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RESEARCH ARTICLE

Mucilage addition increases aggregate stability and tolerance of microbes to soil drying

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Abstract

Background and Aims Polysaccharide-rich mucilage from plant roots alters soil structure and microbial microhabitats, potentially modifying microbial responses to drying and rewetting. Here we tested the hypotheses that mucilage strengthens soil aggregates, increases microbial survival during dry–rewet cycles, and alters respiration responses to drying and rewetting.

Methods To distinguish effects arising from the physical and biophysical properties of mucilage from those due to added carbon, we compared soil amended with 0.1 mg g^{-1} mucilage with soil receiving the same amount of carbon as glucose. Half of the soils were kept well-watered, while half were dried slowly over 24 days, rewet and monitored for a further 20 days. We adopted an integrative approach linking physical properties (aggregate stability) with microbial responses (PLFA) to mechanistically scale from microbial microhabitats to survival and respiration.

Results Mucilage degraded quickly; nevertheless, at minimum water content the extracellular polysaccharide content of mucilage-amended soil was more than twice as large as soils receiving glucose. Wet sieving indicated mucilage amendment and dry-rewet cycle increased aggregate stability. Respiration averaged 12% faster in mucilage-amended than glucose-amended soil. At minimum water content in the dry–rewet treatment, the PLFA pool was 50% larger in mucilage-amended soil, indicating increased microbial survival under water deficit.

Conclusion We conclude that mucilage, for example from plant roots, has complex effects on soil that extend beyond that of a physical binding agent and also include significant effects on microbial survivorship and activity across dry-rewet cycles.

Keywords Birch effect · EPS · Microbial biomass · Microbial survival · Polysaccharide · Respiration · Soil structure

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Introduction

Soils in many natural and managed ecosystems regularly undergo periods of drying followed by rewetting. Drying and rewetting cycles influence microbial activity, biogeochemical cycles including of carbon (C) and nutrients (Fierer and Schimel 2002), and the formation or breakdown of soil aggregates (Dexter 1988). The mechanistic drivers of these effects occur at the small scale of microbial microhabitats, yet they scale up to affect plant growth and ultimately ecosystem productivity via influence on nutrient availability and soil biophysics. The effect of dry-rewet cycles on the C cycle are of special interest owing to their tight link with microbial activity and their global significance. Microbial activity and respiration slow as soil dries, then rapidly increase when soil is rewet with rates of respiration often remaining above those of well-watered controls for one or more days. These large pulses of respiration after re-wetting a dry soil have been known for a century (e.g. Lebedjantzev 1924; Birch 1958, 1964; Bottner 1985) and are known as the "Birch effect". The effects of dry–wet cycles on soil are globally significant because they can account for 25% or more of annual C fluxes in some ecosystems (Reichstein et al. 2002; Carbone et al. 2011; Yan et al. 2014), and can increase cumulative respiration (Fierer and Schimel 2002) and decrease soil C pools (Birch 1958) relative to soils at constant water content.

The effect of drying and rewetting on respiration is underpinned by the activity and population dynamics of soil microbes and substrate availability. In general, prolonged soil drying increases microbial mortality (Kieft et al. 1987; Blazewicz et al. 2014), such that microbial biomass is reduced (Kieft et al. 1987; Warren 2020a) and the surviving microbes may have altered community composition (e.g. Fierer et al. 2003; Barnard et al. 2013; Evans and Wallenstein 2014; de Nijs et al. 2019). After rewetting there is an increase in substrate availability that supports increased microbial activity and respiration. A variable and sometimes small fraction of the increased respiration can be accounted for by microbial osmolytes and extracellular depolymerisation products that accumulate in dry soil and then become available after rewetting (Boot et al. 2013; Warren 2016; Slessarev et al. 2020; Warren and Manzoni 2023). Much of the C respired after rewetting is likely "old C" that is released from physical protection by rewetting physically disrupting soil (Schimel et al. 2011; Homyak et al. 2018; Schimel 2018; Brangarí et al. 2021; Singh et al. 2023). This is supported by studies showing dry-rewet cycles breakdown aggregates (Denef et al. 2001; Cosentino et al. 2006; Kaiser et al. 2015; Singh et al. 2023), with analysis of C content of different-sized aggregates suggesting aggregate breakdown releases C from protection and partially supports the Birch effect (Singh et al. 2023).

Two factors that underpin microbial responses to drying and rewetting, namely soil aggregate strength and microbial microhabitat, are potentially altered by polysaccharide-rich mucilage from plant roots. Roots of most plant species produce mucilage continuously from the root cap, with mucilage often accounting for half of root exudates (Chaboud 1983; Nazari et al. 2022). Mucilage alters the chemical, hydraulic, and physical environment of the rhizosphere (Czarnes et al. 2000; Landl et al. 2021; Nazari et al. 2022; Rosskopf et al. 2022), which leads to direct effects on plants (e.g. water uptake and root growth) and a range of microbially-mediated effects on plants (e.g. via altered nutrient availability) and ecosystems (e.g. soil respiration). At the macroscopic scale, polysaccharide-rich mucilage functions as a binding agent that strengthens soil aggregates, which ought to decrease the amount of old C released by rewetting and consequently lead to a smaller respiration pulse upon rewetting. This reasoning is supported by studies showing incorporating maize root exudate in soils increased soil aggregate stability (Morel et al. 1991) and similar results from experiments with two functional analogues of mucilage, polygalacturonic acid and xanthan. Polygalacturonic acid is a linear polymer of galacturonic acid that closely resembles many plant mucilages, while xanthan is a microbial polysaccharide with a cellulose-like backbone and charged side chains. They both share key functional features of mucilage such as high viscosity, strong water retention, and the presence of acidic groups. When polygalacturonic acid or xanthan are added to soil they increase tensile strength and stability against the disruptive effects of wetting and drying cycles (Czarnes et al. 2000). Likewise, chia seed mucilage stabilises soil (Di Marsico et al. 2018; Naveed et al. 2019), including soil exposed to dry-rewet cycles (Zhong et al. 2021). At the scale of microbial microhabitats, extracellular polysaccharides can increase

water-holding capacity and/or water retention (Roberson and Firestone 1992), enable maintenance of liquid bridges and hydraulic connectivity (Read et al. 2003; Carminati et al. 2017) such that microbes avoid or delay experiencing water deficit by being embedded in biofilm (Bérard et al. 2015; Benard et al. 2023). Collectively these should result in greater activity in drying soil (e.g. via liquid bridges and maintenance of hydraulic connectivity Read et al. 2003; Carminati et al. 2017)such that there is a smaller accumulation of depolymerisation products during drying. In parallel there might be less osmolyte accumulated because microhabitat water potential is higher (less negative), and osmolyte accumulation is driven by negative water potentials (Kempf and Bremer 1998). In both cases, the smaller pool of C accumulated during drying would result in a smaller respiration pulse upon rewetting. Taken together, the ability of polysaccharides to increase soil aggregate strength and facilitate microbial activity and survival under water deficit are expected to decrease the size of the respiration pulse upon rewetting.

Previous studies have examined in isolation how mucilage and related polysaccharides influence soil aggregation, or microbial activity or water relations. As a result, it remains unclear how mucilage-mediated changes in soil physical structure and microbial survivorship interact to shape microbial responses to drying and rewetting, and whether these microscale effects scale up to influence the Birch effect. Here we tested the hypothesis that polysaccharide-rich mucilage a) strengthens soil aggregates, b) enhances survival of microbes under water deficits, and c) alters the response of respiration to dry-rewet cycles. To do this we used a plant-free system to isolate the effects of mucilage on microbes and soil aggregates and respiration without confounding influences from roots, cognisant that in an intact plant-soil system the effect of mucilage on microbes and soil cascade upward to influence processes relevant to plants such as nutrient supply, root penetration and hydraulic continuity between roots and soil. We applied mucilage to soil at 0.1 mg g^{-1} as an intermediate, field-relevant concentration within the broad range of values reported in the literature. For context, our chosen concentration of 0.1 mg g^{-1} is somewhat greater than some estimates (e.g. the theoretical value of 0.056 mg g^{-1}: Holz et al. 2018), but less than other estimates, (e.g. the 1 mg g^{-1} average for 1.5 mm from root surface: Carminati and Vetterlein 2012) and falls within the range of mucilage concentration added to soil previously, e.g. 0.046 to 4.6 mg g^{-1} (Naveed et al. 2018); 1.15 and 5.75 mg g^{-1} (Zhong et al. 2021); 0.02 to 2 mg g^{-1} (Rosskopf et al. 2022). These published estimates of mucilage concentrations span an order of magnitude at least partly because exudates are difficult to quantify reliably in soil and are sensitive to methodological choices (Tiziani et al. 2021; Trevisan et al. 2024). Recognising this uncertainty, we opted to add to soil an intermediate concentration of mucilage. To determine the effect of mucilage on soil, independent of those due to C addition, we compared mucilage-amended soil with soil receiving the same amount of C as glucose. The logic behind this design is that it controls for effects of C addition on, for example, microbial respiration and growth, such that one can attribute the additional effects of mucilage to its polysaccharide structure and associated effects on soil aggregation and microbial microhabitat.

Materials and methods

Soil

The effect of added mucilage on responses to drying and rewetting were examined using soil obtained from a long-term study previously used to examine drying-rewetting responses (Warren 2014, 2016, 2020a; Warren and Manzoni 2023). In June 2009 the intact soil was obtained from A horizon of an abruptic lixisol derived from shale and alluvium. It was planted with perennial native grasses *Themeda triandra* Forssk. and *Microlaena stipoides* (Labill.) R.Br and received adequate water for 14 years. The manipulative experiments described here were carried out under laboratory conditions with approximately 70 kg (dry weight equivalent) of soil collected from 0–15 cm. Visible roots were removed, then the plant-free soil was transported to the laboratory and slowly dried over ten days to 35% of water-holding capacity. The soil had sandy loam texture, pH (H_2O) of 6.5, total N of 0.08%, total C of 0.9%, 0.5 M K_2SO_4-extractable DOC of 22 µg g^{-1}.

Mucilage extraction

Root mucilage is difficult to extract in sufficient quantities for a large-scale soil experiment, so we instead used mucilage from seeds of chia (*Salvia hispanica* L.) because it can be extracted in large quantities and has been commonly used as an analogue of root mucilage (Di Marsico et al. 2018; Naveed et al. 2019; Zhong et al. 2021). Chia mucilage and root mucilage are both dominated by galacturonic acid-rich polysaccharides. Minor differences may occur, such as molecular weight, but their overall polysaccharide composition is sufficiently similar to support the use of chia mucilage as a practical analogue in soil experiments. Chia seed and ultrapure water at 1:20 (mass:mass) ratio were stirred for 2 h at 50 °C and subsequently filtered through cheesecloth. The filtrate (hereafter referred to as mucilage) was free of particulates, viscous and lightly coloured —consistent with expectations for polysaccharide-based mucilage. To determine dry matter content a sub-sample of known volume was dried at 40 °C for 24 h, another subsample was used to determine purity, while the remainder was stored at 2–4 °C for no more than five days before addition to soil. Prior to experimentation we confirmed the purity of mucilage. A subsample was hydrolysed (4 M trifluoroacetic acid), derivatised (methoxyamine-trimethylsilyl) then analysed by GC–MS (Warren 2014), using the same workflow as for EPS that is described in supplementary information. This approach is appropriate for assessing purity of hydrolysed mucilage because it can detect hundreds of water-soluble metabolites from a broad range of compound classes (e.g. small carbohydrates, amino acids, organic acids). The purity of the mucilage was confirmed by GC–MS identifying monosaccharides characteristic of polysaccharide hydrolysates (galactose, glucose, fucose, rhamnose, mannose, arabinose, xylose, galacturonic acid, glucuronic acid) but no other metabolites.

Experimental design

Our experiment compared a well-watered control soil with soil that was slowly dried over 24 days then rewatered and monitored for a further 20 days (Fig. 1). For the well-watered and dry-rewet treatments there were six replicates receiving ~ 0.1 mg g^{-1} mucilage and six replicates receiving the same amount of C as glucose (i.e. mucilage vs glucose * well-watered vs dry-rewet * 6 replicates = 24 mesocosms per experiment). Glucose was used as procedural control because it is a ubiquitous small carbohydrate that lacks the polymeric structure, viscosity, and water retentive properties of mucilage. Three separate experiments were run in parallel to provide samples for 1) measurements of soil respiration with a tuneable diode laser, 2) extraction and chemical analysis, and 3) wet sieving to determine size-distribution of water stable aggregates. The parallel experiments were set up from a single homogenous aliquot of soil at a gravimetric water content of 0.07 g g^{-1} (35% of water-holding capacity). Approx 850 g (dry weight equivalent) of soil was added to each of 72 1.0 L glass mesocosms (Aussie Mason wide mouth jar, Queensland, Australia) at bulk density of 1.35 g mL^{-1}. After one week, half of the mesocosms each received 60.0 mL of 1.5 mg mucilage mL^{-1} and half received 60.0 mL of 1.5 mg glucose mL^{-1}. This resulted in mucilage and glucose concentrations of 0.107 ± 0.003 mg g^{-1} dry soil (mean ± sd, n = 24). Glucose and mucilage both have the same carbon concentration (40% by mass), thus adding equal concentrations of glucose and mucilage ensured mesocosms received the same amount of C irrespective of amendment (36 mg per mesocosm). Solutions were injected evenly throughout the soil using a Cass needle with four radial holes. After addition of mucilage or glucose solution, soils had a gravimetric water content of 0.127 g g^{-1} (63.5% of water-holding capacity).

Two days after adding mucilage or glucose, half of the mesocosms were randomly assigned to the well-watered treatment and half to the dry-rewet treatment. The well-watered treatment was kept at mean gravimetric water content of 0.119 g g^{-1} (59% of water-holding capacity) throughout the 44 days of the experiment. This was achieved by weighing mesocosms every two days and periodically replacing the small amount of water lost via evaporation. Evaporation was minimised by fitting mesocosms with lids that were opened briefly every 1–2 days. The mesocosms of the dry-rewet treatment were allowed to slowly dry over 24 days by keeping open to the laboratory atmosphere. The drying treatment was imposed by allowing water to evaporate from the soil surface, giving rise to the possibility of vertical gradients in water content from top to bottom of mesocosms. We do not think there was

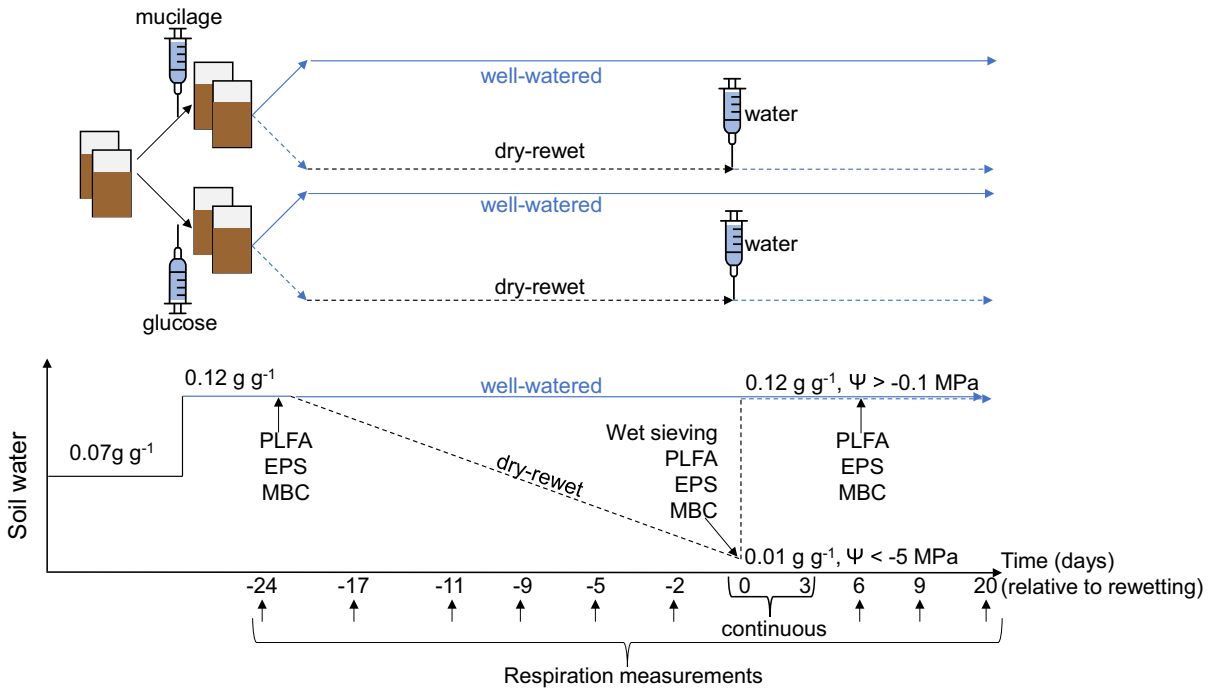


Fig. 1 Experimental design used to examine the effect of mucilage on the dry-rewet response of soil. Semi-dry soil with water content of 0.07 g g^{-1} gravimetric was brought into the laboratory, 850 g (dry weight equivalent) aliquots were installed in glass mesocosms at the approximate bulk density of field soil, let recover for seven days, then they were amended with C as mucilage or glucose which increased soil water to 0.12 g g^{-1}. After two days, half of the glass mesocosms were assigned to a dry-rewet treatment and were allowed to slowly dry over 24 days to a gravimetric water content of around 0.01 g g^{-1} then they were re-wetted to a gravimetric water content of 0.12 g g^{-1}. The remaining glass mesocosms were kept well-watered throughout. In the dry-rewet treatment, soil respiration was measured with a tuneable diode laser system continuously for two hours prior to re-wetting and three days thereafter, while additional measurements of one-hour duration were made on nine occasions (as indicated by arrows on fig). Extracellular polysaccharides (EPS), polar lipid fatty acids (PLFA), and microbial biomass carbon (MBC) were measured in well-watered soils two days after mucilage and glucose had been added to soils (T-24 days), in well-watered and dry-re-wet soils immediately prior to rewetting (T0 days), and on the dry-rewet soils six days after rewetting (T6 days). Wet sieving was used to assess size- distribution of water-stable aggregates on samples collected immediately prior to rewetting (T0). All measurements were made on six replicates, except PLFA were measured on four randomly chosen replicates

significant stratification in water content because there was no visual evidence of abrupt drying fronts and previous experiments showed negligible variation in water content at the top, middle and bottom of mesocosms at peak water stress, likely because soils were dried sufficiently slowly that hydraulic redistribution minimised gradients in water content. At the conclusion of 24 days of drying, the soil water content was 0.011 g g^{-1} (5.5% of water-holding capacity). Soils were subsequently re-wet to the same 0.119 g g^{-1} of the well-watered treatment by injecting water evenly throughout the soil using a Cass needle, then maintained at 0.119 g g^{-1} for the remaining 20 days of the experiment.

The frequency and/or timing of measurements differed between soil respiration, wet sieving and chemical analyses (Fig. 1). For soil respiration it was necessary to obtain many measurements over the time course to get an adequate picture of temporal dynamics and quantify cumulative respiration. For the well-watered treatment, respiration was measured on nine occasions throughout the 44 days of the experiment. For the dry-rewet treatment, measurements were made on the same nine occasions plus we obtained high temporal resolution measurement of re-wetting dynamics by measuring respiration every 7.5 min for two hours before re-wetting and three days after re-wetting. Chemical analyses were less frequent

owing to difficulty and expense, with timing of measurements chosen to maximise information content. Chemical analyses were made: a) on well-watered mesocosms two days after mucilage and glucose had been added (T −24) which was before watering treatments were imposed, b) on well-watered and dry-rewet soils when the dry-rewet soils were at peak water stress (T0), and c) on the dry-rewet treatment six days after rewetting (T6). Six days after rewetting was chosen based on previous studies with the same soil showing that the respiration pulse of re-wet soils has subsided by six days and reverted to the same value as seen in well-watered controls (Warren and Manzoni 2023). The size distribution of water-stable aggregates of well-watered and dry-rewet soils was determined when the dry-rewet soils were at peak water stress (T0).

Soil respiration

CO_2 efflux from the soil headspace (i.e. soil respiration) was quantified with a tuneable diode laser and manifold system (TGA100a, Campbell Scientific, Logan, UT, USA). For well-watered mesocosms daily averages were obtained on eight occasions (T-24, −17, −11, −9, −5, −2, +6, +20 days), while for dry-rewet mesocosms we obtained nine daily averages (T-24, −17, −11, −9, −5, −2, +3, +6, +9, +20 days) and continuous measurements with a 7.5-min resolution for the three days after rewetting mesocosms. To enable sampling of soil headspace the lids of soil mesocosms included inlet and outlet quick-connect bulkhead fittings (Anonymous 2009). Air from outside the laboratory was drawn at 2.5 L min^{-1} into a 10-L buffer volume and humidified to a dew-point of 15 °C (LI-610 dew-point generator, LI-COR, Lincoln, NE, USA), then split into eight streams: six served as inlet streams for the soil respiration mesocosm, one was directed through an empty soil respiration mesocosm and served as the reference, while the eighth stream was a vent to avoid over-pressurising the soil headspace. Air from headspace of the six soil mesocosms, and empty reference soil mesocosm were continuously drawn into the manifold system of the tuneable diode laser at 170 ± 10 mL min^{-1}. The tuneable diode laser was programmed to measure $^{12}CO_2$ of, in turn, two calibration gases (416 µmol mol^{-1} and 612 µmol mol^{-1}), the six soil mesocosms, and empty reference chamber. The manifold system switched between intakes every 45 s, with the CO_2 concentration measured in the last 30 s averaged. Rates of soil respiration were calculated based on tuneable diode laser measurements of (dry) concentrations of $^{12}CO_2$ and flow rate:

$$^{12}CO_2 efflux = Q\left(^{12}C_{out} - {}^{12}C_{in}\right), \quad (1)$$

where Q is the molar flow of air through the soil headspace, C_{out} and C_{in} are concentrations of $^{12}CO_2$ measured in the air exiting the chamber (C_{out}) or entering the chamber (C_{in}, the reference gas). The daily averages each comprised at least eight individual measurements per replicate that were averaged yielding a daily average for each replicate. When dry mesocosms were re-wet, respiration of each mesocosm was measured every 7.5 min for three days. From these data we determined the rate of respiration immediately prior to re-wetting, the time until respiration reached maximum values after rewetting, and the maximum rate of respiration after rewetting. The cumulative soil respiration was generally calculated by linear interpolation between the daily averages, except for dry-rewet soils in the three days after rewetting where we integrated the measurements made at 7.5-min intervals.

Extracellular polysaccharide

The extracellular polysaccharide (EPS) fraction quantified here represents the total extracellular polysaccharide pool recoverable by cation-exchange resin extraction (Redmile-Gordon et al. 2014) and includes both endogenous microbial EPS and any exogenous polysaccharide from the added mucilage. To measure EPS, soils were first extracted at 2–4 °C with 0.01 M $CaCl_2$ to remove water-soluble compounds. The resultant pellet was re-suspended in cold, phosphate-buffered saline, next cation exchange resin (Dowex 50 X8, Na^+ form) was added and the slurry was shaken for 30 min at 2–4 °C, and subsequently centrifuged. The supernatant was precipitated with three volumes of 100% ethanol. The purified EPS was subsequently hydrolysed (4 M trifluoroacetic acid), methoximated-trimethylsilyl derivatives were formed and analysed by GC–MS (Warren 2014), as described in supplementary information.

Microbial biomass carbon

Microbial biomass carbon was measured via chloroform gas fumigation approach with 4.0 g subsamples of fumigated (48 h of vacuum chloroform infiltration) and non-fumigated soil extracted with 20.0 mL of 0.5 M K_2SO_4 by shaking for 30 min at 100 rpm on a flat-bed shaker. Organic carbon in the supernatant was determined spectrophotometrically after dichromate digestion (Cai et al. 2011). Microbial biomass carbon was calculated from the difference between fumigated and non-fumigated extracts using a k_{EC} of 0.45 to convert extractable C into microbial biomass C.

Polar lipid fatty acids

Polar lipid fatty acids (PLFA) were determined on four randomly chosen replicates, essentially as described in Warren (2019). Lipids were extracted using a modified Bligh and Dyer (1959) protocol (Frostegård and Bååth 1996) with 1.0 g fresh soil extracted twice with a 2:1:0.8 (v:v:v) mixture of methanol, chloroform ($CHCl_3$) and citric acid buffer (0.15 M, pH = 4.0) in an ultrasonic bath, then phase separated. To fractionate the lipid extract, 1.0 mL of $CHCl_3$ phase was applied to silica SPE (Discovery DSC-Si SPE, 100 mg) using a method adapted from Mills and Goldhaber (2010). The methanol eluate (containing polar lipids) was dried under nitrogen gas, then derivatised via mild alkaline methanolysis (incubation at 37 °C for 30 min with 0.2 M methanolic KOH and $CHCl_3$). The derivatised samples were dried under nitrogen gas and re-dissolved in hexane that included 25 µg mL^{-1} methyl heptadecanoate-d_{33} (Sigma-Aldrich) as internal standard. The fatty acid methyl esters (FAMES) were quantified via GC–MS (GCMSQP2010Plus, Shimadzu, Kyoto, Japan) fitted with a 5% diphenyl stationary phase (30 m long × 0.25 mm internal diameter × 0.25 mm film thickness; Rxi-5SilMS, Restek, Bellfonte, USA). We identified and quantified FAMES with reference to standards in two commercial FAMES mixtures (37 Fatty acid methyl ester mix and bacterial acid methyl ester mix, Sigma-Aldrich). PLFA were assigned as non-specific, bacterial, or fungal based on Joergensen (2022).

Water-stable aggregate size distribution

Wet sieving was used to determine the size distribution of water-stable aggregates, by adapting the method of Yoder (1936). Samples for wet sieving were collected immediately prior to rewetting of dry soils. This timing was chosen because aggregate size distribution measured at this time provides a reasonable proxy for soil that has undergone a dry-rewet cycle (for dry-rewet treatment) or remained well watered throughout (well-watered treatment). This is because wet sieving involves immersion of soil in water, so for dry soil constitutes a rewetting treatment that induces structural disruption. Measuring soils after experimental rewetting would combine the experimental rewetting effects with those imposed by the sieving procedure, making the results difficult to interpret mechanistically. To wet sieve soils, 25 g (dry weight equivalent) of soil was sieved to 8 mm, distributed evenly onto the upper of three stacked sieves (2000, 250, 53 µm) immersed in water. The sieves were raised and lowered 2–3 cm in the column of water 30 times over two minutes, then the size fractions were rinsed off the sieves and oven dried to determine > 2000 µm (large macroaggregates), 250–2000 µm (small macroaggregates), 53–250 µm (microaggregates) and < 53 µm (silt and clay sized fractions).

Statistics

Univariate statistics were computed with GraphPad Prism 10 (GraphPad Software, Boston, MA, USA) and multivariate statistics with PAST 4.16 (Hammer et al. 2001). For EPS, MBC, summed PLFA and fungal:bacterial PLFA we used unpaired t-tests to examine the effect of amendment (mucilage vs glucose) on parameters. The t-tests were done separately for each timepoint (T-24, T0 and T6) and watering treatment (well-watered and dry-rewet), because soils were destructively sampled such that mesocosms were independent at each timepoint. Soil respiration was measured repeatedly on the same mesocosms, but the primary interest was comparing mucilage vs glucose rather than detailed temporal dynamics. Hence, time series data were summarised into single-value parameters prior to statistical testing. The single-value parameters that captured respiration dynamics and enabled

hypothesis testing were the cumulative amounts of C respired before rewetting, after rewetting, and over the full 44-day experiment. The effects of amendment (mucilage vs glucose) and watering treatment (well watered vs dry–rewet) on these summary parameters were tested using two-way analysis of variance (ANOVA) with Tukey post hoc tests. For the dry-rewet treatment we used unpaired t-test to examine if amendment affected parameters summarising rewetting dynamics, namely, rate of respiration immediately prior to re-wetting, the time taken to reach maximum respiration after rewetting, and the maximum rate of respiration after rewetting. For the size-distribution of aggregates, effects of amendment (mucilage vs glucose) and watering treatment (well watered vs dry–rewet) were assessed with two-way ANOVA with Tukey post hoc test. Ordination was applied to PLFA data to visualise if treatment (well watered vs dry-rewet) and type of C amendment (mucilage vs glucose) affected multivariate patterns of individual PLFA, as might be indicative of shifts in microbial community composition. Ordination was achieved with principal components analysis (PCA) and non-metric multidimensional scaling (NMDS) of absolute concentrations of individual PLFA. Permutational ANOVA (PERMANOVA) was used to examine whether multivariate patterns of PLFA differed with treatment and C amendment. Statistical significance was accepted at $P < 0.05$.

Results

Gravimetric soil water content

Water content of well-watered (WW) mesocosms was 0.115 to 0.125 g g^{-1} throughout the 44 days of the experiment (Fig. 2). For the dry-rewet mesocosms (DR), when water was withheld over 24 days the water content decreased slowly to 0.01 g g^{-1}, rewetting increased water content back to the same 0.12 g g^{-1} of well-watered mesocosms. In both watering treatments there was no visual difference between mesocosms amended with mucilage versus glucose (Fig. 2), with the absence of significant differences confirmed by unpaired t-tests at the key timepoints of T-24, T0 and T6 (all $P > 0.05$).

Respiration

For the well-watered mesocosms, rates of respiration were 30–35 nmol CO_2 g^{-1} h^{-1} in the two days immediately after amendment, decreased by around 50% in the 14 days after amendment, then remained constant at 15- 20 nmol CO_2 g^{-1} h^{-1} for the remainder of the experiment (Fig. 3a). For context, an independent pilot study found that adding glucose or mucilage induced a large but transient increase in respiration, increasing rates 60% above unamended controls before declining to control rates within about 20 days (Fig. S1). In the dry-rewet treatment, rates of respiration decreased progressively as soils dried, reaching a minimum of 1–2 nmol CO_2 g^{-1} h^{-1} immediately prior to re-wetting (Fig. 3a,b). Re-wetting of dry soil led to measurable increases in respiration within the 7.5-min cycle of the measurement system and after 3.5 to 4.5 h reached a maximum 50 to 60 times greater than for dry soil (Fig. 3b). The overall shape and timing of the rewetting response were visually similar for mesocosms amended with glucose versus mucilage (Fig. 3a,b).

Quantitative differences between treatments were assessed using summary parameters (Tables 1 and 2) derived from the respiration time series (Fig. 3a,b). The cumulative respiration over the complete 44-day dry-rewet cycle was greater in mucilage-amended than glucose amended soils (main effect of amendment, $P < 0.0001$), but did not differ between watering treatments (main effect of treatment $P > 0.05$) because slower respiration in the dry-rewet treatment during the drying phase was offset by faster respiration following rewetting (Table 1c). Cumulative respiration during the drying phase (T-24 to T0) and rewetting phase (T0 to T20) were both significantly affected by amendment, treatment and their interaction (2-way ANOVA, all $P < 0.05$). Here we focus on the effect of amendment on cumulative respiration within watering treatments. In well-watered mesocosms, cumulative respiration was 12% greater for mesocosms amended with mucilage than with glucose (Table 1). In dry-rewet mesocosms the cumulative respiration over the complete 44-day dry-rewet cycle was also 12% greater in mucilage-amended than glucose-amended soils, comprising a non significant < 3% difference during the drying phase (Tukey post hoc $P > 0.05$, Table 1a) and a significant 19% difference during the rewet phase (Tukey post hoc

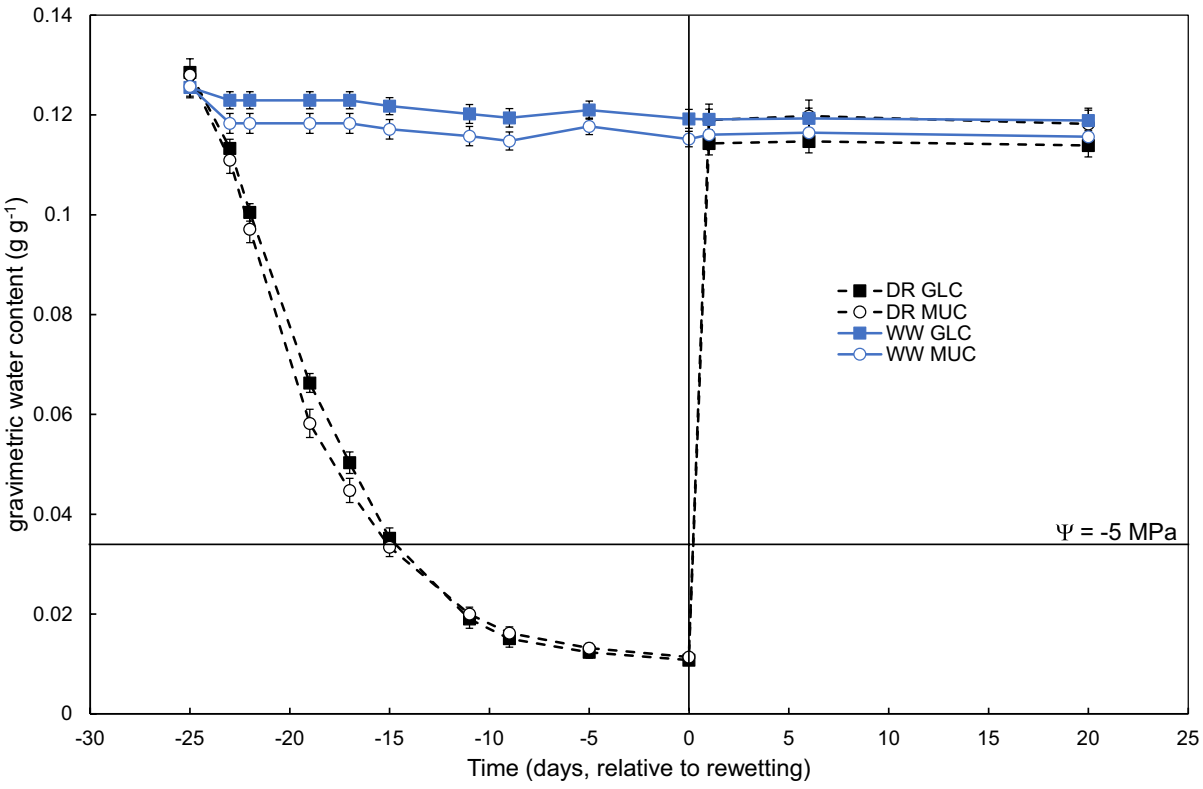
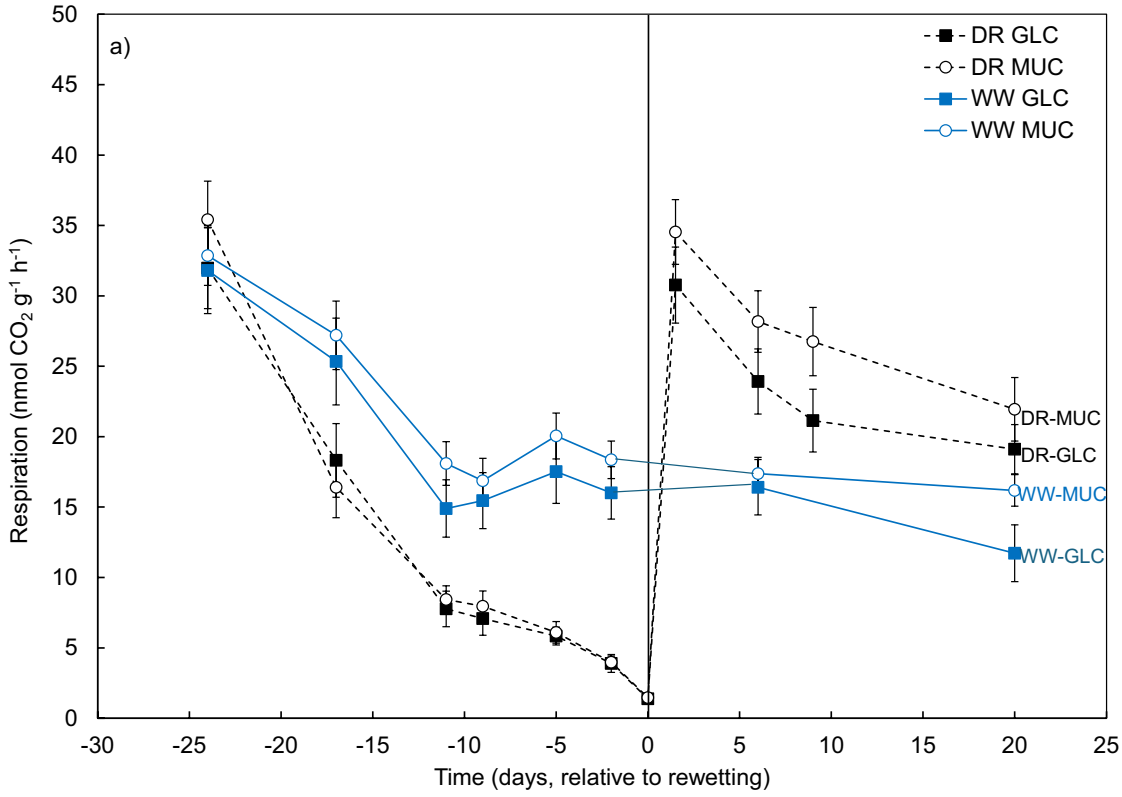


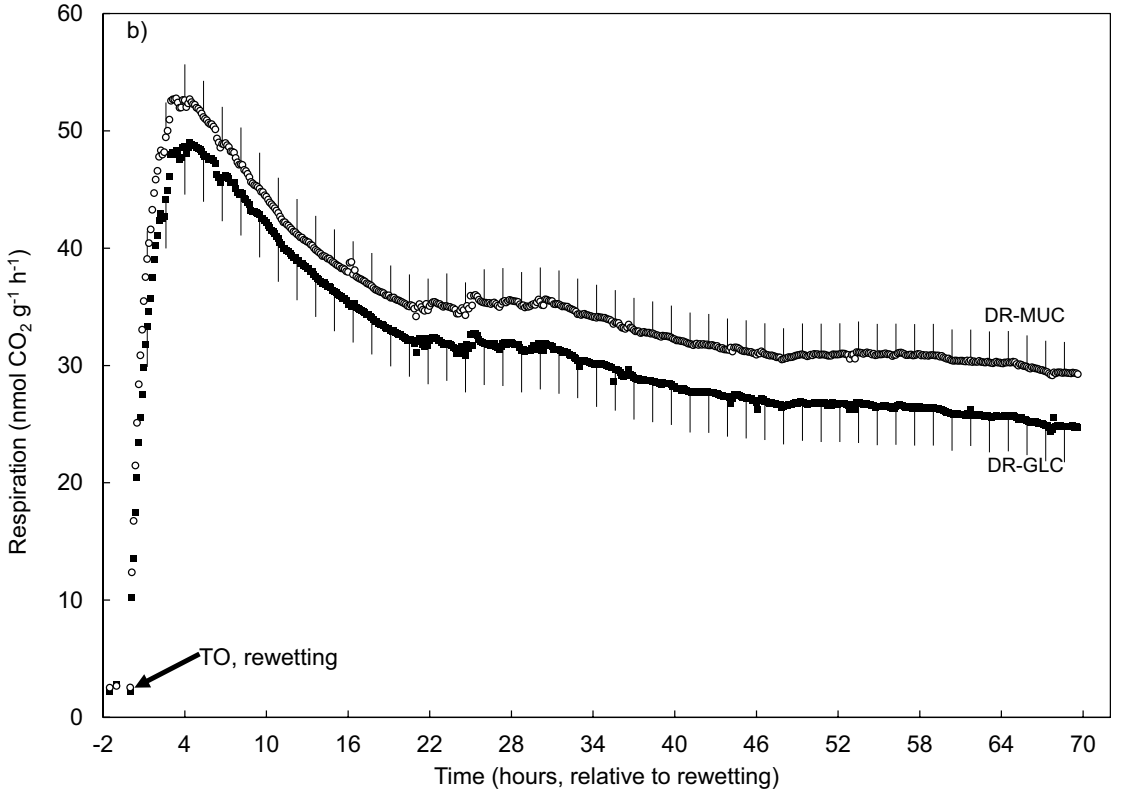
Fig. 2 The time course of soil water content for mesocosms filled with approximately 850 g (dry weight equivalent) soil that were maintained well-watered (WW) or subject to a dry-rewet (DR) cycle. Twenty-four days prior to re-watering, all mesocosms were amended with 0.1 mg $g.^{-1}$ of either glucose (GLC) or mucilage (MUC). Data are mean (SE) of six replicate mesocosms. Soil water content corresponding to −5 MPa was estimated from water release curves based on soil texture (Van Genuchten 1980; Leij et al. 1996)

$P < 0.05$, Table 1b). Summary parameters describing the rewetting response showed no general differences between mesocosms amended with mucilage or glucose (Table 2). There was no difference between mucilage- and glucose-amended mesocosms in the maximum rate of respiration following rewetting, the time taken to reach that maximum, or mean respiration during the three days after rewetting (unpaired t-tests, all $P > 0.05$, Table 2).

Extracellular polysaccharide, microbial biomass carbon, and polar lipid fatty acids

Extracellular polysaccharide (EPS), representing the combined pool of microbial EPS and (residual) added mucilage, was extracted, hydrolysed then quantified by GC–MS. The purity of the extracted EPS was confirmed by GC–MS detecting only the sugar and sugar acids that one would expect for polysaccharide (galactose, glucose, fucose, rhamnose, mannose, arabinose, xylose, galacturonic acid, glucuronic acid). Moreover, the relative abundance of the carbohydrate monomers was similar to previous reports (Fig. S2 and S3). At T-24, which was two days after soils were amended, the EPS content was significantly (unpaired t-test, $P < 0.00001$) greater by around ten-fold in soils amended with mucilage (39 μg g^{-1}) than glucose(3.5 μg g^{-1}) (Fig. 4a). The EPS content of mucilage-amended soils subjected to a dry-rewet treatment decreased to 10.8 μg g^{-1} by the conclusion of the drying cycle (T0) but remained significantly greater than in glucose-amended soils ($P = 0.035$), while by six days after re-wetting EPS content of mucilage-amended soil had decreased further to 4.0 μg g^{-1} and did not differ from glucose-amended soils ($P > 0.05$). In well-watered soils that received mucilage, there


a)
DR GLC
DR MUC
WW GLC
WW MUC
DR-MUC
DR-GLC
WW-MUC
WW-GLC
Respiration (nmol CO2 g-1 h-1)
Time (days, relative to rewetting)


b)
DR-MUC
DR-GLC
TO, rewetting
Respiration (nmol CO2 g-1 h-1)
Time (hours, relative to rewetting)

◂**Fig. 3** Daily averages of respiration (a) and a detailed view of respiration follow re-wetting (b) of soil mesocosms amended with either glucose (GLC) or mucilage (MUC) and kept well-watered (WW) or subject to a dry-rewet cycle (DR). Daily averages (panel a) were determined from measurements of one-hour duration on multiple occasions. Panel b) shows continuous measurements made every 7.5 min for the two hours prior to re-wetting and for three days after rewetting. Data are mean (SE) of six replicate mesocosms. In panel b, ten in every eleven error bars have been removed to reduce visual clutter. Where error bars are shown, they are plotted only in the positive direction for DR–MUC and in the negative direction for DR–GLC. Formal statistical analyses of respiration are in Tables 1 and 2

was a faster decrease in EPS such that by T0 EPS had decreased to 4.4 μg g^{-1} and did not differ from glucose-amended soils ($P > 0.05$). Microbial biomass carbon (MBC) varied from 55 to 80 μg g^{-1} and did not differ significantly between soils amended with mucilage versus glucose ($P > 0.05$, Fig. 4b).

Total PLFA (Fig. 4c) and multivariate relationships among individual PLFA (Fig. 5 and S4) were complex and affected by the type of C amendment (mucilage vs glucose) and between well-watered, dry and re-wet soils. In well-watered mesocosms the total PLFA pool did not differ between mucilage and glucose-amended soils at T-24 or T0, whereas in dry soil at T0 total PLFA was significantly (unpaired t-test, $P = 0.033$) higher by 50% in soils amended with mucilage (16 ± 1 nmol g^{-1}) than glucose (10.6 ± 0.6 nmol g^{-1}) (Fig. 4c). By six days after re-wetting, total PLFA did not differ between soils amended with mucilage versus glucose ($P > 0.05$). Ordination via PCA (Fig. 5) and NMDS (Fig. S4) showed separation of watering treatments and amendments based on multivariate patterns of individual PLFA, with PERMANOVA confirming a significant main effect of watering treatment ($P = 0.0001$) and an interaction between watering treatment and amendment ($P = 0.0049$, Fig. S4). For example, two days after amendment (at T-24) there were already differences in multivariate patterns of individual PLFA between glucose- and mucilage-amended soils, with mucilage amendment associated with larger amounts of two bacterial PLFA (C18:1w7c and C16:1w7c). At peak water stress (T0), mucilage amendment was associated with larger amounts of unspecified microbial PLFA (C16:0, C18:0), fungal PLFA (C18:2w6c, C18:1w9c) and bacterial PLFA (C18:1w7c, Cy C19:0). The ratio of bacterial:fungal PLFA decreased over time from around 1.1 mol mol^{-1} at T-24 to 0.69 mol mol^{-1} at T6, but did not differ between soils amended with glucose versus mucilage at any time-point (unpaired t-tests, all $P > 0.05$, Fig. S5).

Size distribution of water-stable aggregates

There were significant differences between watering treatments and amendments in the size distribution of water-stable aggregates (Fig. 6). The general trend was for aggregation to be greater in soils that received mucilage than those receiving glucose, and in the dry-rewet treatment than the well-watered treatment. These differences in size distribution were reflected in the mean weight diameter (MWD) with 2-way ANOVA showing significant main effects of amendment ($P = 0.0149$) and watering treatment ($P = 0.0008$). Mean weight diameter of mucilage-amended soil was 18% greater than glucose-amended soil in dry-rewet treatment and 12% greater in the well-watered treatment.

Discussion

Mucilage was successfully added to soil, substantially increasing extracellular polysaccharide levels in the dry-rewet treatment (Fig. 4a), while the similar water content and respiration dynamics of mucilage- and glucose-amended soils support use of glucose as procedural control. Using the monosaccharide glucose as procedural control enabled decoupling of effects due to added carbohydrate from the unique physical and biophysical effects of the polysaccharide-based mucilage. Its suitability as a procedural control for added C was supported by mucilage- and glucose-amended soils having similar patterns of respiration and likely similarly rapid rates of degradation (Niedeggen et al. 2024) consistent with both being ubiquitous microbial substrates in soil (Pozzo et al. 2018; Costa et al. 2020). The similar respiration of mucilage- and glucose-amended soils included a two-fold decrease in respiration of well-watered soil in the 15 days after amendment (see inflection at T-11 on Fig. 3) likely due to depletion of added C (compare with time-course of respiration in unamended controls, Fig. S1). Rapid depletion of added mucilage in well-watered soils was confirmed by the measured EPS content decreasing to the same level as glucose-amended

Table 1 Cumulative amount of respired C in mesocosms filled with approximately 850 g (dry weight equivalent) soil that were amended with 0.1 mg g^{-1} of either glucose or mucilage (amendment = 36 mg of C added to each mesocosm) then either maintained well-watered or subject to a dry-rewet cycle (treatment). Data are expressed as mg C respired per mesocosm in a) the 24 days after amendment and immediately prior to re-wetting the dry-rewet treatment (i.e. T-24 to T0 days), b) the 20 days after dry mesocosm were rewet (T0 to T20), and c) the full 44 days of the experiment (T-24 to T20). Data are mean (SE) of six replicate mesocosms. P-values shown are from two-way ANOVA testing the effects of amendment (glucose vs mucilage), watering treatment (well-watered vs dry-rewet), and their interaction. Results of Tukey post hoc indicated by superscript letters wherein different letters indicate $P < 0.05$

	Dry-rewet		Well watered		Two-way ANOVA (P)		
Cumulative respiration (mg C mesocosm^{-1})	Glucose	Mucilage	Glucose	Mucilage	Amendment	Treatment	Interaction
a) T-24 to T0 days	79.9 (1.7)^{a}	82.7 (1.5)^{a}	123.1 (2.1)^{b}	134.9 (1.5)^{c}	0.0005	< 0.0001	0.0199
b) T0 to T20 days	111.4 (1.5)^{a}	132.1 (1.5)^{b}	71.5 (0.9)^{c}	83.2 (1.6)^{d}	< 0.0001	< 0.0001	0.0057
c) T-24 to T20 days	191.4 (4.6)^{a}	214.9 (4.4)^{b}	194.7 (4.9)^{a}	218.0 (3.5)^{b}	< 0.0001	0.4751	0.9820

Table 2 Summary of the rate of respiration measured in dry soil mesocosms in the two hours before re-wetting and three days after re-wetting. Data are the rate of respiration measured in dry soil immediately prior to re-wetting, the maximum rate of respiration measured after re-wetting, and the duration elapsed until the maximum rate was recorded. Also shown is the mean rate of respiration over the three days after re-wetting. Data are mean (SE) of six replicate mesocosms. Differences between glucose- and mucilage-amended soils were assessed using unpaired, two-tailed Student's t-tests

amendment	Dry soil respiration (nmol CO_2 g^{-1} h^{-1})	Maximum respiration after rewetting (nmol CO_2 g^{-1} h^{-1})	Time to maximum respiration (minutes)	Mean respiration 0–3 days after rewetting (nmol CO_2 g^{-1} h^{-1})
Glucose	1.4 (0.3)	49.7 (4.1)	259.2 (34.7)	30.8 (3.2)
Mucilage	1.5 (0.4)	53.1 (3.1)	211.5 (17.4)	34.5 (2.7)
t-test (P)	0.85	0.52	0.25	0.39

soil within 26 days (at T0, Fig. 4a). Slower microbial activity in soil subject to drying (e.g. as indicated by respiration, Fig. 3) resulted in slower degradation of added mucilage such that at the conclusion of the drying cycle (T0), the dry-rewet treatment had more than twice as much EPS as soils receiving glucose. The EPS concentration at the time of rewetting was lower than mucilage concentration sometimes reported for the rhizosphere (Carminati and Vetterlein 2012; Zickenrott et al. 2016; Holz et al. 2018). This concentration is nonetheless realistic because mucilage was applied to the entire soil volume not just the 1–10% that is rhizosphere. In the rhizosphere mucilage is released continuously as roots grow, with this ongoing input likely to reinforce the effects observed in this study.

The activity of microbes, as indicated by respiration, was around 12% faster for soil amended with mucilage than soil receiving the same amount of C as glucose (Table 1), suggesting improved access to substrates. Greater diversity of sugars in mucilage than glucose is unlikely to explain the persistently faster respiration, because respiration time courses (Fig. 3, Fig. S1), cumulative C respired relative to C added (Table 1), and declining EPS pools (Fig. 4) all indicate that added C was largely consumed well before T0. The mechanism is instead more likely to involve polysaccharide enabling closer arrangement of microbes, enzymes and substrates (Burns et al. 2013) and/or faster movement of substrates and products (Zhang et al. 2024) via liquid bridges (Carminati et al. 2017) and maintenance of hydraulic connectivity (Read et al. 2003). The closer arrangement of microbes, enzymes and substrates could be partly a function of polysaccharides promoting adhesion of microbes onto solid surfaces (Tsuneda et al. 2003) and retention of extracellular enzymes (Burns et al. 2013). The mechanisms(s) by which mucilage

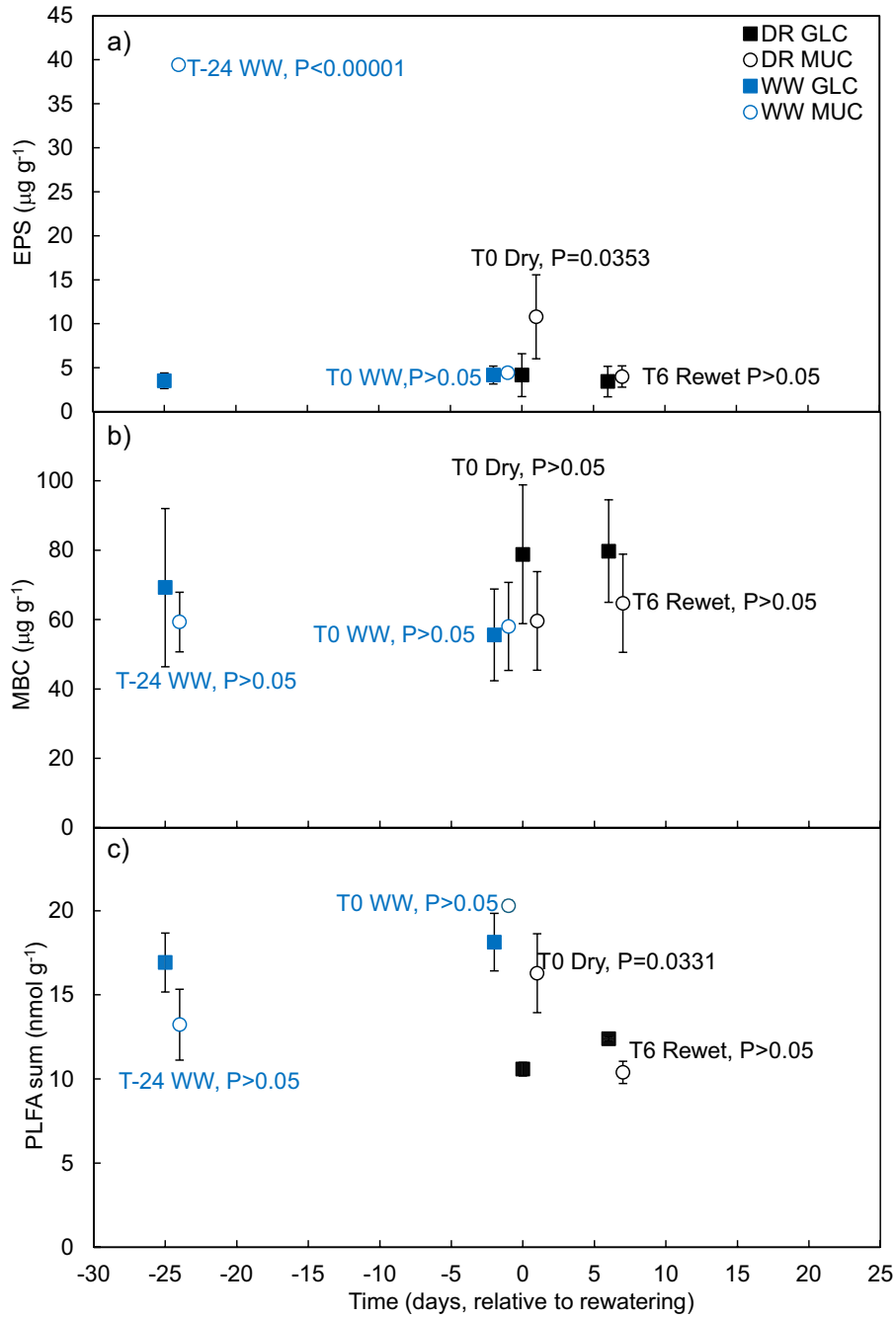


Fig. 4 The **a**) extracellular polysaccharide (EPS), **b**) microbial biomass carbon (MBC), and **c**) summed concentration of polar lipid fatty acids (PLFA sum) of soil mesocosms amended with either glucose (GLC) or mucilage (MUC) and kept well-watered or subject to a dry-rewet cycle. EPS, MBC and PLFA were measured in well-watered soils two days after mucilage and glucose had been added (T-24 days), in well-watered and dry-re-wet soils immediately prior to rewetting (T0 days), and on the dry-rewet soils six days after rewetting (T6 days). To minimize overlap, datapoints for glucose-amended soil have been shifted left by 1 day relative to mucilage-amended soil, while at T0 well-watered datapoints are shifted left of dry-rewet by two days. EPS and MBC data are mean (SE) of six replicate mesocosms, while PLFA data are mean (SE) of four replicate mesocosms. Differences between glucose- and mucilage-amended soils were assessed using unpaired t-tests. t-tests were conducted independently for well-watered soils at T-24, for well-watered and dry soils at T0, and for rewet soils at T6. Statistical significance of differences between glucose and mucilage amendments are indicated on the figure

enhances microbial activity likely require some free water given that in drying soil (Table 1a) and very dry soil (Table 2) rates of respiration did not differ between glucose- and mucilage-amended soils (Fig. 3 and Table 2) perhaps because soil was too dry for functional liquid bridges. While we cannot identify the exact cause, the various explanations for faster respiration of mucilage-amended soil likely involve mucilage altering microbial microhabitats and improving access to substrates.

Mucilage addition increased the ability of microbes to survive soil drying, consistent with our hypothesis and providing further evidence that mucilage improves microbial microhabitats. At the

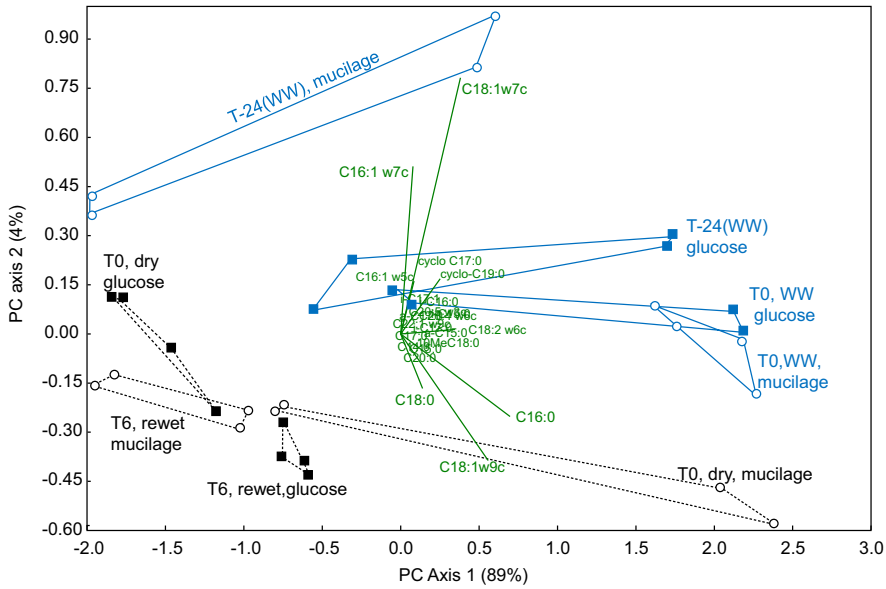


Fig. 5 Principal components analysis (PCA) bi-plot of polar lipid fatty acids (PLFA) of soil mesocosms amended with either glucose (GLC) or mucilage (MUC) and kept well-watered (WW) or subject to a dry-rewet cycle. The biplot combines the score plot, showing how observations (soils) are positioned on the principal components, with a loading plot (green lines), indicating the contribution of each original variable (PLFA) to those components. PLFA was measured two days after mucilage and glucose had been added to soils when all soils were well watered (T-24 days), in well-watered and dry-re-wet soils immediately prior to rewetting (T0 days), and on the dry-rewet soils six days after rewetting (T6 days)

conclusion of the drying cycle, soil amended with mucilage had a 50% larger PLFA pool than soil amended with glucose (Fig. 4c) suggesting that exogenous polysaccharide fosters desiccation tolerance and thus a large microbial population in very dry soil ($\Psi <<$—5 MPa, Fig. 2). In contrast, drying caused a decrease in microbial biomass of glucose-amended soils here (Fig. 4c) and in a previous study with the same soil (Warren 2020b), consistent with multiple independent studies showing that drying decreases microbial biomass (e.g. Sparling et al. 1986; Kieft et al. 1987; Jensen et al. 2003; Wu and Brookes 2005; Bapiri et al. 2010). The protective effect of mucilage in drying soil is consistent with experiments showing polysaccharides foster extreme desiccation tolerance (Zhang and Yan 2012; Krause et al. 2019). Increased microbial survival under water deficit could arise because polysaccharides increase water-holding capacity and or water retention (Roberson and Firestone 1992) allowing microbes to avoid or delay experiencing water deficit by being embedded in biofilm (Bérard et al. 2015; Benard et al. 2023). At the bulk soil scale there was no difference in water content between mucilage- and glucose- amended soil (Fig. 2), implying that any effect of mucilage on water content involves microhabitats becoming hydraulically disconnected from the surrounding soil (Benard et al. 2023). Polysaccharide-rich mucilage modifies water potential gradients and hydraulic conductivity at the pore and root–soil interface scale, slowing local declines in water potential relative to bulk soil as drying progresses (Carminati and Vetterlein 2012). Such hydraulic buffering provides a plausible mechanism by which microbes embedded in mucilage-rich microhabitats experience less severe or delayed water stress. In this context, the rhizosphere has been conceptualised as microbial "mini oasis" embedded within otherwise dry soil (Marasco et al. 2022). Our plant-free system demonstrates that mucilage alone is sufficient to support microbial persistence during drought in the rhizosphere. However, it remains unclear if polysaccharide can keep microbes hydrated in soil that is dry for prolonged periods (McCully and Boyer 1997), or if polysaccharide enables survival by enabling extreme desiccation tolerance (anhydrobiosis) (Crowe et al. 2011; Bosch et al. 2021), or both.

Mucilage addition directly increased water-stable aggregates. Other studies often associated increased

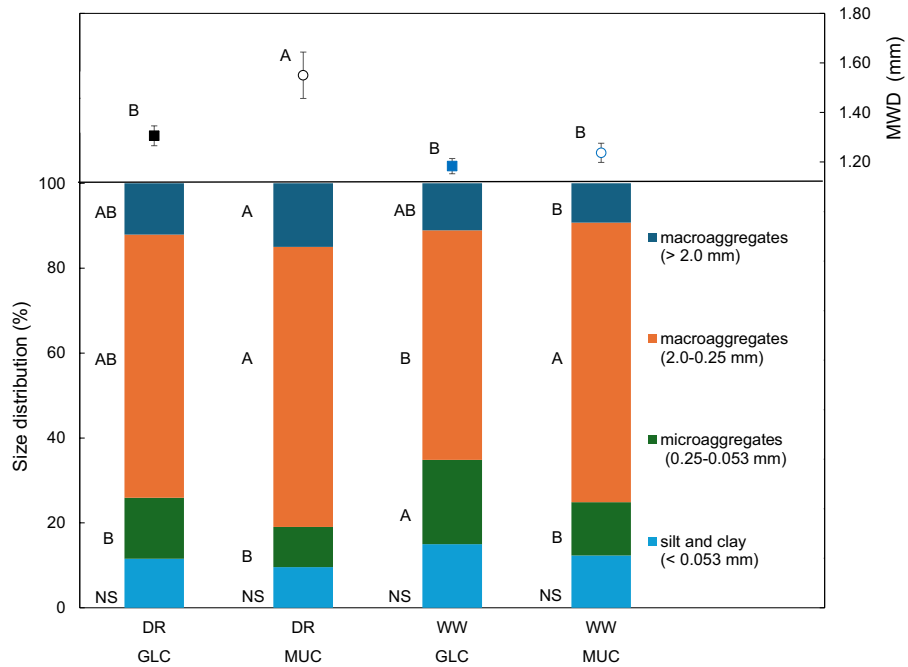


Fig. 6 The size distribution of water-stable aggregates for soil mesocosms amended with either glucose (GLC) or mucilage (MUC) and kept well-watered (WW) or subject to the drying phase of a dry-rewet cycle (DR). The size distribution was measured by wet sieving at T0, which was immediately prior to DR soils being rewet. Mean weight diameter (MWD) was calculated as described by Kemper and Rosenau (1986). Data are means of six replicates. For mean weight diameter, two-way ANOVA assessed the significance of amendment ($P=0.0149$), watering treatment ($P=0.0008$) and their interaction ($P=0.19$). Results of Tukey post hoc are indicated by uppercase letters wherein different letters indicate $P<0.05$

aggregation and/or mechanical stability with roots and/or physical enmeshing/entanglement by fungal hyphae (Cosentino et al. 2006; Baumert et al. 2018), whereas here the system was plant-free and the increased aggregation with mucilage amendment was not specifically associated with fungi. For example, the ratio of bacteria:fungi did not differ between mucilage- and glucose-amended soils (Fig. S5), and mucilage amendment was associated with greater amounts of both fungal PLFA (C18:2w6c, C18:1w9c) and bacterial PLFA (C18:1w7c, Cy C19:0) (Fig. 5). Our data reinforce previous findings that mucilage, and chemically similar extracellular polysaccharide, increase aggregation and/or mechanical stability (Morel et al. 1991; Traoré et al. 2000; Costa et al. 2018; Zhong et al. 2021; Rosskopf et al. 2022) by cementing together soil particles (Carminati et al. 2017). This mechanism is consistent with the role of root mucilage in forming rhizosheaths (Rahim et al. 2024), where soil particles adhere strongly to root surfaces. Increased bond strength (Kreitschitz et al. 2021) and resistance to particle detachment in mucilage-amended systems (Li et al. 2024) provide independent evidence that mucilage can mechanically stabilise soil, and the root-soil interface, through direct particle adhesion.

The response of respiration to drying and rewetting in mucilage- and glucose-amended soil was broadly consistent with a range of other studies (Warren 2014; Leizeaga et al. 2021; Warren and Manzoni 2023), providing confidence that the experimental system captured canonical dry–rewet dynamics. Against this background, our data do not support the hypothesis that mucilage amendment results in a smaller respiration pulse upon rewetting, despite mucilage-amended soil being more physically stable. In this soil pools of osmolytes and depolymerisation products support some of the respiration pulse in the first five hours after rewetting, whereas respiration over longer timescales is likely supported by previously protected "old C" (Warren 2016, 2020a; Warren and Manzoni 2023). Previous

work showed aggregate breakdown releases C from protection and partially support the Birch effect (Singh et al. 2023), yet here the greater aggregate stability afforded by mucilage amendment may not have decreased the amount of C rendered available by rewetting. A complementary explanation is that the respiration pulse is not wholly substrate limited, but also shaped by the larger live microbial biomass of mucilage-amended soil (Fig. 4c) and different community composition (Fig. 5). Drying–rewetting cycles can select for microbial communities that exhibit lower stress and faster post-rewetting respiration (Fierer et al. 2003; Fernandez et al. 2012; Hueso et al. 2012; Barnard et al. 2013; Kakumanu et al. 2013; Kwon et al. 2013; Evans and Wallenstein 2014; Leizeaga et al. 2022) and by analogous reasoning, the larger microbial biomass and altered community composition of mucilage-amended soil may sustain high respiration after rewetting independent of substrate availability. Future studies could disentangle the relative roles of substrate availability and community dynamics by enumerating C in the multiple pools of C that contribute to respiration, including aggregate fractions (Singh et al. 2023), depolymerisation products and osmolytes(Schimel et al. 2011; Slessarev and Schimel 2020; Warren and Manzoni 2023).

Conclusions

Mucilage when added at field-relevant concentrations increased the mechanical stability of soil, increased microbial activity in well-watered soil, and fostered microbial survival in dry soil. The cascading effects of mucilage on soil likely derive from its biophysical role as a liquid bridge and binding agent among soil particles, which promote increased microbial activity and maintain microbial populations in very dry soil. Our results show how plant-derived mucilage shapes microbial functioning and soil physical structure in ways that are relevant for nutrient supply and water relations of plants, and ecosystem-scale C cycling. The effects documented here illustrate how rhizosphere processes such as mucilage secretion could propagate to influence whole-plant performance and ecosystem function under fluctuating moisture conditions.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Anonymous (2009) Application note #127: Measurement of CO_2 Evolution in a Multiplexed Flask System. LI-COR Biosciences, Lincoln, p 12

Bapiri A, Bååth E, Rousk J (2010) Drying-rewetting cycles affect fungal and bacterial growth differently in an arable soil. Microb Ecol 60:419–428

Barnard RL, Osborne CA, Firestone MK (2013) Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME J 7:2229–2241

Baumert VL, Vasilyeva NA, Vladimirov AA, Meier IC, Kögel-Knabner I, Mueller CW (2018) Root exudates induce soil macroaggregation facilitated by fungi in subsoil. Front Environ Sci 6. https://doi.org/10.3389/fenvs.2018.00140

Benard P, Bickel S, Kaestner A, Lehmann P, Carminati A (2023) Extracellular polymeric substances from soil-grown bacteria delay evaporative drying. Adv Water Resour 172

Bérard A, Ben Sassi M, Kaisermann A, Renault P (2015) Soil microbial community responses to heat wave components: drought and high temperature. Clim Res 66:243–264

Birch HF (1958) The effect of soil drying on humus decomposition and nitrogen availability. Plant Soil 10:9–31

Birch HF (1964) Mineralisation of plant nitrogen following alternate wet and dry conditions. Plant Soil 20:43–49

Blazewicz SJ, Schwartz E, Firestone MK (2014) Growth and death of bacteria and fungi underlie rainfall-induced carbon dioxide pulses from seasonally dried soil. Ecology 95:1162–1172

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Boot CM, Schaeffer SM, Schimel JP (2013) Static osmolyte concentrations in microbial biomass during seasonal drought in a California grassland. Soil Biol Biochem 57:356–361

Bosch J, Varliero G, Hallsworth JE, Dallas TD, Hopkins D, Frey B, Kong W, Lebre P, Makhalanyane TP, Cowan DA (2021) Microbial anhydrobiosis. Environ Microbiol 23:6377–6390

Bottner P (1985) Response of microbial biomass to alternate moist and dry conditions in a soil incubated with C-14-labeled and N-15-labelled plant-material. Soil Biol Biochem 17:329–337

Brangarí AC, Manzoni S, Rousk J (2021) The mechanisms underpinning microbial resilience to drying and rewetting – a model analysis. Soil Biol Biochem 162

Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN, Zoppini A (2013) Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol Biochem 58:216–234

Cai Y, Peng C, Qiu S, Li Y, Gao Y (2011) Dichromate digestion-spectrophotometric procedure for determination of soil microbial biomass carbon in association with fumigation-extraction. Commun Soil Sci Plant Anal 42:2824–2834

Carbone MS, Still CJ, Ambrose AR, Dawson TE, Williams AP, Boot CM, Schaeffer SM, Schimel JP (2011) Seasonal and episodic moisture controls on plant and microbial contributions to soil respiration. Oecologia 167:265–278

Carminati A, Vetterlein D (2012) Plasticity of rhizosphere hydraulic properties as a key for efficient utilization of scarce resources. Ann Bot 112:277–290

Carminati A, Benard P, Ahmed MA, Zarebanadkouki M (2017) Liquid bridges at the root-soil interface. Plant Soil 417:1–15

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

Cosentino D, Chenu C, Le Bissonnais Y (2006) Aggregate stability and microbial community dynamics under drying–wetting cycles in a silt loam soil. Soil Biol Biochem 38:2053–2062

Costa OYA, Raaijmakers JM, Kuramae EE (2018) Microbial extracellular polymeric substances: ecological function and impact on soil aggregation. Front Microbiol 9:1636

Costa OYA, de Hollanr M, Pijl A, Liu B, Kuramae EE (2020) Cultivation-independent and cultivation-dependent metagenomes reveal genetic and enzymatic potential of microbial community involved in the degradation of a complex microbial polymer. Microbiome 8

Crowe JH, Crowe LM, Carpenter JE, Petrelski S, Hoekstra FA, Araujo PD, Panek AD (1996) Anhydrobiosis: cellular adaptation to extreme dehydration. Comprehensive Physiology 1445–1477. https://doi.org/10.1002/j.2040-4603.1996.tb01745.x

Czarnes S, Hallett PD, Bengough AG, Young IM (2000) Root- and microbial-derived mucilages affect soil structure and water transport. Eur J Soil Sci 51:435–443

de Nijs EA, Hicks LC, Leizeaga A, Tietema A, Rousk J (2019) Soil microbial moisture dependences and responses to drying–rewetting: the legacy of 18 years drought. Glob Change Biol 25:1005–1015

Denef K, Six J, Bossuyt H, Frey SD, Elliott ET, Merckx R, Paustian K (2001) Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biol Biochem 33:1599–1611

Dexter AR (1988) Advances in characterization of soil structure. Soil Tillage Res 11:199–238

Di Marsico A, Scrano L, Labella R, Lanzotti V, Rossi R, Cox L, Perniola M, Amato M (2018) Mucilage from fruits/seeds of chia (*Salvia hispanica* L.) improves soil aggregate stability. Plant Soil 425:57–69

Evans SE, Wallenstein MD (2014) Climate change alters ecological strategies of soil bacteria. Ecol Lett 17:155–164

Fernandez DA, Roldan A, Azcon R, Caravaca F, Baath E (2012) Effects of water stress, organic amendment and mycorrhizal inoculation on soil microbial community structure and activity during the establishment of two heavy metal-tolerant native plant species. Microb Ecol 63:794–803

Fierer N, Schimel JP (2002) Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biol Biochem 34:777–787

Fierer N, Schimel JP, Holden PA (2003) Influence of drying-rewetting frequency on soil bacterial community structure. Microb Ecol 45:63–71

Frostegård A, Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol Fertil Soils 22:59–65

Hammer Ø, Harper D, Ryan P (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 4:1–9

Holz M, Leue M, Ahmed MA, Benard P, Gerke HH, Carminati A (2018) Spatial distribution of mucilage in the rhizosphere measured with infrared spectroscopy. Front Environ Sci 6. https://doi.org/10.3389/fenvs.2018.00087

Homyak PM, Blankinship JC, Slessarev EW, Schaeffer SM, Manzoni S, Schimel JP (2018) Effects of altered dry season length and plant inputs on soluble soil carbon. Ecology 99:2348–2362

Hueso S, Garcia C, Hernandez T (2012) Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. Soil Biol Biochem 50:167–173

Jensen KD, Beier C, Michelsen A, Emmett BA (2003) Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions. Appl Soil Ecol 24:165–176

Joergensen RG (2022) Phospholipid fatty acids in soil—drawbacks and future prospects. Biol Fertil Soils 58:1–6

Kaiser M, Kleber M, Berhe AA (2015) How air-drying and rewetting modify soil organic matter characteristics: an assessment to improve data interpretation and inference. Soil Biol Biochem 80:324–340

Kakumanu ML, Cantrell CL, Williams MA (2013) Microbial community response to varying magnitudes of desiccation in soil: a test of the osmolyte accumulation hypothesis. Soil Biol Biochem 57:644–653

Kemper WD, Rosenau RC (1986) Aggregate stability and size distribution. In: Klute A (ed) Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods, 2nd edn. Soil Science Society of America Agronomy Monograph No. 9, pp 425–442

Kempf B, Bremer E (1998) Stress responses of *Bacillus subtilis* to high osmolarity environments: uptake and synthesis of osmoprotectants. J Biosci 23:447–455

Kieft TL, Soroker E, Firestone MK (1987) Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biol Biochem 19:119–126

Krause L, Biesgen D, Treder A, Schweizer SA, Klumpp E, Knief C, Siebers N (2019) Initial microaggregate formation: association of microorganisms to montmorillonite-goethite aggregates under wetting and drying cycles. Geoderma 351:250–260

Kreitschitz A, Kovalev A, Gorb SN (2021) Plant seed mucilage as a glue: adhesive properties of hydrated and dried-in-contact seed mucilage of five plant species. Int J Mol Sci 22:1443

Kwon MJ, Haraguchi A, Kang H (2013) Long-term water regime differentiates changes in decomposition and microbial properties in tropical peat soils exposed to the short-term drought. Soil Biol Biochem 60:33–44

Landl M, Phalempin M, Schlüter S, Vetterlein D, Vanderborght J, Kroener E, Schnepf A (2021) Modeling the Impact of Rhizosphere Bulk Density and Mucilage Gradients on Root Water Uptake. Front Agron 3. https://doi.org/10.3389/fagro.2021.622367

Lebedjantzev AN (1924) Drying of soil, as one of the natural factors in maintaining soil fertility. Soil Sci 18:419–448

Leij FJ, Alves WJ, van Genuchten MT, Williams JR (1996) The unsaturated soil hydraulic database. UNSODA 1.0 User's Manual. U.S. Environmental Protection Agency, Cincinnati, p 103

Leizeaga A, Hicks LC, Manoharan L, Hawkes CV, Rousk J (2021) Drought legacy affects microbial community trait distributions related to moisture along a savannah grassland precipitation gradient. J Ecol 109:3195–3210

Leizeaga A, Meisner A, Rousk J, Bååth E (2022) Repeated drying and rewetting cycles accelerate bacterial growth recovery after rewetting. Biol Fertil Soils 58:365–374

Li R, Zhang C, Raffan A, Hallett PD (2024) Plant mucilage increases pull-out resistance of root analogues from soil. Eur J Soil Sci 75

Marasco R, Fusi M, Ramond J-B, Van Goethem MW, Seferji K, Maggs-Kölling G, Cowan DA, Daffonchio D (2022) The plant rhizosheath–root niche is an edaphic "mini-oasis" in hyperarid deserts with enhanced microbial competition. ISME Commun. https://doi.org/10.1038/s43705-022-00130-7

McCully ME, Boyer JS (1997) The expansion of maize root-cap mucilage during hydration. 3. Changes in water potential and water content. Physiol Plant 99:169–177

Mills CT, Goldhaber MB (2010) On silica-based solid phase extraction techniques for isolating microbial membrane phospholipids: ensuring quantitative recovery of phosphatidylcholine-derived fatty acids. Soil Biol Biochem 42:1179–1182

Morel JL, Habib L, Plantureux S, Guckert A (1991) Influence of maize root mucilage on soil aggregate stability. Plant Soil 136:111–119

Naveed M, Brown LK, Raffan AC, George TS, Bengough AG, Roose T, Sinclair I, Koebernick N, Cooper L, Hallett PD (2018) Rhizosphere-scale quantification of hydraulic and mechanical properties of soil impacted by root and seed exudates. Vadose Zone J 17

Naveed M, Ahmed MA, Benard P, Brown LK, George TS, Bengough AG, Roose T, Koebernick N, Hallett PD (2019) Surface tension, rheology and hydrophobicity of rhizodeposits and seed mucilage influence soil water retention and hysteresis. Plant Soil 437:65–81

Nazari M, Bickel S, Benard P, Mason-Jones K, Carminati A, Dippold MA (2022) Biogels in soils: plant mucilage as a biofilm matrix that shapes the rhizosphere microbial habitat. Frontiers in Plant Science 12:798992

Niedeggen D, Rüger L, Oburger E, Santangeli M, Ahmed M, Vetterlein D, Blagodatsky S, Bonkowski M (2024) Microbial utilisation of maize rhizodeposits applied to agricultural soil at a range of concentrations. Eur J Soil Sci 75:e13530

Pozzo T, Higdon SM, Pattathil S, Hahn MG, Bennett AB (2018) Characterization of novel glycosyl hydrolases discovered by cell wall glycan directed monoclonal antibody screening and metagenome analysis of maize aerial root mucilage. PLoS ONE 13

Rahim R, Jahromi OE, Amelung W, Kroener E (2024) Rhizosheath formation depends on mucilage concentration and water content. Plant Soil 495:649–661

Read DB, Bengough AG, Gregory PJ, Crawford JW, Robinson D, Scrimgeour CM, Young IM, Zhang K, Zhang X (2003) Plant roots release phospholipid surfactants that modify the physical and chemical properties of soil. New Phytol 157:315–326

Redmile-Gordon MA, Brookes PC, Evershed RP, Goulding KWT, Hirsch PR (2014) Measuring the soil-microbial interface: extraction of extracellular polymeric substances (EPS) from soil biofilms. Soil Biol Biochem 72:163–171

Reichstein M, Tenhunen JD, Roupsard O, Ourcival JM, Rambal S, Miglietta F, Peressotti A, Pecchiari M, Tirone G, Valentini R (2002) Severe drought effects on ecosystem CO(2) and H(2)O fluxes at three Mediterranean evergreen sites: revision of current hypotheses? Glob Change Biol 8:999–1017

Roberson EB, Firestone MK (1992) Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. Appl Environ Microbiol 58:1284–1291

Rosskopf U, Uteau D, Peth S (2022) Effects of mucilage concentration at different water contents on mechanical stability and elasticity in a loamy and a sandy soil. Eur J Soil Sci 73

Schimel JP (2018) Life in dry soils: effects of drought on soil microbial communities and processes. Annu Rev Ecol Evol Syst 49:409–432

Schimel JP, Wetterstedt JAM, Holden PA, Trumbore SE (2011) Drying/rewetting cycles mobilize old C from deep soils from a California annual grassland. Soil Biol Biochem 43:1101–1103

Singh S, Mayes MA, Kivlin SN, Jagadamma S (2023) How the Birch effect differs in mechanisms and magnitudes due to soil texture. Soil Biol Biochem 179

Slessarev EW, Schimel JP (2020) Partitioning sources of CO_2 emission after soil wetting using high-resolution observations and minimal models. Soil Biol Biochem. https://doi.org/10.1016/j.soilbio.2020.107753

Slessarev EW, Lin Y, Jiménez BY, Homyak PM, Chadwick OA, D'Antonio CM, Schimel JP (2020) Cellular and extracellular C contributions to respiration after wetting dry soil. Biogeochemistry 147:307–324

Sparling GP, Speir TW, Whale KN (1986) Changes in microbial biomass-C, ATP content, soil phospho-monoesterase and phospho-diesterase activity following air-drying of soils. Soil Biol Biochem 18:363–370

Tiziani R, Puschenreiter M, Smolders E, Mimmo T, Herrera JC, Cesco S, Santner J (2021) Millimetre-resolution mapping of citrate exuded from soil-grown roots using a novel, low-invasive sampling technique. J Exp Bot 72:3513–3525

Traoré O, Groleau-Renaud V, Plantureux S, Tubeileh A, Boeuf-Tremblay V (2000) Effect of root mucilage and modelled root exudates on soil structure. Eur J Soil Sci 51:575–581

Trevisan F, Tiziani R, Cesco S, Mimmo T (2024) Timing matters: Exudates collection duration impacts tomato root exudome under nutrient deficiencies. Rhizosphere 30

Tsuneda S, Aikawa H, Hayashi H, Yuasa A, Hirata A (2003) Extracellular polymeric substances responsible for bacterial adhesion onto solid surface. FEMS Microbiol Lett 223:287–292

Van Genuchten MT (1980) A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. Soil Sci Soc Am J 44:892–898

Warren CR (2014) Response of osmolytes in soil to drying and rewetting. Soil Biol Biochem 70:22–32

Warren CR (2016) Do microbial osmolytes or extracellular depolymerisation products accumulate as soil dries? Soil Biol Biochem 98:54–63

Warren CR (2019) Does silica solid-phase extraction of soil lipids isolate a pure phospholipid fraction. Soil Biol Biochem 128:175–178

Warren CR (2020a) Pools and fluxes of osmolytes in moist soil and dry soil that has been re-wet. Soil Biol Biochem 150

Warren CR (2020b) Soil microbial populations substitute phospholipids with betaine lipids in response to low P availability. Soil Biol Biochem 140

Warren CR, Manzoni S (2023) When dry soil is re-wet, trehalose is respired instead of supporting microbial growth. Soil Biol Biochem 184

Wu J, Brookes PC (2005) The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. Soil Biol Biochem 37:507–515

Yan LM, Chen SP, Xia JY, Luo YQ (2014) Precipitation regime shift enhanced the rain pulse effect on soil respiration in a semi-arid steppe. PLoS ONE. https://doi.org/10.1371/journal.pone.0104217

Yoder RE (1936) A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses. Agron J 28:337–351

Zhang Q, Yan T (2012) Correlation of intracellular trehalose concentration with desiccation resistance of soil *Escherichia coli* populations. Appl Environ Microbiol 78:7407–7413

Zhang M, Wu Y, Qu C, Huang Q, Cai P (2024) Microbial extracellular polymeric substances (EPS) in soil: from interfacial behaviour to ecological multifunctionality. Geo-Bio Interfaces 1

Zhong X-l, Li J-t, Naveed M, Raffan A, Hallett PD (2021) A laboratory study to disentangle hydrological, mechanical and structural mechanisms of soil stabilization by plant mucilage between eroding and depositional zones of a slope. Eur J Soil Sci 72:125–140

Zickenrott I-M, Woche SK, Bachmann J, Ahmed MA, Vetterlein D (2016) An efficient method for the collection of root mucilage from different plant species—a case study on the effect of mucilage on soil water repellency. J Plant Nutr Soil Sci 179:294–302