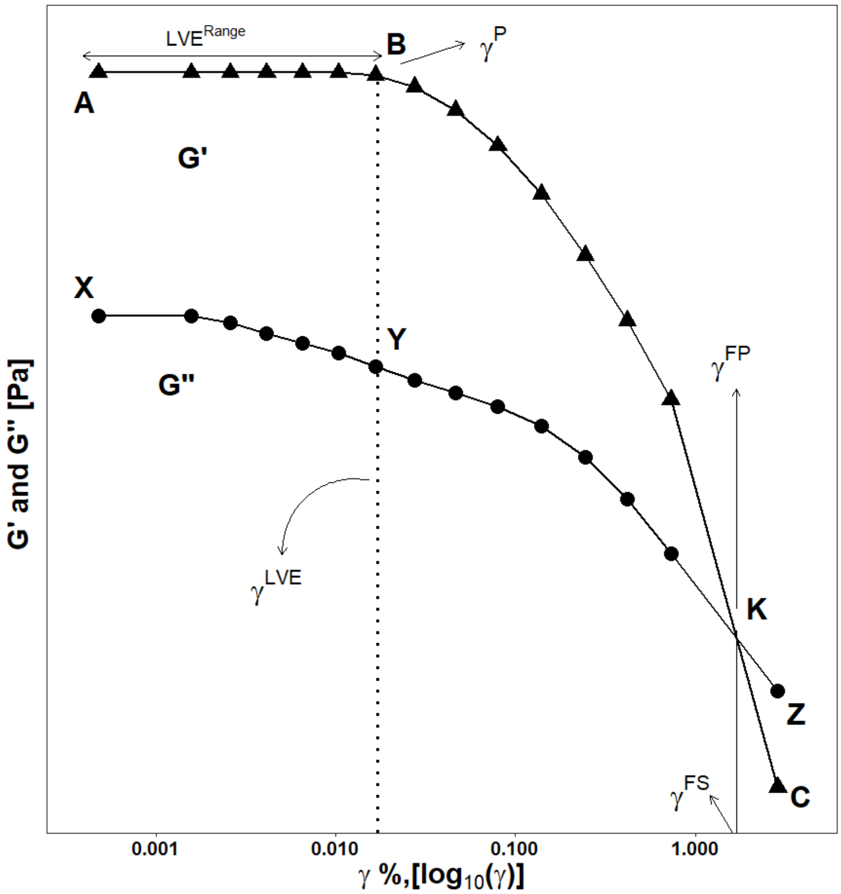

Figure 1: Schematic diagram of soil amplitude sweep test showing the stress-strain curve (G′ and G′′): G′ is storage modulus (A, B, C), G′′ is loss modulus (X, Y, Z), γ (%), is oscillation strain. The yield point γ^{P} (B) is the point on a stress-strain curve that indicates the limit of elastic behavior (A, B) and the beginning of plastic behavior (B, C). The normal stress (γ^{LVE}) and flow stress (γ^{FS}) are the corresponding stress for the limits of elastic (B) and plastic behavior (K); the flow point γ^{FP} indicates the points where the soil begins to flow like liquid while the Linear ViscoElastic Range LVE^{Range} (A, B) describes the soil elasticity.

The Linear ViscoElastic Range (LVE^{Range}) is the range of deformation stress where G′ and G′′, are constant (Schramm, 2006). The LVE^{Range} is determined between points A - B and X - Y, with point B at the limit of deformation (Markgraf *et al.*, 2006; Holthusen *et al.*, 2010). Within the LVE^{Range}, no significant change in the soil's internal structure occurs, the soil structure will deform elastically and will return to its original shape when the applied stress is removed. The point of intersection (K) between the curves G′ and G′′ defines the flow point where the elastic and viscous components are equal. The value tanδ (G"/G') indicates the relative degree of energy dissipation or damping of the material. The elastic stress was plotted as a function of oscillation strain (Naveed *et al*., 2018). The peak elastic stress was denoted as yield stress and the corresponding strain was denoted as yield strain as suggested by Walls *et al*. (2003). The yield stress is the onset of soil structural collapse, which generally lies between the linear viscoelastic range (LVE^{Range}) and flow point.

Statistical analysis

The experiment was set up as a three-way factorial design with two levels of root exudates (0 and 14.4 mg C g^{-1} soil), and two levels of the $\delta^{13}C$-barley residue amendment (0 and 0.44 mg C g^{-1} soil) and two levels of soil mineralization (0 and 12 days). Each treatment had six replicates. Statistical analyses were done using the R statistical computing language (R Core Team, 2020). The response variable satisfied normality tests and the data were analysed as an N-factor analysis of variance (N-way-ANOVA) or repeated ANOVA for respiration data, at a significant level of $P < 0.05$. When the F statistic from the ANOVA showed that the mean effect of the treatments was significant, a pairwise comparison of means with Tukey HSD tests was used to indicate when arithmetic means of various properties differed significantly between the treatment factors at $P < 0.05$ level.

Results

Soil mineralisation

Daily mean CO_2 emitted over 12 days showed no significant variations among the treatments (Figure 2a). The isotopic ^{13}C-CO_2 emitted was larger on day 3 for the $\delta^{13}C$-barley residue treatment and day 7 for the root exudate + $\delta^{13}C$-barley residue interaction and both treatments followed a steady decline until the incubation ended (Figure 2b). After incubation, the amount of remaining $\delta^{13}C$-barley residue in the soil was not significantly different between $\delta^{13}C$-barley residue treatment and root exudate + $\delta^{13}C$-barley residue treatment (Figure 2c).

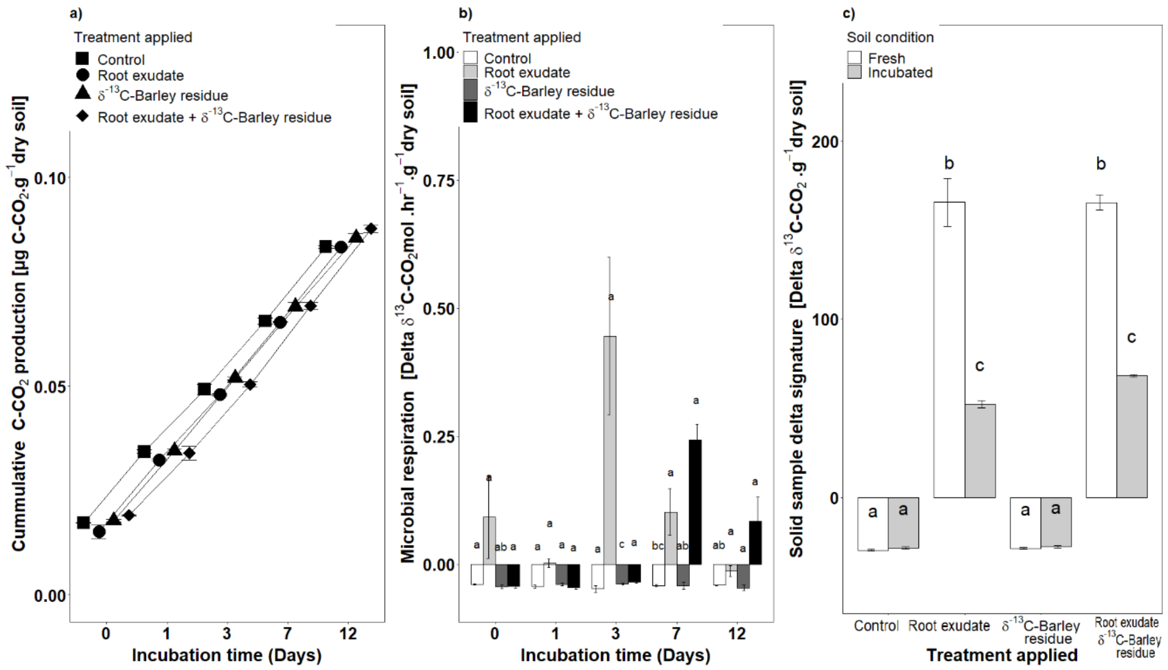

Figure 2: Microbial activities on root exudate and δ[13]C-barley residue sandy loam soil (a) rate of decomposition were determined for CO_2 (µg C-CO_2 .g soil^{-1} .hour^{-1}), (b) cumulative mineralization, (c) respired isotopic ^{13}C-CO_2 signal and (d) depleted isotopic ^{13}C-CO_2 signal for solid sample.

Soil water retention

The water content of soil treated with root exudate compounds was significantly lower whereas the water contents of soils treated with δ^{13}C-barley residue and root exudate + δ^{13}C-barley residue treatments were significantly higher compared to the unamended soil (Table 1). The water content of fresh soil samples at -2 kPa water potential was initially increased by δ^{13}C-barley residue and root exudate + δ^{13}C-barley residue treatments ($P < 0.05$), but incubation of the soil samples resulted in a decrease in water content for all the treatments except the for the control treatment ($P < 0.05$) (Table 1).

Table 1. Mean values of interaction effects for root and δ^{13}C-barley residue on gravimetric water content (*w*) and delta isotopic signature (δ) for sandy loam soil (< 500 µm).

		δ^{13}C **signature on soil solid**		**θg or w(g)**	
Soil	**Treatments**	**LSMean**	**Group**	**LSMean**	**Group**
Fresh	Control	-29.613	a	0.416	b
	δ^{13}C-barley residue	165.545	c	0.500	d
	Root	-28.566	a	0.343	a
	Root exudate + δ^{13}C-barley residue	165.397	c	0.465	c
Decompose	Control	-28.225	a	0.495	cd
	δ^{13}C-barley residue	52.310	b	0.473	cd
	Root	-27.599	a	0.333	a
	Root exudate + δ^{13}C-barley residue	68.350	b	0.402	b

LSMean = Least Squares Mean, θg or w = gravimetric water content

Soil rheological behaviour

With the application of fresh treatment, the dynamic viscosity (the resistance to movement of one layer of soil over another) for soils treated with root exudate at -2 kPa matric potential was significantly ($P < 0.05$) decreased compared to that of the control soil (Figure 3a). At 0.1% oscillation strain, the soil structural resistance (dynamic viscosity) following the application of fresh root exudate compounds was 205% less than that of control soil (Figure 3a). While soils treated with δ^{13}C-barley residue alone (Figure 3b) and root exudate + δ^{13}C-barley residue (Figure 3c) were not significantly different from that of untreated soil (Figures 3b and, 3c). The yield stress (stress at structural collapse) for soils with root exudate treatment and the combination of root exudate + δ^{13}C-barley residue treatment was significantly ($P < 0.05$) lower compared to the unamended soil (Figure 4a and 4c). The yield stress for soils with root exudate treatment and the combined treatment of root exudate + δ^{13}C-barley residue was 276% and 273% lower compared to that of untreated soil, respectively (Figure 4). Similarly, storage modulus (G'), loss modulus (G"), and tanδ (G"/G') for soil treated with root exudate compounds were significantly lower compared to the control/unamended soil and δ^{13}C-barley residue with or without root exudate compounds treatments (Table 2).

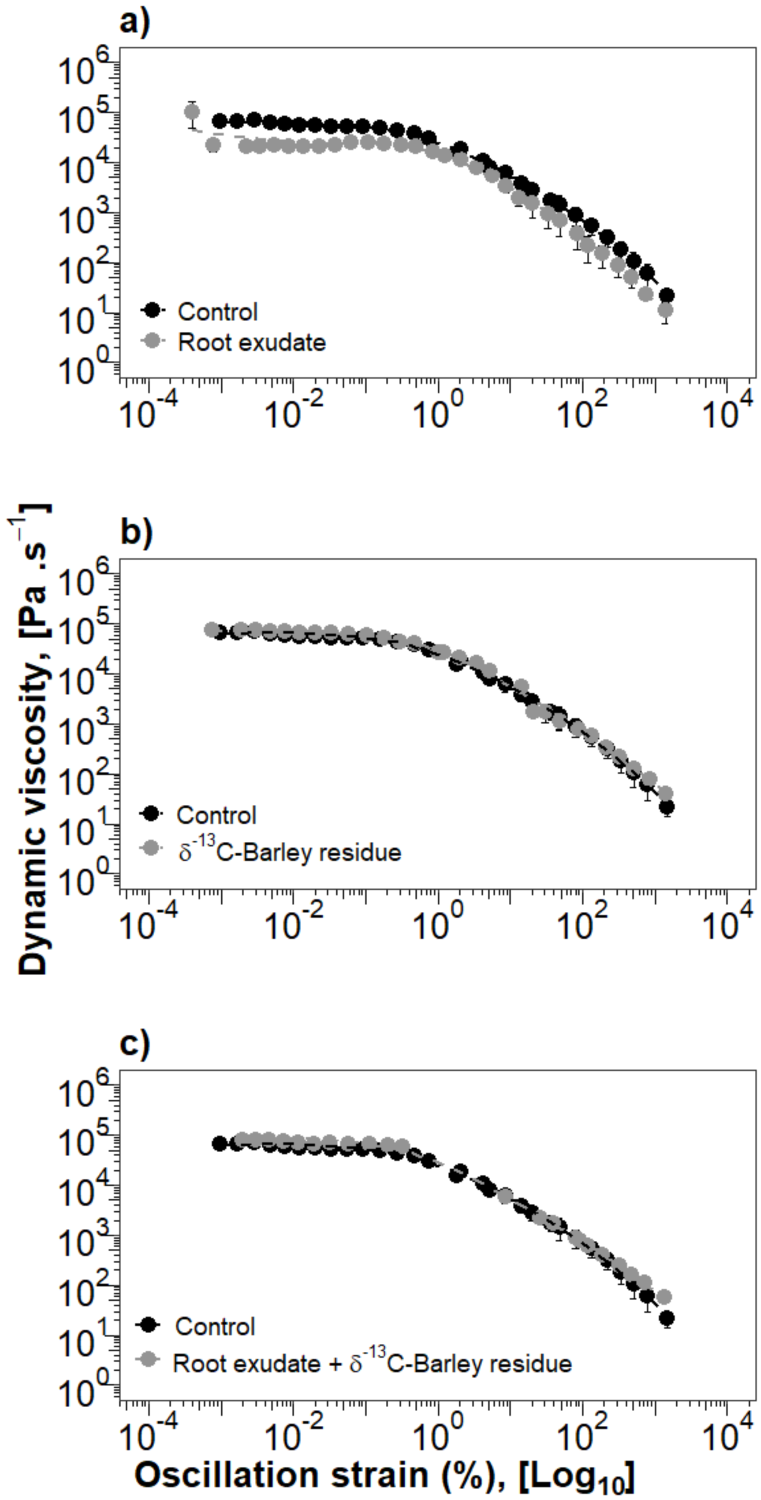

Figure 3: Dynamic viscosity, η (Pa.s), as a function of oscillation strain for different treatments before decomposition (fresh). Dynamic viscosity for root exudate treatment is significantly lower compared to the control.

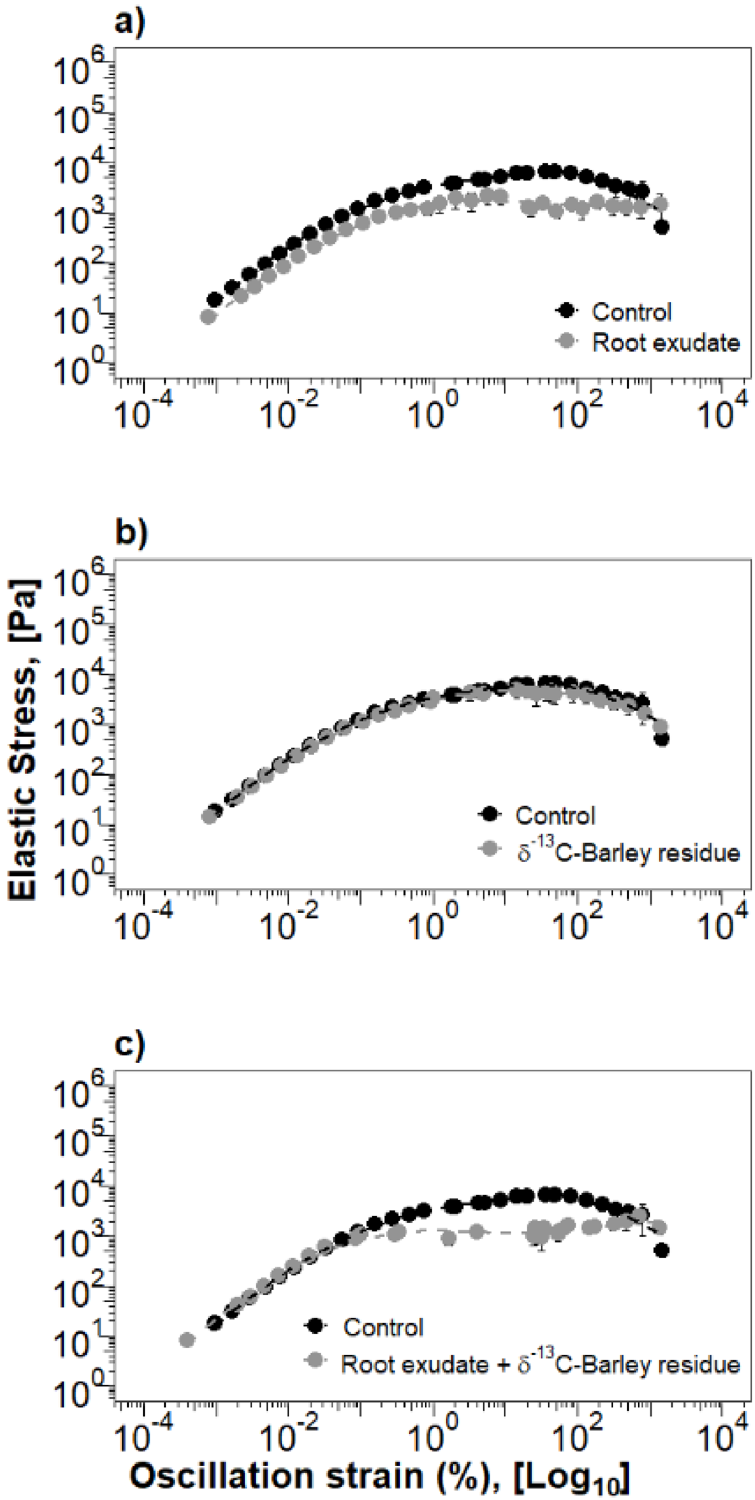

Figure 4: Yield stress, γ or σ_y (Pa), as a function of oscillation strain for different treatments before decomposition (fresh). Yield stress for root exudate treatment and root exudate + δ^{13}C-barley residue treatment is significantly lower than the control treatment.

Table 2. Mean values of interaction effects for root exudate and δ^{13}C-barley residue on rheology properties for sandy loam soil (< 500 µm).

		Rheology parameters									
		γ (Pa)		η (Pa.s)		**G″** (Pa)		**G′** (Pa)		**Tan δ** **(-)**	
Soil	**Treatments**	**LSMean**	**Group**	**LSMean**	**Group**	**LSMean**	**Group**	**LSMean**	**Group**	**LSMean**	**Group**
Fresh	Control	6175	a	51324	a	380262.33	a	1985221.67	a	0.2002	a
	δ13C-barley residue	4791	a	59375	ac	437163.50	ac	1934243.33	ac	0.2257	ac
	Root	2237	b	24936	b	285325.58	b	1477315.00	b	0.1877	b
	Root exudate + δ13C-barley residue	1753	b	68131	c	469927.33	ac	2120630.00	ac	0.2214	ac
Decompose	Control	3560	bc	65233	acd	482593.67	ac	1819866.67	ac	0.2601	c
	δ13C-barley residue	4959	ac	67410	c	524966.33	ac	1856661.67	ac	0.2796	c
	Root	4512	ac	82523	d	641410.17	c	2322755.00	c	0.2760	c
	Root exudate + δ13C-barley residue	4039	ac	79027	cd	605223.83	c	2120450.00	ac	0.2848	c

LSMean = Least Squares Mean, **γ** or σ_y = Yield stress, η = Dynamic viscosity, G″ = Loss modulus, and, G′ = Storage modulus

Root exudate compounds initially weaken the soil by reducing its resistance to deformation (Figures 3a and 4), but after 12 days of incubation and microbial decomposition, the soil structural resistance deformation stress was quickly increased for soils treated with root exudate compounds (Figures 5b). After decomposition, the dynamic viscosity (η) at 0.1% oscillation strain for soil treated with root exudate compounds increased ($P < 0.05$) by 331% compared to that of pre-decomposition (Figures 6). In a similar manner, soils treated with root exudate compounds showed an increase ($P < 0.05$) in yield stress after decomposition. The yield stress for soils treated with root exudate compounds alone and root exudate + δ^{13}C-barley residue treatments was increased by 201% and 230% after decomposition as compared to that of pre-decomposition, respectively (Figures 7b and 7d). Both dynamic viscosity and yield stress for the control/unamended soil and δ^{13}C-barley residue treated soil were not significantly different after decomposition/incubation (Figures 6 and 8). A comparison of dynamic viscosity and yield stress for all samples after decomposition/incubation, did not show significant differences between the treatments (Figures 8).

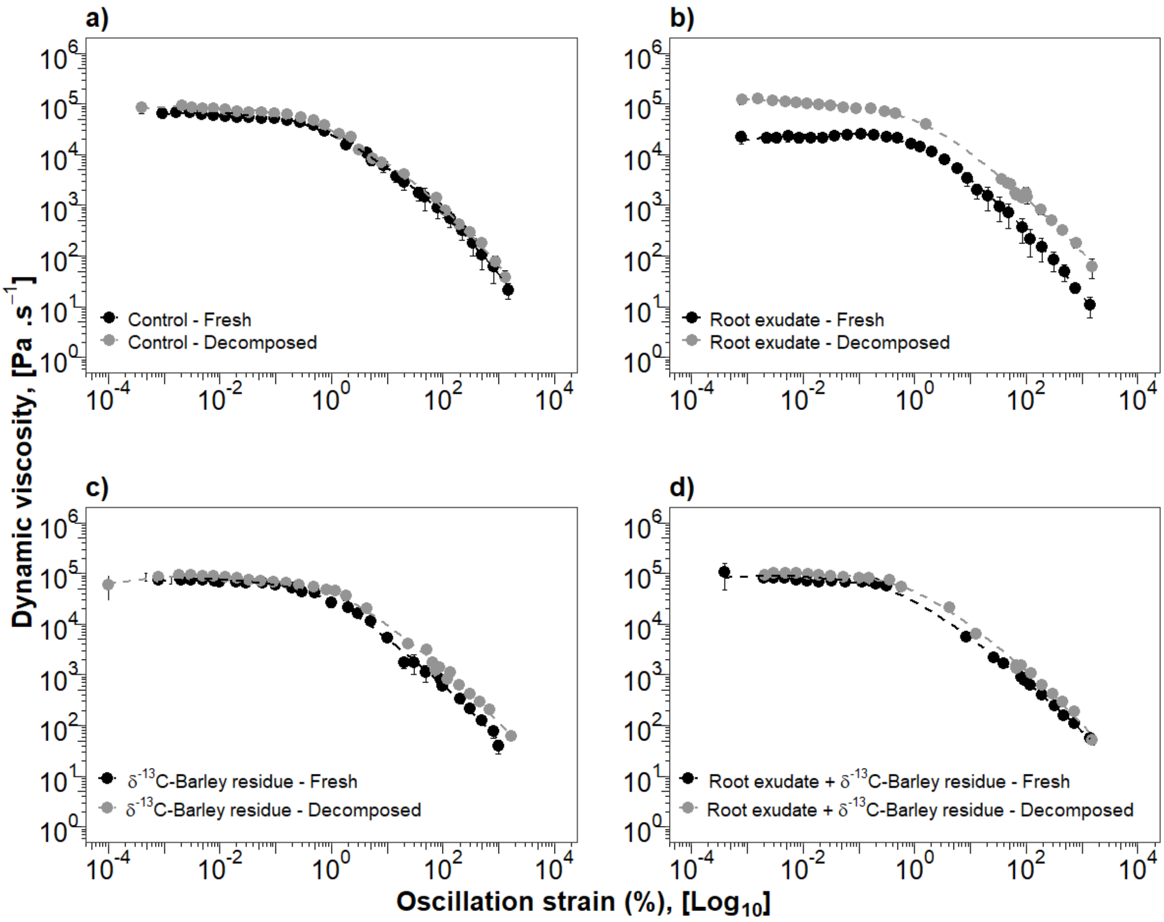

Figure 5: Dynamic viscosity, η (Pa.s), at 0.1% oscillation strain before and after decomposition for different treatments.

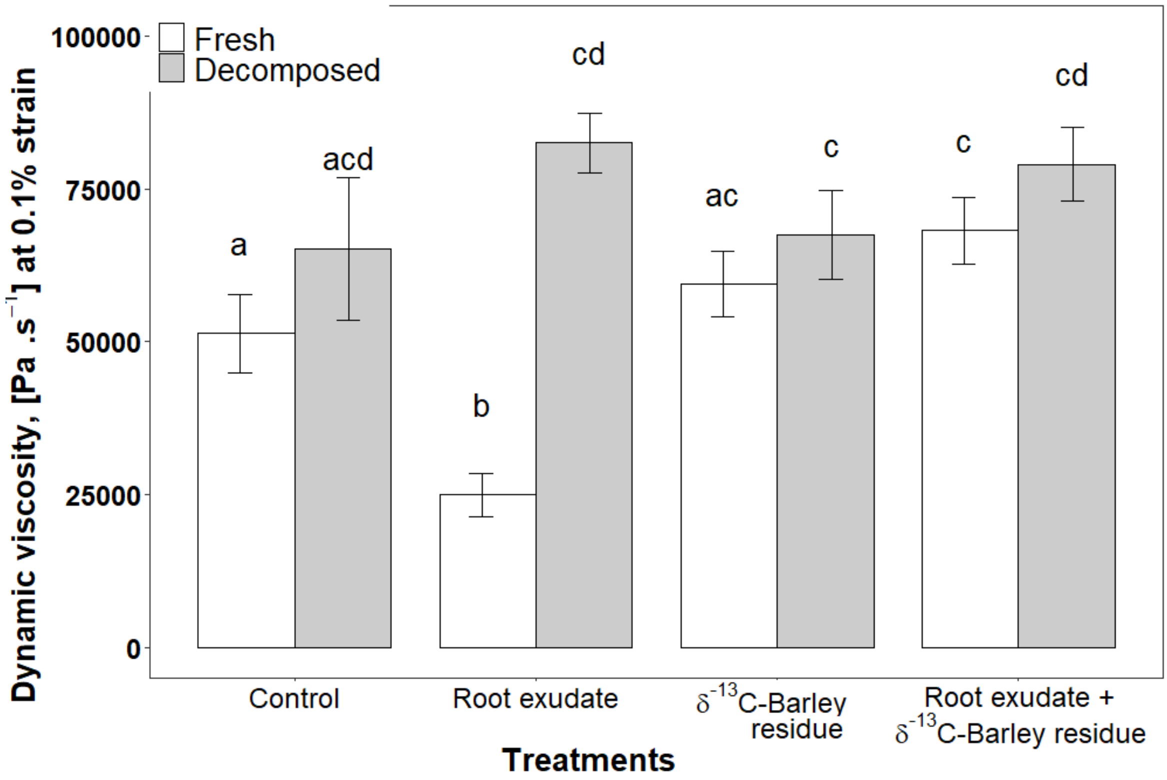

Figure 6: Dynamic viscosity, η (Pa .s-1), as a function of oscillation strain for different treatments (comparison between before and after decomposition).

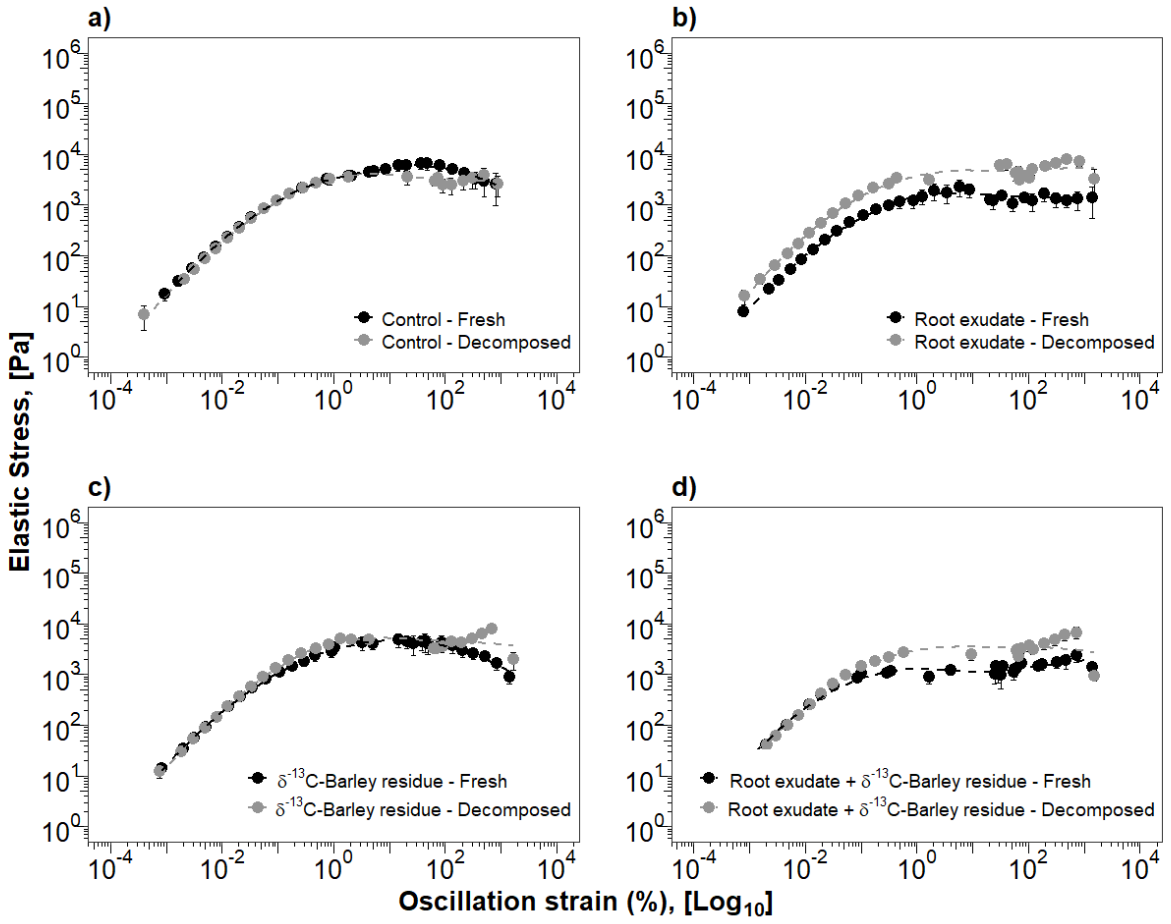

Figure 7: Yield stress, σ_y (Pa), as a function of oscillation strain for different treatments (comparison between before and after decomposition).

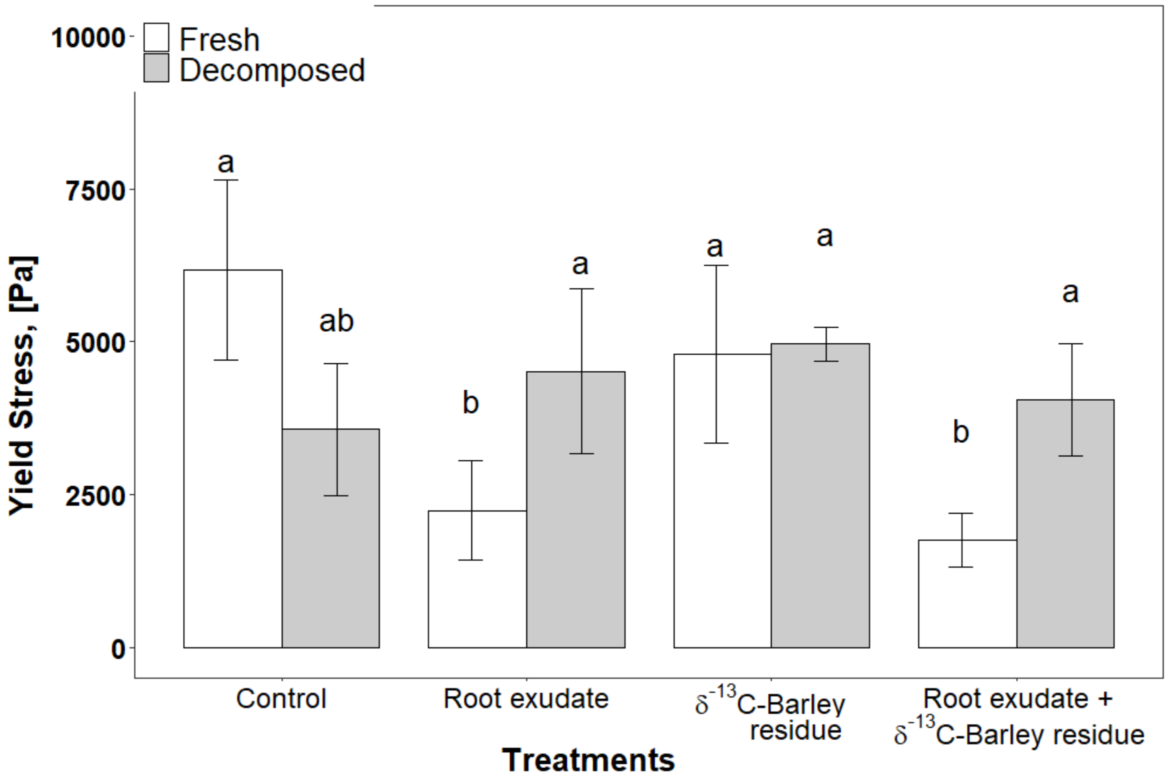

Figure 8: Yield stress, σ_y (Pa), before and after decomposition for different treatments.

Discussion

Soil mineralisation

Daily mean CO_2 emitted over 12 days showed no significant variations among the treatments. A possible reason is that sieving the soil to a particle size of less than 500 µm liberated so much of the native carbon that our amendments were insignificant. Another possible reason could be the state of the soil microbial community before the treatments since the soil was air-dried to about 1% moisture content. The isotopic ^{13}C-CO_2 emitted was significantly higher for the $\delta^{13}C$-barley residue treatments with and without root exudate compounds reflecting the impact of readily available carbon on the mineralization.

Soil water retention

The soil with root exudate treatment retained significantly less water compared to unamended soil and soil amended with $\delta^{13}C$-barley residue. Relatively larger amounts of organic acids and fewer free and polysaccharide-derived sugars present in the root exudate compounds could have been the reason for the decreased water retention in the soil (Naveed *et al.*, 2017). This suggests that root exudate compounds act as surfactants reducing the surface tension of the soil water and consequently the amount of water retained at any matric potential. These results are in agreement with Naveed *et al.*, (2019) for the case of barley root exudate. These authors reported that soil with barley root exudate amendment retained significantly less water whereas the soil with maize root exudate and chia seed exudate amendments retained significantly more water than the unamended soil. Our results are also in agreement with Whalley *et al*. (2005) who reported that the rhizospheres of both maize and barley tended to be drier at a given matric potential than the bulk soil. In contrast, various other studies have observed that microbial polysaccharides and root mucilages increase water content by acting as a hydrogel (Ahmed *et al*. 2014; Moradi *et al*. 2012). This reflects that soil water retention in the rhizosphere depends on the physico-chemical characteristics of root exudates and their origin. There is a need to understand how this rhizosphere water dynamics influenced by root exudates would impact root water uptake. Kroener *et al*. (2014) showed that mucilage increased the rhizosphere water content, however, simulations were conducted with chia seed mucilage with high viscosity. In this study, soils amended with δ^{13}C-barley residue and root exudate + δ^{13}C-barley residue retained significantly higher water compared to the unamended soil (Table 2). This is in agreement with several studies carried out on the impact of organic amendments on soil water retention (Ankenbauer and Loheide, 2017; Blanco-Canqui *et al*., 2015; Naveed *et al*., 2014; Olness and Archer, 2005). After 12 days of incubation, retention of soil water slightly decreased compared to pre-incubation values for all the soil amendments. This is logical and might be caused by the loss of soil organic matter due to mineralization.

Soil rheological behaviour

The dynamic viscosity and yield stress measured at -2 kPa matric potential for soil treated with root exudate compounds were significantly lower compared to that of unamended soil. This is despite significantly lower water content for the root exudate-treated soil. This suggests that the addition of root exudate compounds dispersed soil by changing interparticle bonding. The possible reason could be anions of organic acids, present in large amounts in root exudate compounds, might be adsorbed onto the mineral soil particles, which might in turn increase the net negative charge of clays and result in greater clay dispersibility (Shanmuganathan & Oades, 1983). This is in agreement with Naveed *et al*., (2017) who reported that barley root exudate significantly dispersed the soil at the onset. The dispersion of soil due to the amendment of root exudate compounds could potentially promote root growth and increase the release of nutrients and carbon from the soil through the exposure of new particle surfaces. However, the dispersion of soil due to root exudate compounds is not universal and depends on the physical and chemical characteristics of the root exudate compounds (Naveed *et al*., 2017, Traoré *et al*., 2000; Morel *et al*., 1991). For example, Naveed *et al*., (2017) reported that maize root exudates and chia seed mucilage gelled the soil at the onset. Similarly, Barré and Hallett (2009) show that root exudate compound polygalacturonic acid (PGA) increased the viscosity of clays considerably. The dynamic viscosity and yield stress for soil treated with δ^{13}C-barley residue were not significantly different from that of unamended soil. A possible reason is that the reactivity of the δ^{13}C-barley residue due to its surface area increased the soil structural resistance and its susceptibility to deformation stress.

Incubation had a greater impact on soil rheological behaviour for the soil amended with root exudate compounds. The initial decrease in dynamic viscosity and yield stress at the onset of the root exudate amendment was recovered after 12 days of incubation. Both dynamic viscosity and yield stress were not significantly different among the treatments after the incubation period. The observed increase in dynamic viscosity and yield stress for root exudate amendment following the incubation period suggests that microbial decomposition stabilizes the soil, and the observed role of root exudate compounds to disperse soil was rather short-lived. The resistance to displacement increased for the soils following incubation, which may be associated with microbial activity. Furthermore, the weak mechanical characteristics of fresh soils were reversed for all treatments after microbial decomposition, and agree with findings by Naveed *et al*. (2018) who showed that incorporation of barley root exudate in soil resulted in an immediate weakening and dispersion of aggregates, followed by an increase in aggregation due to microbial decomposition. Brax *et al*. (2020) showed that the mineralisation of organic carbon compounds in soils affects interparticle bonding by substituting ions in exchange sites. Liang *et al*. (2006) and Omari *et al*. (2017) found that the increases in cation substitution at charge surfaces are driven by microbial activities which markedly increase the absorption of organic matter to particle surfaces, resulting in meaningful increases in the absorption of negatively charged cations followed by a drop in zeta-potential, a key indicator of the stability of colloidal dispersions (Hanaor *et al*., 2012). In addition, Alazigha *et al*. (2018) found that changes in cationic exchange properties at clay particle surfaces from microbial decomposition of organic carbon prompted flocculation, which could be the reason for the stability of soil amended with root exudate compounds after incubation. It may have been that the complex compounds produced from microbial decomposition of organic compounds had a greater impact on soil stabilization and may account for the large increases obtained in dynamic viscosity for soils amended with root exudate after decomposition. This suggests that microbial decomposition increased soil resistance and exhibited a greater range in yield stress than the range found on fresh soils (Figure 8). tanδ (G''/G') represents the quality or stiffness of soil structure following the application of stress. tanδ increased on incubation/decomposition irrespective of the treatment (Table 2). This means that following decomposition soil aggregate and structural resistance to deformation stress is enhanced.

Conclusions

The impact of root exudate compounds and $\delta^{13}C$-barley residue on the micro-mechanical properties of soils at -2 kPa matric potential enhances our understanding of the processes driving soil structure formation and stabilization in the rhizosphere by serving as a model system to understand the impact of plant root exudate, decomposition dynamics and the rhizosphere formation pathway under wet conditions. Our results highlight the significant effect of root exudate on soil structural stability, through reduced rate of micro-mechanical behavior before microbial decomposition of these substrates. Specifically, the yield stress and dynamic viscosity for soil treated with root exudate compound at the onset, reflect the weakening and dispersion effect of root exudates on soil aggregates which is important for root growth and access to protected nutrients within the aggregates, although this effect is reversed following decomposition of the exudate. The values for soil micro-mechanical properties observed following the application of root exudate + $\delta^{13}C$-barley residue treatment alone at both stages of decomposition are important as it shows that root exudate compounds and the associated soil microbial activities are essential to predict how soils amended with organic residue may respond structurally to mechanical stress.

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