The rheology of rhizosphere formation by root exudates and soil microbes

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Abstract
One of the major inputs driving the formation of soil structure is exudation from plant roots. We used approaches from rheology to test the hypothesis that root exudates initially disperse soil, thus easing root penetration and releasing nutrients, followed by gelling (i.e. aggregation) if the compounds are transformed by soil microbes. Soils were amended with 0, 1.5 and 15 mg/g soil of root-exudate compounds consisting of a mix of sugars, amino acids and organic acids and incubated for 13 days at either 2ºC, to reduce microbial action, or 16ºC. Incubation at 2ºC significantly suppressed respiration rates compared to 16ºC, suggesting microbial processes were impaired. A parallel plate rheometer was used to quantify the rheological behaviour of the incubated soils amended with root-exudate compounds. The drop in flow point (stress where breakdown occurs) at 2ºC incubation and rise in flow point at 16ºC incubation suggests dispersion by the root exudates followed by gelling if root exudates are transformed by microbes.

Key Words
Rhizosphere, rheology, root-exudates, microbiology, aggregation.

Introduction
Plant root exudates and their mineralization by soil microbes drive the formation of the distinct structural zone around roots termed the rhizosphere (McCully 1999). The rhizosphere has enhanced particle aggregation, microbial biomass and water retention compared to bulk soil, which is critical to the functioning of plants as these processes impact fluxes of nutrients, water and oxygen (Hinsinger *et al.* 2009). Our research is examining how root exudates and their transformation by soil microbes influence the rheological behaviour of wet soil. Rheology is the study of the flow of matter and it should help quantify the underlying processes driving soil structure formation and stabilisation. We hypothesise that root exudates initially disperse soil, thus easing root penetration and releasing nutrients. Soil microbes then transform exudates into biological glues that gel soil. To test this hypothesis we measured the rheological behaviour of soils equilibrated to a range of water contents and amended with root-exudate compounds. Incubations of the soil at 2ºC and 16ºC were used to control microbial activity.

Methods
In this preliminary paper we report data from one soil, but data on more soils will be collected before the World Congress of Soil Science 2010. A Eutric Cambisol derived from undifferentiated sandstone, with 71% sand, 19% silt and 10% clay, pH (H_2O) 6.2, 1.9% C and 0.07% N was sampled from 0-100 mm depth. It was from Bullion Field, situated at the Scottish Crop Research Institute, Dundee. The soil was air-dried and then passed through a 400 μm sieve.

Soil was first pluviated dry into 3 rings and then water retention at 0, -5, -10, -20 and -50 kPa potentials was measured on a tension table. From these data we selected a water content of 0.43 g/g because it occurred at a water potential (-10kPa) and water filled pore space (75%) that would minimise water and aeration stresses to microbes.

Larger air-dry soil samples of 600 g were then wet to 0.23 g/g^1 using a fine mist and incubated at 16ºC for 2 weeks to allow the microbial community to re-establish and to mineralise carbon released from drying. The sample was then divided into three and amended with 0, 1.5 or 15 mg C/g soil of root-exudate compounds. The compounds were a mix of sugars, amino acids and organic acids that are found in natural root exudates (Paterson *et al.* 2007). Soil subsamples were further subdivided and then sealed in 1 litre Kilner jars fitted with a Suba-seal in the lid to allow gas sampling. Three replicates of each amendment rate were incubated at 16ºC, which is the typical local soil temperature in summer, and another three replicates at 2ºC to suppress microbial activity. The jars were vented regularly and carbon dioxide measured in the headspace on days 1,

1 – 6 August 2010, Brisbane, Australia. Published on DVD.

3, 7, 10 and 13 with a syringe sample injected into a gas chromatograph. After 13 days incubation, the soil from each jar was divided into subsamples to measure rheology and microbial properties. Only respiration and rheological data will be presented here.

The rheology subsamples were subdivided and wet to water contents ranging from no added water (stiff, aggregated soil) to a flowing liquid. The samples were mixed thoroughly and placed in a sealed bag for 24 h at 2°C to improve equilibration and minimize biological activity. A Haake MARS Parallel Plate Rotational Rheometer (Thermo Scientific, Waltham, MA, USA) using 35 mm diameter serrated stainless steel plates and a 2 mm gap setting provided measurements of rheological behaviour. Samples less than 10 g were needed. A stress controlled amplitude sweep test with an oscillation frequency of 50Hz and stress ramp from 0.1 to 10^4 Pa was used. Data on zero-shear viscosity and flow-point were used to describe rheological behaviour. Zero-shear viscosity is the resistance to flow of an unstressed material. With increasing stress, shear-thinning decreases the viscosity of materials like soil until structural collapse occurs at the flow-point. Soil water content was measured for each sample tested in the rheometer.

The viscosity and flow point were plotted against water content for all experimental treatments. Linear regression with groups was used to assess differences between treatments following a log-log transformation. Analysis of variance was used to assess differences in respiration rates between treatments.

Results and Discussion

The amount of added root-exudate compounds and incubation temperature had significant effects on soil respiration ($P<0.001$; Figure 1). Incubation at 2ºC resulted in about 3% of the respiration at 16ºC for the largest amendment rate. From these results, the microbial transformation of root-exudate compounds at 2ºC was only a small fraction of the total, leaving most of the root-exudate compounds in their original form. The impact of the root-exudate compounds alone can thus be taken as the 2ºC results and microbially transformed root-exudate compounds as the 16ºC results. For the 15 mg/g amendment rate, root-exudate compounds caused a large reduction in Flow Point at 2ºC and a large increase at 16ºC ($P<0.001$). This suggests that root-exudates cause dispersion followed by gelling once the compounds are transformed by soil microbes. These data are different from Barré and Hallett (2009), who found the model root exudate polygalacturonic acid gelled soils, but this is likely due to the different chemical properties of the exudates. They also found that a fungal exudate gelled soil, which corresponds with our results.

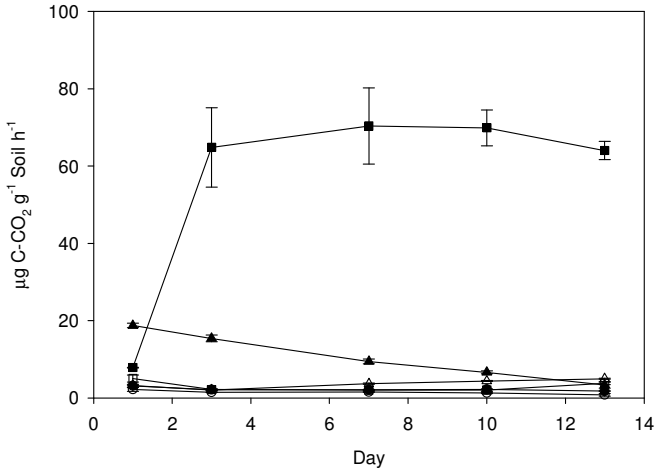

Figure 1. Respiration rate for soils amended with 0 (circle), 1.5 (triangle) and 15 (square) mg/g soil of root-exudate compounds incubated at 2ºC (hollow) and 16ºC (solid).

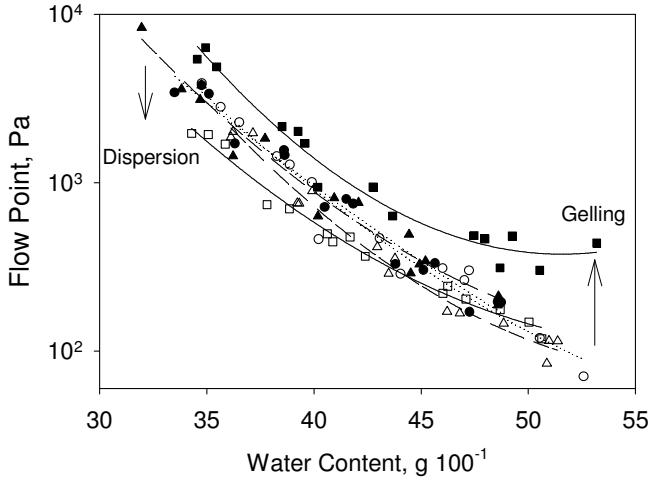

Figure 2. Flow Point for soils amended with 0 (circle), 1.5 (triangle) and 15 (square) mg/g soil of root-exudate compounds incubated at 2ºC (hollow) and 16ºC (solid).

The added root-exudate corresponds to daily (1.5 mg/g soil) and total (15 mg/g soil) amounts that would be expected from a growing root (Paterson *et al.* 2007). Moreover, the complexity of the compounds provides a realistic surrogate for real root-exudates that are very difficult to collect in the volumes required for mechanical testing. However, future research will collect rhizosphere soil and attempt to harvest real root exudates to test whether similar effects are found. Barré and Hallett (2009) found the impact of exudates on rheology was dependent on clay mineralogy and soil texture, and Tarchitzky and Chen (2002) showed the rheology of dilute soil suspensions was pH dependent, so both will be tested using exudates and soil water contents more typical of natural conditions.

Conclusion

We have demonstrated that root exudates initially disperse soil followed by gelling once transformed by soil microbes. Quantification of the underlying mechanical process using rheology provides data that will be useful in understanding and modeling the development of rhizosphere soil structure. The initial dispersion by root-exudate compounds may lead to particle reorientation that eases root penetration, releases previously inaccessible nutrients and the formation of new aggregates. Microbial transfromation of root-exudate compounds gels soils, which along with accentuated cycles of wetting drying at the root-soil interface, forms the very stable and structurally distinct zone of soil termed the rhizosphere.

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