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Plant versus microbial controls on soil aggregate stability in a seasonally dry ecosystem

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ABSTRACT

The formation of water-stable macroaggregates in soil is beneficial for many reasons, including carbon (C) sequestration, nutrient retention, and erosion control. A mix of biotic (e.g., plant C input, microbial activity) and abiotic factors (e.g., water, mineral interactions) contribute to form macroaggregates. However, in order to better model and manage soil macroaggregates, we need to know more about the relative contributions of these mechanisms. Previous experiments to separate microbial and abiotic mechanisms have been hampered by the need to add sterilant dissolved in water, thus limiting our ability to draw conclusions about the role of soil moisture in controlling aggregation and preventing conclusions about dry soil. Our first goal was to quantify the contribution of plant growth (and fresh plant C inputs) by continuously removing plants for 2 years in a seasonally dry grassland. Our second goal was to quantify microbial vs. abiotic contributions to macroaggregate formation under a range of soil moisture conditions by using chloroform vapor to sterilize soil without adding water or destroying soil structure. In the field, regardless of dry season length, removing plants reduced the average size of soil aggregates by 22–33%, which was primarily driven by a shift from large macroaggregates (2–9 mm diameter) to small macroaggregates (0.25–2 mm). In the laboratory, in sterile soils macroaggregate production increased with the moisture content. The resulting physicogenic aggregates appeared planar and angular at both macro- and micro-scales. In contrast, biogenic aggregates were formed most at intermediate moisture levels and were spherical. Our results suggest that—even in dry climates—soil macroaggregates are preserved by the presence of even dead plant roots, but are engineered by live microbes.

1. Introduction

Water-stable aggregates are beneficial in soil for many reasons, including carbon (C) sequestration, nutrient retention, aeration, and erosion control, as well as providing microenvironments for soil biodiversity and an improved seed bed for plants (Tisdall and Oades, 1982; Gupta and Germida, 1988; Six et al., 2000; Briar et al., 2011; Kong et al., 2011; Peng et al., 2015). Because water (rain, dry/rewet events, etc.) causes one of the most common disturbances to soil structure, it is desirable to both maintain the water-stability of existing aggregates and promote the formation of larger aggregates. Although microaggregates (53–250 μm diameter) store C longest, macroaggregates (>250 μm) generally contain more C (Elliott, 1986; Jastrow et al., 1996; Six et al., 2000) because the binding agents are enriched in C; macroaggregates also act as incubators encouraging the formation of microaggregates (Jastrow, 1996). Furthermore, the organic compounds that help bind soil aggregates may themselves be recalcitrant and contribute to long-term C storage (Cheshire, 1977; Foster, 1981; Degens and Sparling, 1995).

Despite the importance of soil aggregates in providing essential ecosystem services, the mechanisms that control their formation and persistence have not been completely elucidated. This is particularly true for arid and semiarid ecosystems where earthworms and other macroinvertebrates tend to be less abundant (Lavelle et al., 1997), and where seasonal drying make structural resistance to rewetting even more important. Notably lacking is an appreciation of the relative roles of biotic vs. abiotic drivers of aggregate formation. This was pointed out over two decades ago, "methods for assessing soil structure do not usually differentiate between biotic and abiotic factors (Oades, 1993)" and the call for an "explicit incorporation of biology" continues today (Keil and Mayer, 2014). Existing theories suggest that some mix of abiotic (e.g., water, organic-mineral interactions) and biotic factors (e.g., plant C input, microbial activity) is needed to form and maintain water-stable macroaggregates (Six and Paustian, 2014). However, in order to better model and manage soil aggregation, we need to identify and better quantify the relative contributions of these mechanisms.

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The formation of water-stable macroaggregates by abiotic mechanisms (i.e., "physicogenic aggregates") is generally thought to play a secondary role compared to biology (i.e., "biogenic aggregates"), at least for temperate soils with 2:1 clay minerals. For example, in a recent review on organic-mineral interactions, Keil and Mayer (2014) noted that, "while inorganic glues can cause aggregation in some environments, it is clear that biologic processes and their attendant organic glues dominate aggregate formation." Abiotic mechanisms are expected to primarily form microaggregates, which may require more time than macroaggregates to develop but persist longer in the environment (Tisdall and Oades, 1982; Lützow et al., 2006). The most important abiotic mechanisms that bind soil aggregates are generally considered to be ligand exchange and polyvalent cation bridging (Six et al., 2004; Bronick and Lal, 2005; Keil and Mayer, 2014). Ligand exchange dominates in acidic soils because of abundant oxides. Acidic hydroxyl groups of organic matter are exchanged with hydroxyl groups on mineral oxide surfaces and cemented into place, thus creating water-stable connection points within aggregates. Cation bridging—typically involving calcium, magnesium, or aluminum—dominates in neutral and basic soils. Positively charged cations can connect soil particles by bridging the gap between negatively charged clay surfaces and negatively charged organic compounds (Oades, 1988; Muneer and Oades, 1989; Clarholm et al., 2015). If enough connections are made, then a water-stable aggregate is formed. Although drying–rewetting cycles are known to form aggregates (Six et al., 2004), water's role in promoting abiotic "bridging reactions" is largely unknown because of the confounding influences of biology.

Beneficial effects of biology on soil aggregate stability are well-documented (Oades, 1993; Tisdall, 1994; Degens, 1997; Six et al., 2004). Plants play two main roles in the formation of soil aggregates, particularly macroaggregates (Denef et al., 2001). First, plant roots physically enmesh soil into aggregates (Tisdall and Oades, 1982; Oades and Waters, 1991; Degens, 1997; Six et al., 2004). Reflecting this, root length and plant abundance can relate positively to aggregate stability (Roberson et al., 1991; Haynes and Francis, 1993). Second, plants affect aggregate formation through organic residues that enter the soil (e.g., litterfall, root sloughing and dieback) and mix with mineral particles. These residues can act as a "nucleus" for aggregate formation by feeding microbes that secrete organic glues (De Gryze et al., 2005; Cosentino et al., 2006). Although the qualitative roles of plants are well known, their quantitative role is lesser known, particularly in seasonally dry ecosystems.

Microbes—whether they are inside macroinvertebrates or exist as free-living bacteria and fungi—also engineer soil aggregates. Soil microbial biomass relates positively to aggregate formation (Degens, 1997; Keim and Kandeler, 1997; Ghani et al., 2003; Cosentino et al., 2006), a pattern explained by two mechanisms. First, fungi are known to enmesh aggregates similarly to plant roots (Tisdall and Oades, 1982), particularly in sandy soils (Chenu, 1989; Chantigny et al., 1997; De Gryze et al., 2005). Removing fungi using fungicide reduces macroaggregate formation (Denef et al., 2001). Second, bacteria and fungi produce extracellular polymeric substances (EPS) that act as glues to connect soil aggregates as a possible strategy for accessing distant resources and creating a more stable environment in which to live (Roberson and Firestone, 1992; Chenu and Roberson, 1996; Flemming and Wingender, 2010). Microbes on the outer surface of aggregates may be in an ideal location to connect neighboring aggregates *if* they can access C substrates to make EPS (Holden, 2011; Schimel and Schaeffer, 2012). Production of EPS is likely a deeply rooted life-history trait of microbes used to remain hydrologically connected to resources in dry soil (Schimel and Schaeffer, 2012). Although EPS have been studied for the past five decades (e.g., Cheshire, 1977; Foster, 1981), extracting and purifying microbial EPS from soil is still not straightforward (Redmile-Gordon et al., 2014).

The first goal of our study was to investigate plant growth and C input as a driver of soil aggregate stability in a seasonally dry grassland. Although much has been learned in the laboratory, there is a lingering need to identify mechanisms that form and preserve water-stable aggregates in field soils (Degens, 1997). To quantify the legacy of fresh C inputs on soil aggregate stability, our approach was to create a gradient of plant influence by continuously removing grasses and forbs. Do plant C inputs during the growing season sustain water-stable aggregates through the dry season? And do plant–aggregate interactions depend on the length of the dry season? We expected that water addition (i.e., no dry season) would accelerate the decomposition of plant binding agents and therefore decrease the average size of water-stable aggregates, particularly when combined with plant removal.

Our second goal was to quantify the contribution of microbes to macroaggregate formation on the drier side of the soil moisture spectrum. Previous experiments to separate biotic and abiotic mechanisms have been hampered by the need to add sterilant dissolved in water (Molope et al., 1987; Hu et al., 1995; Bossuyt et al., 2001; Denef et al., 2001), thus preventing conclusions about dry soil. We instead fumigated with chloroform ($CHCl_3$) vapor to continuously kill microbes without water. By separating biotic and abiotic processes on the drier side of the soil moisture spectrum, we gain a clearer understanding of whether microbes help engineer dry soil and whether effects of biotic and abiotic processes are additive. We hypothesized that the combination of ample access to substrates and strong incentive to increase hydrologic connectivity causes microbes to allocate more C to soil binding agents at intermediate moisture (i.e., moderate stress).

2. Materials and methods

2.1. Site description

We studied a seasonally dry grassland at the University of California Sedgwick Reserve near Santa Ynez, California, USA (370 m ASL, 34.7120 °N, 120.0388 °W). The site experiences a Mediterranean-type climate with hot dry summers and cooler wet winters. The mean annual precipitation and temperature are 380 mm and 16.8 °C, respectively. Roughly 90% of annual precipitation falls between November and April. The Water Year (WY) officially begins on 1 October and ends on 30 September. During the 2 y of the study, annual precipitation was roughly 50% below average (175 mm in WY 2013 and 201 mm in WY 2014).

The soil was keyed as a thermic *Pachic Haploxeroll* with silty clay loam texture, pH 6.0, 2.2% C, and 0.21% N. The soil is derived from the Paso-Robles formation, which is poorly consolidated alluvial material formed from montmorillinite that eroded from nearby Monterey Shale deposits, hence the prevalence of 2:1 clays. The soil also contains some amount of Franciscan Complex minerals including ultramafics (e.g., serpentinite), sandstone, and chert. Vegetation is dominated by nonnative Mediterranean annual grasses; primarily *Bromus diandrus*, *Bromus hordaceous*, and *Avena fatua*.

2.2. Field treatments

2.2.1. Plant C input

To create a gradient of plant influence (i.e., both direct effects of root enmeshment and indirect effects of labile C input), grasses and forbs were removed by hand as soon as possible after germination continuously for 2 y. Plots (2 m by 1 m) were selected in December 2012 based on similar initial plant cover and composition; plots were seggregated into three blocks and all treatments were replicated in each block. The site was relatively flat (<2% grade) to minimize lateral flow of water and dissolved C between treatments. All plots were oriented with the longer 2-m side spanning from north to south and marked at the corners with stakes. Plots were spaced at least 1 m apart to minimize edge effects and "sharing" of water between plots.

To manipulate plant C input, we began on 14 December 2012 by removing all existing plant litter (i.e., thatch) from the soil surface gently by hand. Thatch removal was done to minimize any confounding effects of older litter so that each treatment would have similar initial conditions. Based on a comparison with additional plots where thatch was not

removed, there was not a statistically significant effect of thatch removal on soil water content (0–10 cm deep) or temperature (5 cm deep) during the dry season ($P > 0.05$).

We created four levels of plant removal: 0%, 30%, 60%, and 90%. For the 0% plant removal treatment, plots were not altered after the initial thatch removal. For the 30% plant removal treatment, roughly one-third of the aboveground biomass was gently weeded by hand to minimize soil disturbance. Roots were removed too if they easily slid out of the ground without disturbing soil. To achieve 30% plant removal, the 2-m side of the plot was divided into six 33-cm sections with each section roughly equal to three hand-widths. In a random, curvy fashion, one hand-width of plants was pulled from each section, resulting in roughly a one-third removal of aboveground biomass across the entire plot. For the 60% plant removal treatment, two hand-widths of plants were pulled from each section, resulting in roughly a two-third removal of aboveground biomass. For the 90% plant removal treatment, we tried to remove all plants. However, we refrain from referring to the treatment as "100% removal" because of germination between site visits. The plant removal treatments were maintained every 7–10 days during the growing season and as needed during the dry season. Plant removal during the dry season was needed when wind blew litter onto plots and when plants germinated prematurely in the irrigated plots. However, premature germination during summer was surprisingly rare. Our goal was to alter plant C input during the growing season, but not to extend the length of the growing season. Edge effects of root growth from outside the plots were minimized by clearing a ~30-cm perimeter around every plot using a motorized weed whacker and garden sheers. The plant C treatments were maintained through the end of WY 2014.

2.2.2. Dry season length

The four levels of plant C input were crossed with two levels of dry season length ($N = 3$). The dry season in California typically lasts six months; from May until October. For the purpose of assessing plant effects on water-stable aggregates, we aimed to compare the extremes of water availability: extended dry season vs. no dry season. The dry season length was extended until January (i.e., ~9 months total) using rainout shelters (2.5 m by 1.2 m) that intercepted all water before it reached the soil. Each shelter was built from clear corrugated polycarbonate roof panels (Suntuf, Palram Americas, Kutztown, Pennsylvania) attached to a metal frame with two support legs on the higher corners. The higher side of the shelter (~30 cm tall) was oriented on the east side of the plot and the water drained to the lower, west side (~5 cm tall). Each shelter was secured in the plot using metal rebar at the corners. Probably because the shelters were sufficiently ventilated at the sides (i.e., the 1-m side of the plots), we did not observe any consistent effect of the shelters on soil temperature (e.g., greenhouse effect) or soil moisture (e.g., dew accumulation). To extend the WY 2013 dry season, shelters were in place from 7 October 2013 (one week before the first rain event) until 30 January 2014.

The "no-dry-season" treatment was designed to prevent severe micro-scale hydrologic disconnectivity during the typical 6-month dry season (Parker and Schimel 2011). The predetermined threshold between "moist" and "dry" soil was 10% volumetric water content (VWC), equivalent to approximately 9% gravimetric water content and −10 MPa water potential (Priester et al., 2013). Based on previous measurements near our site (Fierer et al., 2005), 10% VWC is associated with a marked decline in soil respiration, suggesting that microbes rapidly lose access to C substrates as soils dry below this water content; consistent with the −14 MPa threshold for diffusion determined by meta-analysis (Manzoni et al., 2012). Therefore, we eliminated the dry season for 2 y by irrigating plots roughly biweekly, starting as soon as soils reached the 10% VWC threshold in the spring. Irrigation continued until the first rain event in the fall. In 2013, irrigation began on 14 May and lasted until 6 November with a total of twelve irrigation events. In 2014, irrigation began on 23 May and lasted until 21 October with a total of ten irrigation events. Each irrigation event consisted of adding 30 L of local well water to each plot using a backpack sprayer with a nozzle to minimize soil disturbance. This amount of water was equivalent to 1.5 cm per plot per irrigation event. Because of the sprayer's 15 L capacity and the desire to allow time for infiltration, the 30 L water was added in two 15 L "doses" spaced roughly 1 h apart. Based on field measurements of soil moisture using a portable MiniTrase Time Domain Reflectometer (Soil Moisture Equipment Corporation, Santa Barbara, California), each irrigation event moistened soils in the top 10 cm to ~25% VWC. Then, on average, soils dried 1% VWC per day, particularly during the middle of the summer when temperatures soared and winds gusted. Thus, irrigation was required every 2 weeks to prevent drying below 10% VWC. Because this study was performed during a severe multiyear drought, it should be noted that the 180 mm of total water added in 2013 and 150 mm in 2014 nearly doubled annual precipitation.

2.3. Field measurements to assess plant controls

2.3.1. Soil sampling

Surface soils (0–10 cm) were collected from field plots on 30 January 2014 and 9 September 2014. On the first date after 1 y of treatments, soils were only sampled from the long-dry-season treatment (across all levels of plant removal) to assess the legacy effect of plants on soil structure after 9 months without precipitation. On the second date after 2 y of treatments, soils were sampled from both the long-dry-season and no-dry-season treatments to assess the persistence of plant effects and the role of soil moisture.

On both dates, we collected one 5-cm diameter soil core from near the center of each plot. Due to the hardness of dry soil, a steel corer and sledgehammer were required to extract the soils, but we tried to minimize disturbance to soil structure by hammering as few times as possible and by gently sliding the soil out of the corer. Soil cores were transferred to individual sealed plastic containers that were rigid to prevent disturbance to soil structure during transport to the laboratory at the University of California Santa Barbara. Field moist soils were first sieved to <9.5 mm to remove large rocks. Any visible plant leaves, stems, or roots were removed by hand. Soil clods and aggregates >9.5 mm were gently broken along natural planes of weakness and then passed through the sieve. Soils were then stored at room temperature (20–22 °C) and air dried for 2 weeks to create a similar starting point before rewetting. This was particularly important in September 2014 when we compared dry soils to irrigated soils.

2.3.2. Aggregate separation

Water-stable soil aggregates were separated by wet sieving into different size classes according to Elliott (1986). Air-dried soils (<9500 μm) were gently mixed with a spoon and a representative subsample was weighed (40 g) into sealed specimen cups until sieving. The soil in each cup was gently poured and spread across a 2000 μm sieve that was placed inside a larger circular plastic tub to hold water. Slaking—or the breakdown of soil aggregates by air pressure buildup—began by slowly pouring deionized water into the plastic tub (i.e., not directly onto the sieve) to gently rewet the aggregates from the bottom-up. Enough water was added to completely submerse all aggregates on the sieve. Aggregates were left undisturbed to slake for 5 min before the sieve was manually moved up and down 3 cm, 50 times for 2 min to allow smaller aggregates to fall through the sieve into the plastic tub. The aggregates that remained on the sieve were gently transferred to a clean, pre-weighed aluminum pan using a squeeze bottle with deionized water. The soil solution that passed through the 2000 μm sieve was then gently poured onto a 250 μm sieve in a second plastic tub, and the 2 min wet-sieving procedure was repeated. The fraction of aggregates between 250 μm and 2000 μm was transferred to a separate aluminum pan. Finally, the soil solution that passed through the 250 μm sieve was poured onto a 53 μm sieve and the 2 min wet-sieving procedure was repeated. All sieves

and tubs were thoroughly brushed and rinsed with water between samples. The soil fractions between 53 μm and 250 μm and <53 μm were transferred to separate aluminum pans. Water was evaporated from the aggregates by placing the four pans from each soil sample into a drying oven at 60 °C for 72–96 h. After drying, any remaining rocks and plant litter were removed from the largest size class using forceps. By subtracting the pan weight from the oven-dry soil in the pan, we calculated the percentage of soil in different size classes. We henceforth refer to water-stable aggregates greater than 2000 μm diameter as "large macroaggregates." Aggregates between 250 μm and 2000 μm are "small macroaggregates" and between 53 μm and 250 μm are "microaggregates." Particles <53 μm are referred to as "clay and silt."

2.3.3. Complementary belowground measurements

To characterize the efficacy of the plant removal treatment as well as field patterns of C and N availability and initial conditions for the 6-month laboratory experiment, we measured soil volumetric water content, plant root biomass, soil C and N pools, and soil microbial biomass.

Soil volumetric water content (VWC) was monitored 0–10 cm deep using a portable MiniTrase Time Domain Reflectometer (Soil Moisture Equipment Corporation, Santa Barbara, CA) with stainless steel waveguides. On 24 sampling dates between 12 March 2013 and 22 September 2014, the waveguides were inserted into the soil in the center of each plot between 10:00 and 12:00. The irrigated plots (i.e., no dry season treatment) were typically measured one week after water addition in 2013 and two weeks after water addition in 2014. Thus, the measurements of soil moisture in the no-dry-season treatment represent the drier extent of conditions experienced in these plots.

Root biomass was measured using wet-sieving (Fogel, 1983). Intact soil cores (5 cm diameter, 10 cm deep) were collected from all plant removal treatments (N = 3) at the end of the first growing season on 24 May 2013. In the laboratory, we first weighed each soil core and then subsampled (10 g) to calculate and correct for soil water content. The remaining soil was gently transferred to a 1 L canning jar. Enough deionized water was added to the beaker to completely cover the soil (~300 ml). The jar was sealed with a lid and placed on a rotary shaker table at 180 rpm for 5 min to gently separate roots from mineral soil. After shaking, mineral soil was allowed to settle for 5 min while roots and other organic matter floated to the top. The floating material was then poured onto a 500 μm sieve and gently rinsed with water using a squeeze bottle. Using forceps, all root fragments were transferred from the sieve to a clean, pre-weighed aluminum pan. The remaining soil in the jar was shaken and sorted for two additional cycles to extract as many roots as possible. Any organic matter that was not obviously a root was not transferred to the pan. Pans were dried at 60 °C for 48 h to calculate the dry weight of roots in each core. Root biomass was expressed as mg g^{-1} soil.

Soil C and N availability in 0% and 90% plant removal plots (long-dry-season treatment only) was estimated in January 2014 using three approaches. First, total C and N concentrations in oven-dried soils were measured using an elemental combustion system (NA 1500 Series 2, Fisons Instruments, Ipswich, United Kingdom). Second, water-extractable C (organic) and N (inorganic and organic) were measured by shaking (180 rpm) 8 g of soil in 32 ml of deionized water in a 50-ml centrifuge tube for 3 h. After shaking, the tubes were centrifuged (3000 *g*, 15 min) and the supernatant (10 ml) was pipetted into a clean 12-ml centrifuge tube and stored at −20 °C until analysis on a total organic carbon analyzer (TOC-V CSN, Shimadzu Scientific Instruments, Columbia, Maryland). Finally, the most labile pool of soil C was estimated by the respiration rate following rewetting. In a 125-ml canning jar equipped with an airtight butyl rubber septum, 10 ml of deionized water was added to 5 g of soil and the slurry was shaken (180 rpm) for 2.5 h. The headspace carbon dioxide (CO_2) concentration was measured 0.5, 1.5, and 2.5 h after adding water. Each gas sample (1 ml) was collected using a glass syringe with side-port needle, and then immediately measured using a non-dispersive infrared gas analyzer (LI-820, LiCor, Inc., Lincoln, Nebraska). Rates of CO_2 production were expressed as μg CO_2-C per gram of oven-dry soil equivalent per hour.

Soil microbial biomass was compared across field and laboratory treatments by substrate-induced respiration (Anderson and Domsch, 1978). Because we used glucose as the sole substrate, this assay is henceforth referred to as "glucose-induced respiration." The procedure for glucose-induced respiration was almost identical to the wet-up respiration assay described in the previous paragraph. The only difference was that a glucose solution (50 mg glucose dissolved in 1 ml ultrapure water) was added to the soil slurry before sealing the jars. The amount of glucose added (10 mg g^{-1} soil) was based on an optimum found by West and Sparling (1986). Glucose is known to be a straightforward indicator of biological activity in this soil (Blankinship et al., 2014).

2.4. Laboratory experiments to assess microbial mechanisms

To separate biotic and abiotic mechanisms that form water-stable macroaggregates, we manipulated microbial biomass and soil moisture in the laboratory. The first experiment lasted six months to simulate the length of a typical dry season, allowing ample time for aggregate formation (e.g., De Gryze et al., 2005). The second follow-up experiment lasted one week to see how quickly the treatment effects appeared.

2.4.1. Long-term sterilized soil incubations

We began by collecting soils (0–10 cm) on 30 January 2014 from the long-dry-season treatment, both in plots with plants (0% removal) and without plants (90% removal). Soils from all the plots with plant C input were combined and homogenized to create a single composite sample. Similarly, all soils without recent plant C input were combined and homogenized. The two composite samples were sieved (9.5 mm) and air-dried in the laboratory for two weeks to normalize any differences in soil moisture. Large rocks and roots were removed by hand.

To quantify macroaggregate formation, soil incubations began with only microaggregates and other particles (i.e., silt and clay) with a diameter less than 250 μm. Dry-sieving was first used to separate the <250 μm fraction. The >250 μm fraction (i.e., macroaggregates) was disaggregated using a coffee grinder (~1 min per sample) and then shaken through the 250 μm sieve. The grinding efficiency was roughly 90% (i.e., ~10% of macroaggregates could not be disaggregated). Soil subsamples (N = 3) from both treatments were used to measure initial levels of gravimetric water content, water-extractable organic C, and microbial biomass.

For six months, soils were exposed to all combinations of three treatments (N = 3): fresh C input (with vs. without plants); moisture (5%, 10%, 15%, 20% of water-holding capacity); and microbial biomass (live vs. sterile). The water-holding capacity (WHC) of ground soil was 0.75 g H_2O g^{-1} soil, as measured by saturation on Whatman Grade 1 filter paper followed by 24 h of draining under 100% humidity. A soil water content of 5% WHC is typical during the summer dry season and 20% WHC is typical during the winter wet season; 10% and 15% WHC represent the intermediate drying phase during spring. Ground air-dry soils (<250 μm) at 5% WHC were weighed (45 g) into 50-ml glass beakers. The depth of soil in each beaker was ~4 cm. For the 10%, 15%, and 20% WHC treatments, deionized water was added using a spray bottle with a fine mist. Our aim was to gently rewet the soil while vertically distributing the water as evenly as possible. The 45 g soil subsample was added as four ~11.25 g layers (gently agitating the beaker to level soil between layers) with a certain number of sprays (~0.1 g H_2O per spray) preceding each layer. For the 10% WHC treatment, there were four sprays per layer (i.e., 16 sprays total). For 15% WHC, there were eight sprays per layer (32 sprays total). For 20% WHC, there were 12 sprays per layer (48 sprays total). Based on visual observation, the discrete layers of water diffused and disappeared within one week. After manipulating soil moisture, each beaker was immediately placed into individual 1 L canning jars and sealed with airtight lids to prevent drying. The 10%, 15%, and 20% WHC treatments also included 5 ml of

additional water that was pipetted to the bottom of the jar (i.e., outside of the beaker) to maintain 100% humidity in the jar to minimize evaporation from the soil during the incubation. Our intent was to investigate discrete, constant levels of soil moisture rather than rewetting cycles.

To kill soil microbes without adding water, half of the incubation jars were continuously fumigated with ethanol-free chloroform ($CHCl_3$). In contrast to the continuous sterilization of $CHCl_3$ vapor, other common sterilants (e.g., sodium azide, mercuric chloride, Captan fungicide, and oxytetracycline bactericide) must be added in combination with water and may also require repeated applications to prevent microbial regrowth. In a fume hood, the jars assigned to the sterilized treatment were opened and a 12 ml vial with $CHCl_3$ was carefully placed inside next to the beaker. The jars were then sealed using lids with Teflon tape wrapped around the rubber seal to prevent corrosion. Using a vacuum pump, we evacuated each jar and allowed air to rush back in to enhance $CHCl_3$ movement into soil micropores.

The jars were incubated in a vented, temperature-controlled room in the dark for six months at 30 °C. This temperature is common during the dry season in surface soils at our field site. Jars in the live treatment were vented weekly to prevent CO_2 buildup and oxygen depletion. Jars in the sterilized treatment, which are known to have very low rates of CO_2 production (Blankinship et al., 2014), were vented monthly in the fume hood and refilled with fresh $CHCl_3$ as needed. At the end of the six months, we vented the jars, wiped off excess water on the outside of the beaker, and recorded the final weight of each beaker of soil to ensure proper gravimetric water content. Using a spatula, a 5 g subsample was carefully scraped off the top corner of the soil in the beaker and immediately (<1 h) used to estimate microbial biomass by glucose-induced respiration, as described earlier. The remaining soil (~40 g dry weight) was placed in a drying oven at 60 °C for 16 h to quickly dry soils before aggregate separation. For the aggregate separation procedure, it is important that all soils begin at a similar, dry state so that the air pressure buildup is comparable following rewetting (Haynes and Beare, 1997). Previous tests showed that 16 h was a sufficient amount of time to dry soils from all moisture treatments. The goal was to maximize the drying rate in order to minimize any microbial regrowth after $CHCl_3$ removal. After recording the dry soil weight, a fine scalpel was used to gently transfer and spread soil from the beaker onto a 250 μm sieve. Water-stable macroaggregates that remained on the sieve after 5 min of slaking and 2 min of wet-sieving were transferred to aluminum pans, dried, and weighed as described earlier. We calculated the percentage of soil mass that was incorporated into water-stable macroaggregates. Because our aim was to quantify the formation of macroaggregates from microaggregates, silt, and clay, we did not distinguish between large (>2000 μm) and small macroaggregates (250–2000 μm) in the laboratory experiment.

2.4.2. Short-term sterilized soil incubations

Results from the 6-month incubations motivated a shorter-term, follow-up experiment to see how quickly the effects of water and microbes appear. Is it really necessary to run an incubation for 6 months to observe the formation of macroaggregates by water and microbes? The procedure for the short-term incubations was the same as for the long-term incubations, except that: (i) incubations lasted one week; (ii) soils were collected on 9 September 2014; and (iii) only soils from plots with plants were included.

2.5. Aggregate imaging

We imaged aggregates after seeing differences in the shape of biotically and abiotically formed aggregates with the naked eye. Macroscopic images of oven-dried water-stable aggregates were captured using a point-and-shoot camera (PowerShot ELPH 310 HS, Canon Inc., Tokyo, Japan). Microscopic images were captured using an Environmental Scanning Electron Microscope (ESEM) at the Micro-Environmental Imaging and Analysis Facility at University of California Santa Barbara. The system included an XL30 ESEM (FEI Company, Hillsboro, Oregon) with field emission gun, patented gaseous secondary electron detector, and temperature-controlled Peltier stage to control condensation (400 Pa). We imaged air-dry, water-stable macroaggregates isolated from fresh soils collected on 2 February 2015 in plots with and without plants (N = 4). We also imaged air-dry and rewetted biogenic (live, 10% WHC) and physicogenic (sterilized, 20% WHC) macroaggregates isolated at the end of the laboratory incubations (N = 4).

2.6. Statistical analyses

The mean weight diameter for each soil sample was calculated by multiplying the mean diameter of each size class (5750, 1125, 151.5, and 26.5 μm) by the proportion of soil mass in each size class and then adding the four products. JMP 12 software (SAS Institute, Cary, North Carolina) was used for all statistical tests. Effects of field plant removal on aggregate size were analyzed using both linear regression (with four levels of plant removal on *x*-axis) and pooled *t*-tests at an alpha level of 0.05 to compare plots with plants (0% removal) to plots without plants (90% removal). No transformations were required. Effects of soil moisture in the laboratory were identified using Tukey HSD post hoc tests at an alpha level of 0.05 for each combination of microbial biomass and plant removal treatments.

3. Results

3.1. Effects of plant removal: field experiment

Removing 1 year of plant growth caused an 87% decrease in root biomass, 35% decrease in the respiration pulse upon rewetting, and four-fold increase in water-extractable N (Table 1). Root biomass in the 30% and 60% plant removal plots was 1.9 (±0.8) and 1.3 (±0.6) mg g^{-1} soil, respectively, which was intermediate to the 0% and 90% plant removal plots, but more similar to 90% removal. Soil moisture was similar between the 0%, 30%, and 60% plant removal treatments; but soils in the 90% plant removal treatment tended to be wetter during the dry season, particularly early in the dry season and in 2013 (Fig. 1). In 2014, all plant growth was stunted by the lack of late-fall and early-winter precipitation. In a one-way ANOVA, statistically significant differences ($P < 0.05$) between 0% and 90% plant removal lasted from 22 March until 2 July in 2013, and from 5 May until 5 June in 2014. Statistically significant differences in soil moisture between the long dry season and no dry season treatments lasted from 24 May until 18 December in 2013 and from 5 June until 22 September in 2014. There was not a statistically significant effect of plant removal on soil microbial biomass

Table 1
Effect of 1 year of plant removal on belowground properties (0–10 cm deep) in a seasonally dry grassland near Santa Ynez, California. All variables were measured near the end of the 2013 dry season to characterize initial conditions for the laboratory soil incubations. Parentheses show standard errors (N = 3). Asterisks (*) indicate significant difference between 0% plant removal ('with plants') and 90% plant removal ('without plants') in a pooled *t*-test at an alpha level of 0.05.

Response variable	Units	With plants	Without plants	p-value
Root biomass	mg roots g^{-1} soil	6.0 (1.4)	0.8 (0.3)	0.024*
Wet-up respiration	μg CO_2-C g^{-1} soil h^{-1}	5.7 (0.1)	3.7 (0.4)	0.0064*
Glucose-induced respiration	μg CO_2-C g^{-1} soil h^{-1}	6.6 (0.1)	6.9 (0.2)	0.32
Water-extractable organic C	μg C g^{-1} soil	151.4 (12.4)	206.5 (23.4)	0.11
Water-extractable N	μg N g^{-1} soil	11.7 (1.1)	48.8 (10.3)	0.023*
Total C	mg C g^{-1} soil	21.6 (1.2)	24.9 (1.5)	0.12
Total N	mg N g^{-1} soil	1.9 (0.1)	2.3 (0.2)	0.12

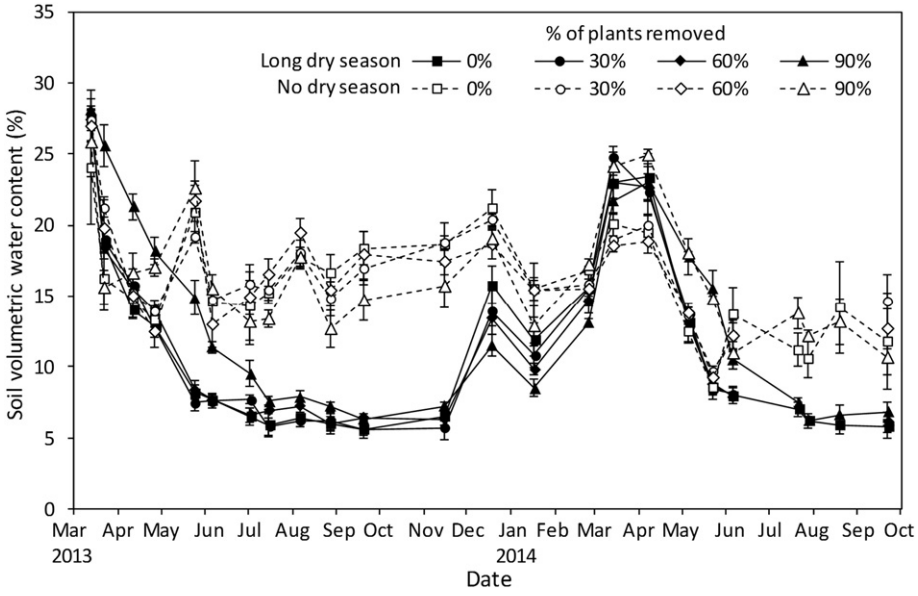

Fig. 1. Soil volumetric water content (0–10 cm deep) in field plots with varying levels of plant removal and irrigation. Symbols show mean and standard error bars. The long dry season plots experienced ambient precipitation. In order to prevent soils from drying below 10% volumetric water content, the no dry season plots were irrigated approximately biweekly between 14 May and 6 November in 2013 and between 23 May and 21 October in 2014.

(as estimated by glucose-induced respiration), total water-extractable organic C, or total C and N.

Removing plants reduced the average size of water-stable soil aggregates by 22–33% regardless of dry season length or duration of plant removal (Fig. 2). All linear regressions of plant removal and the mean weight diameter of soil aggregates were statistically significant ($P < 0.05$). The negative effect of plant removal on the average size of water-stable aggregates was primarily driven by a shift from large macroaggregates to small macroaggregates and microaggregates (Fig. 3). This pattern did not depend on dry season length. In the long-dry-season plots, 2 years of plant removal decreased the soil mass in large macroaggregates by 13.1%, which was redistributed into small macroaggregates (+7.3%), microaggregates (+4.5%), and the silt and clay fraction (+1.3%). In the no-dry-season plots, plant removal decreased the fraction of large macroaggregates by 23.1%, which was primarily distributed into small macroaggregates (+18.1%), but also microaggregates (+3.4%) and silt and clay (+1.6%).

Large macroaggregates collected late in the dry season from plots with plants generally showed larger pores than plots without plants (Fig. 4). Also, the binding effects of dead roots and fungal hyphae were easy to find when plants were present. In plots without plants, however, we did not observe any obvious plant-derived detritus in macroaggregates.

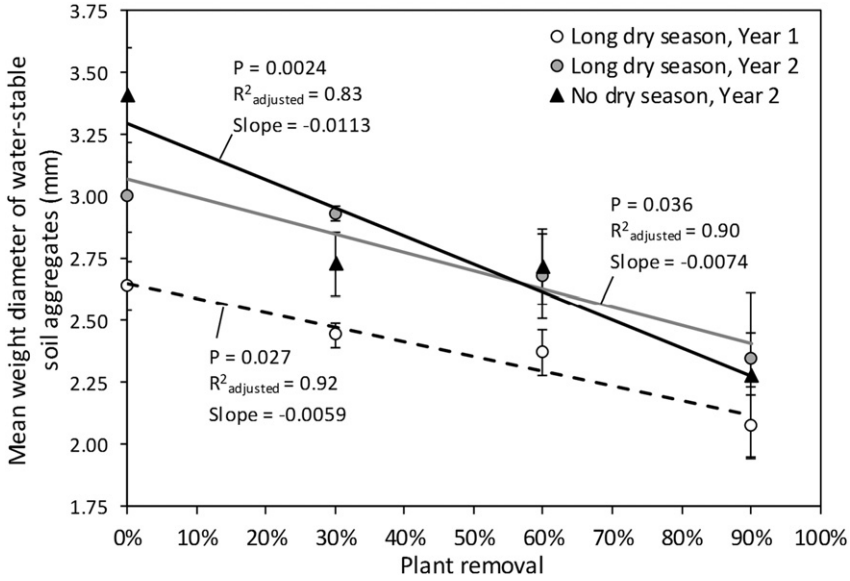

Fig. 2. Linear regressions showing the influence of grassland plant removal on the average size of water-stable soil aggregates. 'Long dry season, Year 1' soils (*dashed line*) were collected in January 2014 after 9 months without precipitation and 1 y of plant removal treatments established near Santa Ynez, California. 'Long dry season, Year 2' soils (*gray line*) were collected from the same plots in September 2014 after six months without precipitation and 2 y of plant removal. 'No dry season, Year 2' soils (*black line*) were collected in September 2014 from different plots exposed to 2 years of plant removal and biweekly irrigation to prevent soil desiccation. Points show the mean and standard error for each combination of plant removal, water availability, and year ($N = 3$).

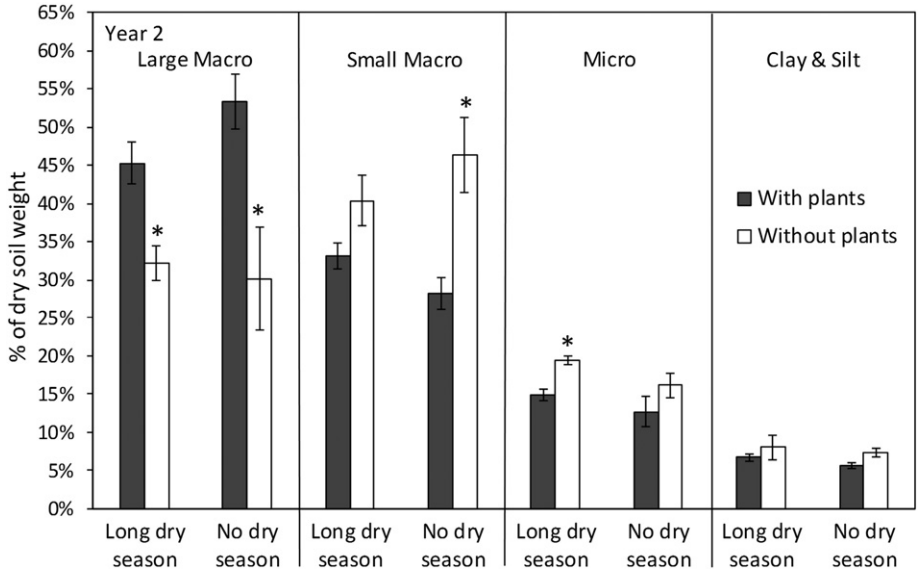

Fig. 3. Effect of 2 years of grassland plant removal (0% vs. 90% removal) on the size distribution of water-stable soil aggregates. 'Long dry season' soils were exposed to ambient conditions with typical summer dryness. 'No dry season' soils were irrigated biweekly during the summer to prevent drying. Large macroaggregates ('large macro') were 2000–9000 μm in diameter. Small macroaggregates ('small macro') were 250–2000 μm. Microaggregates ('micro') were 53–250 μm. Clay and silt were less than 53 μm. Columns and bars show means and standard errors (N = 3). Asterisks (*) indicate statistically significant effect of plant removal in a pooled *t*-test at an alpha level of 0.05.

3.2. Effects of microbe removal: lab experiment

After six months of continuous $CHCl_3$ fumigation in the laboratory, soil microbial biomass (as estimated by glucose-induced respiration) in the sterilized treatment was 82–99% lower than the live treatment without $CHCl_3$, regardless of soil moisture or plant treatments (Fig. 5). In the sterilized treatment, water-stable macroaggregate formation increased with increasing soil moisture, both after six months (Fig. 6) and one week (Fig. 7). After one week, an average of 0.5% of soil mass was incorporated into macroaggregates under the driest condition and

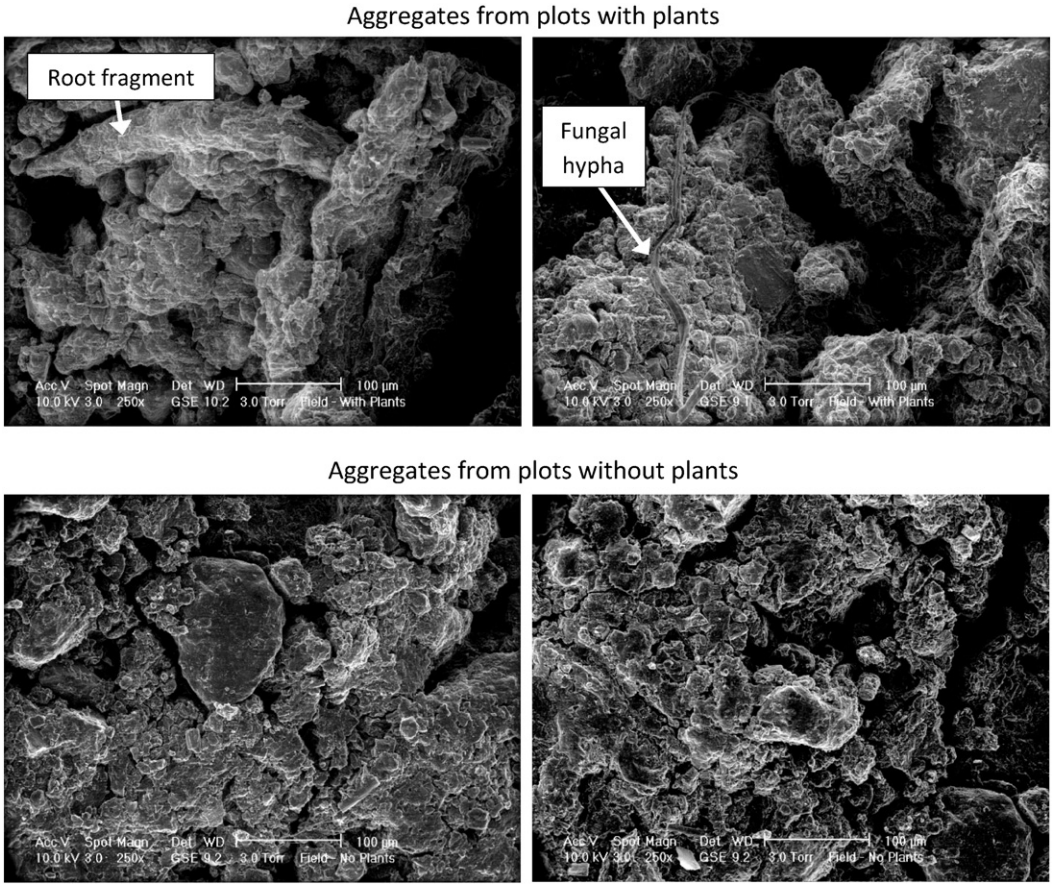

Fig. 4. Environmental scanning electon microscope (ESEM) images of "field-fresh" water-stable macroaggregates in grassland soils with plants (*top*) and in plots where plants were removed by hand for 2 years (*bottom*). Scale bars at the bottom of each image show 100 μm.

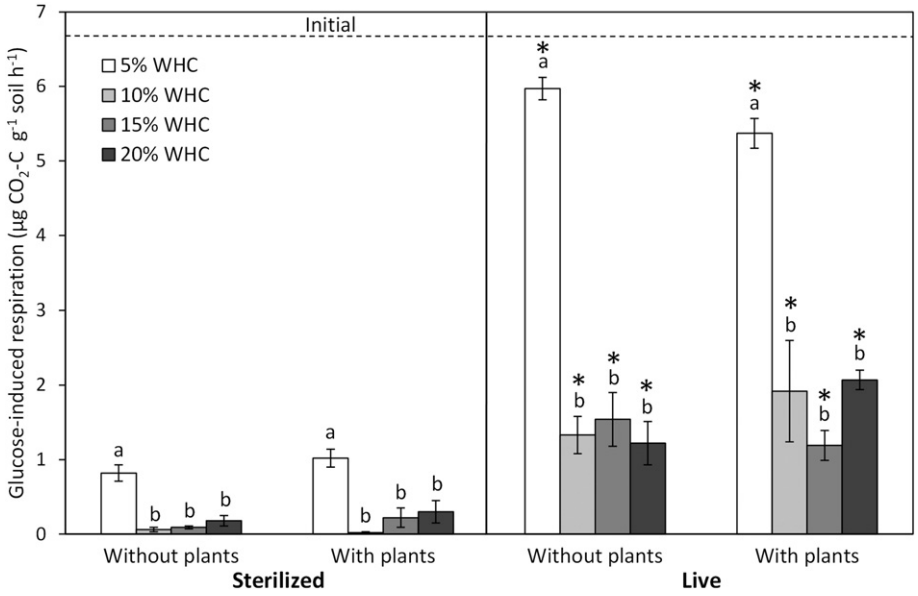

Fig. 5. Soil microbial biomass estimated by glucose-induced respiration after six months of laboratory exposure to all combinations of three treatments: (i) moisture (5%, 10%, 15%, and 20% of soil water-holding capacity (WHC)); (2) fresh carbon input (with and without plants); and (3) sterilization (with and without chloroform). 'Sterilized' soils were continuously fumigated with chloroform vapor and 'live' soils had no chloroform. Columns and bars show means and standard errors (N = 3). Letters indicate effects of altered soil moisture for each treatment combination using Tukey HSD post hoc tests at an alpha level of 0.05. An asterisk (*) indicates significant difference between live and sterile treatments in *t*-test at an alpha level of 0.05 for each combination of moisture and plant C input. Dashed line shows the initial level of glucose-induced respiration for all treatments at the beginning of the 6-month incubations.

2.5% under the wettest condition. After six months, 0.4% of soil mass was incorporated into water-stable macroaggregates under the driest condition and 23% of soil mass under the wettest condition. The effect of moisture did not depend on recent plant C input.

In the live treatment, macroaggregate formation after six months was greatest in soils at intermediate moisture (Fig. 6). Regardless of plant removal, 12–17% of soil mass was incorporated into macroaggregates under intermediate soil moisture, representing a 13-fold increase compared to sterilized soil at 10% WHC and a two-fold increase compared to sterilized soil at 15% WHC. Under the driest condition (i.e., 5% WHC), macroaggregate formation in the live treatment was similar to the sterilized treatment. Under the wettest condition (i.e., 20% WHC), however, macroaggregate formation declined by 75% in the live treatment as compared to the sterilized treatment. The 1-week follow-up experiment showed very little macroaggregate formation in the live treatment, with a slight increase from 0.5% of soil mass under the driest condition to 0.8% under the wettest condition.

The macroaggregates that formed when soil was sterile and moist were visually distinct from macroaggregates formed when soil was live and dry, both at macro- and micro-scales (Fig. 8). The large macroaggregates formed under sterile conditions—henceforth referred to as physicogenic aggregates—tended to be flat with jagged, angular edges. These physicogenic aggregates showed stacks of silt and microaggregates with a greater prevalence of angular micro-structures and fewer spherical micro-structures. The macroaggregates formed under live conditions—henceforth referred to as biogenic aggregates—tended to be spherical with rounded edges. These biogenic aggregates showed a greater prevalence of spherical micro-structures and generally larger pores.

4. Discussion

By systematically removing plants and microbes, we aimed for a more integrative understanding of the biotic and abiotic processes that form and preserve water-stable aggregates on the drier side of the soil moisture spectrum. The gradient of plant removal in the field allowed us to quantify the role of plants in preserving water-stable aggregates. At the same time, sterilizing soils in the laboratory allowed us to determine the role of microbes in forming macroaggregates compared to abiotic processes alone.

Plants were important for stabilizing large macroaggregates; the plots without plants had a lower proportion of large macroaggregates despite being wetter on average. We are uncertain why the mean weight diameter of aggregates was larger in the second year of the field study; perhaps this was because the first year had less precipitation overall and an extremely dry second half of the growing season which may have stunted the growth of soil macroaggregates. We did not measure root or aboveground plant biomass in both years, so we cannot confirm this mechanism. Without summer irrigation, soil lost from the large macroaggregates tended to distribute evenly into small macroaggregates and microaggregates. With irrigation, soil lost from large macroaggregates was primarily distributed into small macroaggregates. Therefore, plants—directly or indirectly—appear to have connected small macroaggregates to make large macroaggregates. However, at least after two years, there was little evidence that fewer small macroaggregates formed, suggesting that microbes and abiotic processes played a stronger role than plants in stabilizing small macroaggregates. We did not sample the field treatments often enough to characterize seasonal variation in aggregate size, but we can reasonably assume that few aggregates were formed during the middle and later parts of the dry season when plants were dead and microbes were hydrologically disconnected from their resources. The aggregates we isolated late in the dry season were likely formed during the previous wet season and spring dry-down period. Our results indicate that dead, plant-associated binding agents—primarily roots—persist through the dry season, probably because the binding agents were physically inaccessible to other microbial decomposers. The chemical resources and physical soil structure that plants depend on during wet periods can be sustained during dry periods. Therefore, at least in the field, the legacy of dead plants on soil structure was greater than we expected.

The preservation of large macroaggregates by plants was enhanced by irrigation rather than weakened. We hypothesized that water addition would accelerate decomposition of plant binding agents and therefore decrease the average size of water-stable soil aggregates, particularly when combined with plant removal. Instead, water addition actually increased the formation of large macroaggregates *if* plants were present,

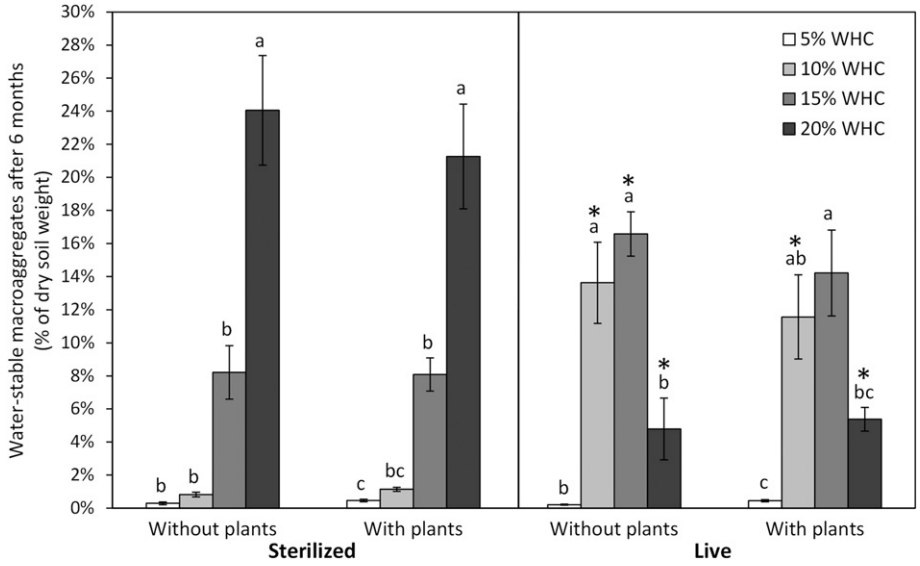

Fig. 6. Effect of soil moisture on the formation of water-stable macroaggregates (>250 μm) as a function of microbial biomass and plant carbon input in a six-month incubation experiment. All treatments began with no macroaggregates and were maintained in the laboratory at 5%, 10%, 15%, and 20% of soil water-holding capacity (WHC). 'Sterilized' soils were continuously fumigated with chloroform vapor and 'live' soils had no chloroform. Soils 'without plants' were collected from field plots where plants were removed for 1 y, and soils 'with plants' were from unmanipulated plots. Columns and bars show means and standard errors (N = 3). Letters indicate effects of altered soil moisture for each treatment combination using Tukey HSD post hoc tests at an alpha level of 0.05. An asterisk (*) indicates significant difference between live and sterile treatments in *t*-test at an alpha level of 0.05 for each combination of moisture and plant C input.

perhaps due to increased activity of roots and mycorrhizae. Therefore, plant–aggregate interactions depended on dry season length. Surprisingly, the combination of two years of plant removal and water addition did not cause a collapse in soil structure (although it did cause some surface crusting). If the soil binding agents were readily soluble and decomposable by microbes, then water addition should have destroyed macroaggregates. Instead, water favored macroaggregate formation, perhaps by increasing fungal enmeshment or by allowing microbes to access C substrates to make EPS glues. However, effects of irrigation on soil aggregate stability were small compared to plant removal.

The role of microbes in engineering dry soil became clearer in the laboratory. After incubating soils for six months, the positive effect of microbes on macroaggregate formation was indeed most prominent

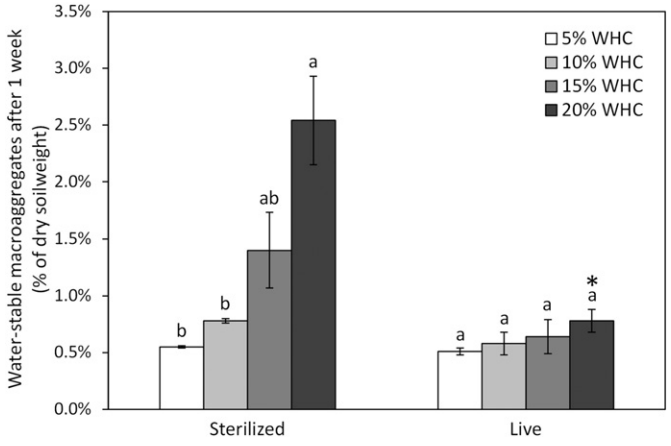

Fig. 7. Effect of soil moisture on the abiotic and biotic formation of water-stable macroaggregates after a one-week incubation experiment. All laboratory treatments began with recent plant carbon inputs and no macroaggregates. Soils were maintained at 5%, 10%, 15%, and 20% of water-holding capacity (WHC). 'Sterilized' soils were continuously fumigated with chloroform vapor whereas 'live' soils had no chloroform. Columns and bars show means and standard errors (N = 3). Letters indicate effects of soil moisture (or lack thereof) for each sterilization treatment using Tukey HSD post hoc tests at an alpha level of 0.05. An asterisk (*) indicates significant difference between live and sterile treatments in *t*-test at an alpha level of 0.05 for each combination of moisture and plant C input.

at intermediate levels of soil moisture. This pattern was not evident after one week suggesting that aggregate formation by both microbes and abiotic processes requires months to occur. It is also quite possible that soil microbial communities were negatively affected by the extreme disturbance of sieving and grinding, and one week was insufficient for communities to recover. At 10% of soil water-holding capacity (WHC), macroaggregate formation increased ten-fold in the presence of live microbes compared to sterile conditions. We hypothesize that under the driest conditions (5% WHC), which was approximately −40 MPa for this soil (Priester et al., 2013), microbes could not access C to be active (and hence to make EPS) or to engage any other mechanism they may be using to produce aggregates. There was also apparently insufficient water to form oxide "cements" and cation bridges (which are likely active in the sterile, moist treatment). However, if provided a small amount of water (10% WHC), equivalent to adding 40 ml of water per kg soil, perhaps C substrates were able to diffuse to microbes with incentive to connect the dry soil matrix to access more resources, either via fungal hyphal growth or bacterial EPS production (Roberson and Firestone, 1992; Ophir and Gutnick, 1994; Alvarez et al., 2004). The 10% WHC treatment was equivalent to a soil water potential of −10 MPa which is wetter than the −14 MPa threshold required for diffusion (Manzoni et al., 2012). Regardless of whether the pattern was driven by fungi or bacteria, our results indicate that relatively little water was required to allow microbes to form macroaggregates.

The small amount of water required to initiate the formation of biogenic macroaggregates has at least three far-reaching implications. First, this pattern suggests that as long as there is enough moisture to encourage measurable microbial activity, microbes will act to create aggregates. Second, small precipitation events in arid and seasonally-dry ecosystems may have big effects on soil structure by activating microbes. Third, if we want to encourage native soil microbes to produce beneficial effects in building soil structure in drought-prone agriculture and other management contexts (e.g., restoration after wildfire, reduced windblown dust), then irrigation water can be conserved. A relatively small cost in water may have large benefits for restoring soil structure.

Although abiotic processes did not appear to be important for macroaggregate formation under the driest conditions, abiotic processes mattered in wetter soil. The positive effect of water on aggregate

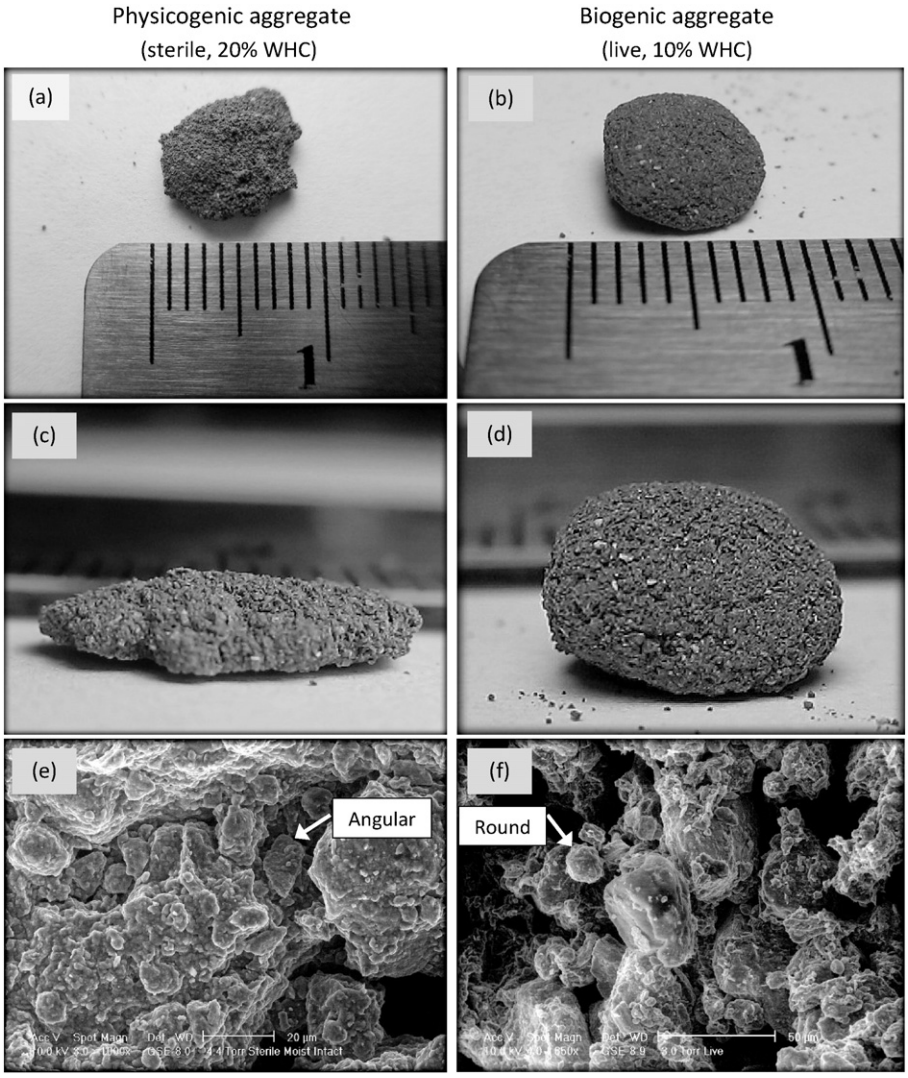

Fig. 8. Images of water-stable macroaggregates formed during 6-month soil incubations under sterile moist conditions (*left side*) and live dry conditions (*right side*). Overhead macroscopic views show the jagged, angular shape of the abiotically formed aggregates (*a*) and the round shape of the biotically formed aggregates (*b*; scale shows 1 cm with mm increments). Side macroscopic views show the planar shape of physicogenic aggregates (*c*) and the three-dimensional, spherical shape of biogenic aggregates (*d*). Environmental scanning electron microscope (ESEM) images show the microstructure of physicogenic (*e*; scale bar shows 20 μm) and biogenic aggregates (*f*; scale bar shows 50 μm).

formation was detectable after one week and after six months abiotic forces had aggregated >20% of the soil mass—in fact more soil was in macroaggregates in the sterile 20% WHC condition than was in aggregates in any of the live soil treatments. Perhaps water was required to allow for mixing of oxide cements and arranging of cation bridges. In the case of cation bridges, water likely plays a role in transporting cations to ideal locations to connect clay surfaces with organic matter. If cations and organic matter must diffuse to build connections among particles, then both water and time may be required to develop water-stable physicogenic aggregates.

Surprisingly, under the wettest conditions simulated in the laboratory (20% WHC), which is typical during the wet season, microbes suppressed macroaggregate formation by 75% compared to sterilized soil and by 62% relative to the live soil at 15% WHC. One hypothesis for this decrease might be that fungi are primarily responsible and that they prefer drier conditions (Cornejo et al., 1994; Treseder et al., 2010; Yuste et al., 2011), in which case maybe 20% WHC would be too wet. This mechanism, however, would not explain the discrepancy between the live and sterilized treatments. There is no obvious mechanism by which *less* fungi would interfere with abiotic binding agents under wet conditions. The discrepancy indicates that biology was somehow interfering with the chemical reactions that form physicogenic macroaggregates. Alternative hypotheses would include: (1) microbes invested less C in EPS production under the wettest conditions because soil was more hydrologically connected; and (2) microbes consumed organic matter or somehow otherwise prevented connections between organic matter and cations. Fungi can translocate calcium and magnesium (Clarholm and Skyllberg, 2013), and both bacteria and fungi exude low-molecular-mass organic acids that may destabilize cation bridges by chelating the cations (Clarholm et al., 2015). Although the mechanism(s) still needs to be identified, our results reveal that microbes do not necessarily benefit soil aggregate stability.

We did not expect such small effects of field plant removal in the laboratory soil incubations. As opposed to the direct effects of root enmeshment and/or exudates in the field, the laboratory measurements were intended to isolate the indirect effects of plant litter via microbial priming. Neither abiotic nor biotic patterns of macroaggregate formation depended on the presence of fresh root residues. Therefore, as stated earlier, mechanisms of abiotic aggregate binding do not necessarily involve fresh plant litter. Furthermore, biotic patterns were also independent of plant removal suggesting that microbes did not depend on fresh plant C to make EPS; the most accessible C during drought was probably C associated with the mineral soil. This interpretation agrees with the hypothesis that labile C (i.e., plant C) has a transient effect on aggregate

stability whereas recalcitrant C (i.e., mineral-soil C) has less intense but longer-lasting effects (Abiven et al., 2009).

Our results also suggest that the shape of a macroaggregate may provide information about how it was formed (Oades, 1993; Pulleman et al., 2005; Velasquez et al., 2007). Physicogenic macroaggregates formed under sterilized, moist (20% WHC) conditions appeared planar and angular at both macro- and micro-scales. In contrast, biogenic macroaggregates formed under live, dry (10% WHC) conditions appeared spherical with rounded edges. Perhaps the planar structure of physicogenic aggregates reflects the underlying unidirectional binding of clay platelets, whereas the spherical structure of biogenic aggregates reflects the random, multidirectional binding of microbial glues and hyphae. Therefore, macroaggregate shape might be helpful in monitoring the success of field efforts to improve soil aggregate stability using native or introduced microbes. However, it remains unclear how the shape and origin of macroaggregates relates quantitatively to soil C sequestration, nutrient retention, or erosion control.

To conclude, on the drier side of the soil moisture spectrum, when there is not enough water for the abiotic formation of water-stable macroaggregates, biology can engineer macroaggregates. Dead plant roots can indeed help sustain large macroaggregates during the dry season, whereas microbes sustain both small and large macroaggregates. Therefore, both dead plants and live microbes have roles in maintaining structure in dry soils. There are clearly non-additive interactions between the biotic and abiotic processes that form beneficial water-stable macroaggregates in soil, but these interactions require water. In drier soil, microbiology dominates.

Acknowledgments

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References

Abiven, S., Menasseri, S., Chenu, C., 2009. The effects of organic inputs over time on soil aggregate stability—a literature analysis. Soil Biol. Biochem. 41, 1–12.
Alvarez, H.M., Silva, R.A., Cesari, A.C., Zamit, A.L., Peressutti, S.R., Reichelt, R., Keller, U., Malkus, U., Rasch, C., Maskow, T., Mayer, F., Steinbüchel, A., 2004. Physiological and morphological responses of the soil bacterium *Rhodococcus opacus* strain PD630 to water stress. FEMS Microbiol. Ecol. 50, 75–86.
Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem. 10, 215–221.
Blankinship, J.C., Becerra, C.A., Schaeffer, S.M., Schimel, J.P., 2014. Separating cellular metabolism from exoenzyme activity in soil organic matter decomposition. Soil Biol. Biochem. 71, 68–75.
Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. Appl. Soil Ecol. 16, 195–208.
Briar, S.S., Fonte, S.J., Park, I., Six, J., Scow, K., Ferris, H., 2011. The distribution of nematodes and soil microbial communities across soil aggregate fractions and farm management systems. Soil Biol. Biochem. 43, 905–914.
Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. Geoderma 124, 3–22.
Chantigny, M.H., Angers, D.A., Prévost, D., Vézina, L.-P., Chalifour, F.-P., 1997. Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems. Soil Sci. Soc. Am. J. 61, 262–267.
Chenu, C., 1989. Influence of fungal polysaccharide, scleroglucan, on clay microstructures. Soil Biol. Biochem. 21, 299–305.
Chenu, C., Roberson, E.B., 1996. Diffusion of glucose in microbial extracellular polysaccharide as affected by water potential. Soil Biol. Biochem. 28, 877–884.
Cheshire, M.V., 1977. Origins and stability of soil polysaccharide. J. Soil Sci. 28, 1–10.
Clarholm, M., Skyllberg, U., 2013. Translocation of metals by trees and fungi regulates pH, soil organic matter turnover and nitrogen availability in acidic forest soils. Soil Biol. Biochem. 63, 142–153.
Clarholm, M., Skyllberg, U., Rosling, A., 2015. Organic acid induced release of nutrients from metal-stabilized soil organic matter—the unbutton model. Soil Biol. Biochem. 84, 168–176.
Cornejo, F.H., Varela, A., Wright, S.J., 1994. Tropical forest litter decomposition under seasonal drought: nutrient release, fungi and bacteria. Oikos 70, 183–190.
Cosentino, D., Chenu, C., Bissonnais, Y.L., 2006. Aggregate stability and microbial community dynamics under drying-wetting cycles in a silt loam soil. Soil Biol. Biochem. 38, 2053–2062.
De Gryze, S., Six, J., Brits, C., Merckx, R., 2005. A quantification of short-term macroaggregate dynamics: influences of wheat residue input and texture. Soil Biol. Biochem. 37, 55–66.
Degens, B.P., 1997. Macro-aggregation of soils by biological bonding and binding mechanisms and the factors affecting these: a review. Aust. J. Soil Res. 35, 431–459.
Degens, B.P., Sparling, G.P., 1995. Repeated wet-dry cycles do not accelerate the mineralization of organic C involved in the macro-aggregation of a sandy loam soil. Plant Soil 175, 197–203.
Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Soil Biol. Biochem. 33, 1599–1611.
Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J. 50, 627–633.
Fierer, N., Chadwick, O.A., Trumbore, S.E., 2005. Production of CO_2 in soil profiles of a California annual grassland. Ecosystems 8, 412–429.
Flemming, H.-C., Wingender, J., 2010. The biofilm matrix. Nat. Rev. 8, 623–633.
Fogel, R., 1983. Root turnover and productivity of coniferous forests. Plant Soil 71, 75–85.
Foster, R.C., 1981. Polysaccharides in soil fabrics. Science 214, 665–667.
Ghani, A., Dexter, M., Perrott, K.W., 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biol. Biochem. 35, 1231–1243.
Gupta, V.V.S.R., Germida, J.J., 1988. Distribution of microbial biomass and its activity in different soil aggregate sizes classes as affected by cultivation. Soil Biol. Biochem. 20, 777–787.
Haynes, R.J., Beare, M.H., 1997. Influence of six crop species on aggregate stability and some labile organic matter fractions. Soil Biol. Biochem. 29, 1647–1653.
Haynes, R.J., Francis, G.S., 1993. Changes in microbial biomass C, soil carbohydrate composition and aggregate stability induced by growth of selected crop and forage species under field conditions. J. Soil Sci. 44, 665–675.
Holden, P.A., 2011. How do the microhabitats framed by soil structure impact soil bacteria and the processes they catalyze? In: Ritz, K., Young, I. (Eds.), The Architecture and Biology of Soils: Life in Inner Space. CAB International, Oxfordshire, pp. 1–62
Hu, S., Coleman, D.C., Beare, M.H., Hendrix, P.F., 1995. Soil carbohydrates in aggrading and degrading agroecosystems: influences of fungi and aggregates. Agric. Ecosyst. Environ. 54, 77–88.
Jastrow, J.D., 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. Soil Biol. Biochem. 28, 665–676.
Jastrow, J.D., Miller, R.M., Boutton, T.W., 1996. Carbon dynamics of aggregate-associated organic matter estimated by carbon-13 natural abundance. Soil Sci. Soc. Am. J. 60, 801–807.
Keil, R.G., Mayer, L.M., 2014. Mineral matrices and organic matter. Treatise Geochem. Org. Geochem. 12, 337–359.
Keim, R., Kandeler, E., 1997. Stabilization of aggregates by the microbial biomass as affected by soil texture and type. Appl. Soil Ecol. 5, 221–230.
Kong, A.Y.Y., Scow, K.M., Córdova-Kreylos, A.L., Holmes, W.E., Six, J., 2011. Microbial community composition and carbon cycling within soil microenvironments of conventional, low-input, and organic cropping systems. Soil Biol. Biochem. 43, 20–30.
Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P., Heal, O.W., Dhillion, S., 1997. Soil function in a changing world: the role of invertebrate ecosystem engineers. Eur. J. Soil Biol. 33, 159–193.
Lützow, M.V., Kögel-Knaber, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temerate soils: mechanisms and their relevance under different soil conditions—a review. Eur. J. Soil Sci. 57, 426–445.
Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: results from meta-analysis. Ecology 93, 930–938.
Molope, M.B., Grieve, I.C., Page, E.R., 1987. Contributions of fungi and bacteria to aggregate stability of cultivates soils. J. Soil Sci. 38, 71–77.
Muneer, M., Oades, J.M., 1989. The role of Ca-organic interactions in soil aggregate stability. I. Laboratory studies with ^{14}C-glucose, $CaCO_3$ and $CaSO_4 \cdot 2H_2O$. Aust. J. Soil Res. 27, 389–399.
Oades, J.M., 1988. The retention of organic matter in soils. Biogeochemistry 5, 35–70.
Oades, J.M., 1993. The role of biology in the formation, stabilization and degradation of soil structure. Geoderma 56, 377–400.
Oades, J.M., Waters, A.G., 1991. Aggregate hierarchy in soils. Aust. J. Soil Res. 29, 815–828.
Ophir, T., Gutnick, D.L., 1994. A role of exopolysaccharides in the protection of microorganisms from desiccation. Appl. Environ. Microbiol. 60, 740–745.
Parker, S.S., Schimel, J.P., 2011. Soil nitrogen availability and transformations differ between the summer and the growing season in a California grassland. Appl. Soil Ecol. 48, 185–192.
Peng, X., Horn, R., Hallett, P., 2015. Soil structure and its functions in ecosystems: phase matter and scale matter. Soil Tillage Res. 146, 1–3.
Priester, J.H., Ge, Y., Chang, V., Stoimenov, P.K., Schimel, J.P., Stucky, G.D., Holden, P.A., 2013. Assessing interactions of hydrophilic nanoscale TiO_2 with soil water. J. Nanoparticle Res. 15, 1899. http://dx.doi.org/10.1007/s11051-013-1899-4.
Pulleman, M.M., Six, J., Uyl, A., Marinissen, J.C.Y., Jongmans, A.G., 2005. Earthworms and management affect organic matter incorporation and microaggregate formation in agricultural soils. Appl. Soil Ecol. 29, 1–15.

Redmile-Gordon, M.A., Brookes, P.C., Evershed, R.P., Goulding, K.W.T., Hirsch, P.R., 2014. Measuring the soil-microbial interface: extraction of extracellular polymeric substances (EPS) from soil biofilms. Soil Biol. Biochem. 72, 163–171.

Roberson, E.B., Firestone, M.K., 1992. Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. Appl. Environ. Microbiol. 58, 1284–1291.

Roberson, E.B., Firestone, M.K., Sarig, S., 1991. Cover crop management of polysaccharide-mediated aggregation in an orchard soil. Soil Sci. Soc. Am. J. 55, 734–739.

Schimel, J.P., Schaeffer, 2012. Microbial control over carbon cycling in soil. Front. Microbiol. 3, 348. http://dx.doi.org/10.3389/fmicb.2012.00348.

Six, J., Paustian, K., 2014. Citation classic XII: aggregate-associated soil organic matter as an ecosystem property and measurement tool. Soil Biol. Biochem. 68, A4–A9.

Six, J., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. 32, 2099–2103.

Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res. 79, 7–31.

Tisdall, J.M., 1994. Possible role of soil microorganisms in aggregation in soils. Plant Soil 159, 115–121.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33, 141–163.

Treseder, K.K., Schimel, J.P., Garcia, M.O., Whiteside, M.D., 2010. Slow turnover and production of fungal hyphae during a California dry season. Soil Biol. Biochem. 42, 1657–1660.

Velasquez, E., Pelosi, C., Brunet, D., Grimaldi, M., Martins, M., Rendeiro, A.C., Barrios, E., Lavelle, P., 2007. This ped is my ped: visual separation and near infrared spectra allow determination of the origins of soil macroaggregates. Pedobiologia 51, 75–87.

West, A.W., Sparling, G.P., 1986. Modifications to the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents. J. Microbiol. Methods 5, 177–189.

Yuste, J.C., Peñuelas, J., Estiarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., Pujol, M., Sardans, J., 2011. Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Glob. Chang. Biol. 17, 1475–1486.