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Engineering rhizobacterial community resilience with mannose nanofibril hydrogels towards maintaining grain production under drying climate stress

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ABSTRACT

Ongoing impacts of climatic change especially moisture stress remain a global challenge to agricultural production and food security. Such abiotic effects directly influence soil microbial communities. Previously we demonstrated that polymeric hydrogels, able to provide specific interactions with soil microbial communities, enhance the dynamics and selectivity of ingress and colonisation of plant beneficial bacteria at soil microcosm scales. We now show that small quantities of osmotic hydrogels containing mannose nanofibrils specifically situated in close proximity to developing root zones provide enhanced microbial ingress, colonisation and continuous wetting pathways when contacting developing wheat root systems. These effectively create extensions to the natural rhizosphere sustaining significant beneficial rhizobacterial communities when under moisture stress. Sequencing studies, on wheat production soils undergoing a season of low average rainfall clearly showed microbial abundance increases and taxa selectivity in the rhizosphere-hydrogel regions compared to controls. Here wheat yields increased by about 20% with hydrogel addition compared to controls, which represented a 15% increase in the overall average yield in the region. This represents the first reported demonstration that developing rhizospheres may be readily engineered to consistently select their own distinct microbiome as nodes of functional microbial abundance significantly benefiting grain yield during abiotic stress. Thereby suggesting new opportunities to maintain grain yields during periods of drying climate via these fibrillar hydrogels.

1. Introduction

Increasing the yield of water-limited grain crops has become an important challenge in maintaining agricultural production during periods of climatically induced abiotic stress (Ray et al., 2015; Lesk et al., 2016). Among the opportunities to improve drought tolerance, engineering beneficial plant growth-promoting bacterial communities (PGPB) interacting at the root-soil interface has become a strategic research focus (Joshi et al., 2018; Orozco-Mosqueda et al., 2018). Recently, Hartman and Tringe reviewed the relative contributions of these microbial communities to their plant host (Hartman and Tringe, 2019); highlighting feedback between the soil rhizosphere and the root endosphere in achieving plant health and productivity (York et al., 2016). Additionally, Naylor and Coleman-Derr reviewed mechanisms underlying how drought affected the efficacy of PGPB communities in terms of an interplay between: drought soil properties – drought induced plant phenotype – shifts in soil and root microbiome functionality as well as altered root exudation processes (Naylor and Coleman-Derr, 2017). Priming with inoculated PGPB communities retrieved from plant roots coevolved in harsh climates has been shown to enhance severe drought stress tolerance increasing both biomass and survivorship of wheat seedlings thereby suggesting a route to improving water use efficiency (Timmusk et al., 2014). Finally, Compant et al. (2019) reviewed the ecology, functions and emerging applications of plant

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growth and stress resilience, noting the importance of knowledge-driven selection and delivery of microbial strains and consortia in achieving these. The study reported here harnesses an autogenous selectivity of the formulated hydrogels to support these outcomes.

Polymeric hydrogels are widely studied for agricultural production particularly in horticulture and forestry where they principally function as water retention additives and control release agents for pesticides and nutrients (Teodorescu et al., 2009; Guilherme et al., 2015), although their application to broad-acre grain crop production is yet to be explored. The incorporation of polysaccharides into polymeric hydrogels has received significant research attention where they provide soil with slower nutrient release and increased water retention (Montesano et al., 2015; Bortolin et al., 2016; Zhang et al., 2017). Recently, Ramli reviewed the applications, underlying mechanisms and plant growth responses of slow release fertilizer hydrogels including the combined synthetic and natural polymeric hydrogels as either co-polymers or interpenetrating networks (Ramli, 2019). Such composite hydrogels aim at the synergistic effects of increased biodegradability and increased water absorbency (Li et al., 2007). These composite hydrogels have included carbohydrate fibres as the biopolymer component which in the case of jute fibres crosslinked with polybutylacrylate hydrogel (when incubated under soil degradation conditions) reduced weight loss to about 67% compared to jute alone (Sahoo et al., 2005). Wilhelm et al. (2019) have recently detailed the bacterial contributions to lignocellulose degradation using metagenomic and stable isotope probing finding that gram-negative bacteria dominated lignocellulose degradation while fungi were more prominent in cellulose degradation.

Previously, we have detailed the impact of confining polymer hydrogel networks on the dynamics of bacterial ingress and colonisation and showed that synthetic hydrogels with their controllable chemical and structural functionality provide well-defined microenvironments for bacterial populations such as inoculants (Truong et al., 2015). Subsequently, we have shown that polymeric hydrogels with specific polysaccharide functionality selectively enhance plant beneficial colonisation in soil microcosms, suggesting new autogenous routes to stress tolerance (Pham et al., 2017). It is well known that pathogenic bacterial adhesion is directed to specific mannose functionality through their surface protein adhesins (Pieters, 2007) which form the basis of targeted anti-adhesion therapies not reliant on antibiotics. Additionally, Lee et al. (2012) showed that self-assembled multivalent mannose-nanofibrillar structures readily promote bacterial adhesin binding and that their dimension was an important determinant of activity. More recently, De Cesare et al. (2019) demonstrated that rhizobacterium *Burkholderia terricola* preferentially attached to poly (ε-caprolactone)-based polymer nanofibers that had been pre-coated with adsorbed organic material derived from both the nutrient broth and bacterial exudates when conditioned fibres had diameters in the range of approximately 100 nm.

Here, we use mechanistic soil microcosm studies outlining model microbial ingress into polymeric hydrogels containing lignocellulose (where the nanofibril component provides mannose functionality together with the high specific surface area and aspect ratio) to demonstrate a selective increase in beneficial microbial abundance. In subsequent broad-acre field evaluation we show that, small quantities of nanofibrillar-polymeric hydrogel in close proximity to developing roots were colonised by rhizobacteria providing plant-growth promoting functionality. We then show that this biphasic microenvironment of nanofibrils within hydrogel macropores results in a significant increase in wheat grain yield under climatic water stress conditions beyond that seen when these hydrogel constructs were absent.

2. Materials and methods

2.1. Polymeric hydrogel composition

Polyacrylic acid (PAA) and its equivalent with lignocellulose fibrils (PAA-L) were compared at two scales of preparation. Potassium (K) PAA was prepared in the laboratory as previously described (Truong et al., 2015; Pham et al., 2017) while the corresponding PAA-L was synthesized according to the scheme detailed in Fig. 1. Additionally, larger quantities of the corresponding crosslinked PAA-L were provided by BASF SE (Ludwigshafen, Germany) as the K salt for field studies. PAA-L provided nanofibril surfaces designed for microbial attachment as well as increased biodegradable carbon. The micro- and nano-structures of the PAA and PAA-L hydrogels were determined by field emission cryo-SEM (FEI NOVA nanoSEM, FEI Co., Hillsboro, Oregon). Samples were prepared *in-situ* using the Gatan Alto 2500 pre-chamber followed by fracture to provide free-break surfaces and low temperature sublimation. Micromechanical measurements of hydrogels were performed with a stress-controlled rheometer (DSR 200, Rheometrics, Paulsboro NJ) as detailed in the Supplementary Information.

2.2. Microbial ingress using pure cultures and soil microcosms

Topsoil samples (0–10 cm) were collected from the semi-arid central 'wheat belt' of Western Australia near Badgingarra, Pingelly and Dandaragan (Tables S–1, Fig. S–5). Sites were selected to represent coarse-textured sandy soils where plant available water holding capacity has been identified as a major limitation to wheat yield (Lawes et al., 2009). Two soils (Dandaragan and Pingelly) were selected to represent typically wettable soils having molarity of ethanol droplet (MED) values of 0.4 (King, 1981), while Badgingarra with a MED of 2.4 represented a significantly more hydrophobic soils prevalent in some areas of the Western Australian wheat belt. Prior to microcosm experiments, soils were dried at 40 °C and sieved to their fine earth fraction (<2 mm) before establishing the *ex-situ* microcosms.

To determine the capacity of the hydrogels (PAA and PAA-L) to support microbial ingress and colonisation, studies were conducted in pure cultures and soil microcosms. Model soil bacteria (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Janthinobacterium lividum*) were employed to determine microbial ingress, viability and colonisation of the PAA and PAA-L polymer networks using LIVE/DEAD BacLight Bacterial Viability stains (ThermoFisher Scientific) and confocal laser scanning microscopy (Fluoview FV1000-1X81, Olympus, Japan). Bacterial populations were quantified as 3-dimensional z-stacks starting at the hydrogel interface (x, y plane) together with the integrated fluorescence intensity profile of both the live and dead bacteria, as described previously (Truong et al., 2015; Pham et al., 2017). Microbial ingress from soils was carried out in cylindrical microcosms of 15 g soil capacity, in which a single granule of hydrogel was placed in the centre. Preparation of the microcosms and quantification of bacterial numbers using confocal scanning laser microscopy are detailed in the Supplementary Information.

2.3. Field studies

Field trials were conducted at Pingelly in 2013, Badgingarra in 2014 and Dandaragan in 2014 to assess the impact of functionalised hydrogels on the soil microbial colonisation and wheat yield. Treatments included control plots (no amendment) and plots to which hydrogels were added (20 kg per ha in 2013 and 10 kg per ha in 2014) in furrow and approximately three cm below the seed. Treatments were applied in a randomised block design using a small plot air seeder where each plot was 2 m × 12 m having eight rows of crop. Plots were replicated fourfold for Pingelly and tenfold for Badgingarra and Dandaragan. Around each block a buffer strip was seeded. Trials were managed according to best district practice, including seeding method, fertiliser application, and date of sowing. At 4 and 12 weeks post-seeding, plants were retrieved from the soil and hydrogels collected from the roots to conduct microbial community analysis. At maturity, plants were harvested and grain yields determined.

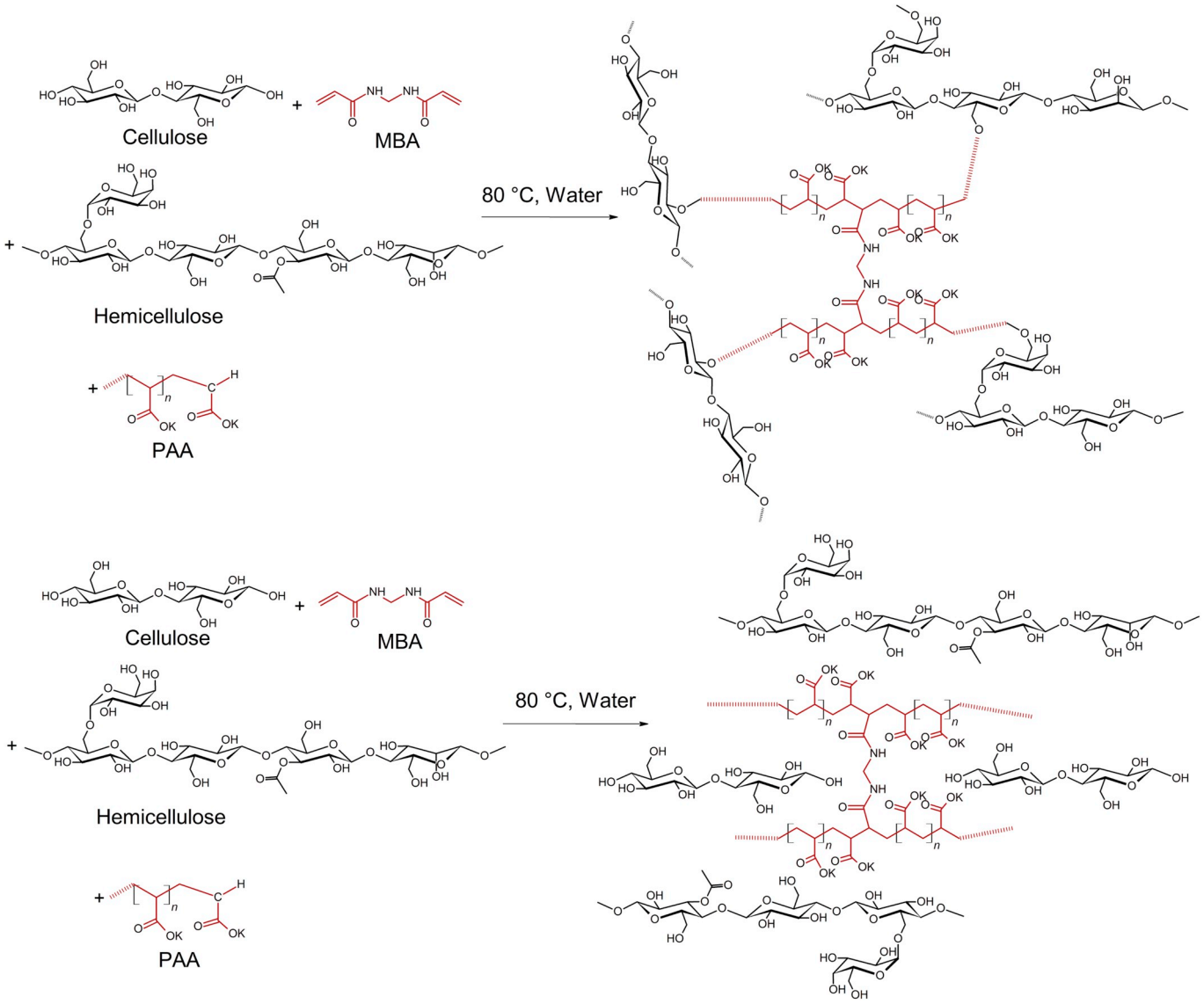

Fig. 1. Modes of inclusion of cellulose and hemicellulose components during the formation of PAA–lignocellulose (PAA-L) where the extent conjugation depends on reactivity of lignocellulose components.

2.4. Microbial community analysis

DNA was extracted from the hydrogels and soils at a distance from the hydrogels (>20 mm) using the PowerSoil® DNA Isolation Kit (MoBio, Carlsbad, CA) following the manufacturer's protocol. The extracted DNA was quantified using a fluorescence approach (Qbit® 2.0 Fluorometer, Invitrogen) and diluted to a concentration of 1 ng μL^{-1} before PCR amplification of bacterial 16S rRNA genes. PCR amplification was carried out on multiplex samples according to the detailed Supplementary Information. Next generation sequencing was performed at the Lotterywest State Biomedical Facility Genomics (UWA) using the Ion Torrent Personal Genome Machine (Life Technologies, Mulgrave, Australia) as described by Rothberg et al. (2011) and Whiteley et al. (2012) using 400 base pair chemistry.

2.5. Sequence and statistical analysis

Sequences obtained were analysed using QIIME (Caporaso et al., 2010). Multiplexed, barcoded sequences were split and only sequences with a minimum quality score of 20 containing no mismatches in the barcode used, forward and reverse primers were retained. Chimeras were removed using USEARCH (Edgar, 2010). All sequences were clustered into Operational Taxonomic Units (OTUs) based on 97% sequence similarity and taxonomy was assigned using the greengenes database version 13.5 (McDonald et al., 2011). Sequences were rarefied to 2020 reads per sample for in situ field studies to allow for comparison between samples with different sequencing depth (Gihring et al., 2012). Statistical analysis of the community structure was performed at the family level using the software PRIMER-E (Clarke and Gorley, 2015). Relative abundance data were log-transformed prior to conducting PERMANOVA with significant differences expressed as Monte Carlo P values, and SIMPER analysis based on Bray-Curtis similarity. Furthermore, PCoA based on weighted UniFrac distances (Lozupone et al., 2007) and Chao1 indices were produced using QIIME (Caporaso et al., 2010). Sequence data was submitted to the European Nucleotide Archive under study accession PRJEB27874. Metagenomic functions were predicted *in silico* to detect potential plant-growth promoting traits of the hydrogel microbiome (Langille et al., 2013). Four plant-growth promoting traits were identified that were upregulated in PAA-L hydrogel when compared to soil and rhizosphere microbiomes.

2.6. Quantitative PCR

To quantify the abundances of 16S rRNA genes, a qPCR master containing 10 μL GoTaq qPCR Master Mix (Promega, Australia), 0.1 μL of forward (EUB 338, 20 μM) and reverse primer (EUB518, 20 μM), 5.8 μL PCR-grade H2O, 2.0 μL BSA (50 μg μL^{-1}), and 2.0 μL template DNA (1 ng μL^{-1}) was prepared. qPCR was conducted using the ViiA™ 7 Real-Time PCR System (Applied Biosystems, Carlsbad, CA) under the following conditions: initial denaturing 95 °C for 15 min, 40 cycles of denaturing at 95 °C for 1 min, annealing at 53 °C for 30 s, elongation at 72 °C for 1 min followed by fluorescence measurement at 72 °C for product quantification (Fierer et al., 2005). A subsequent melting point analysis confirmed product specificity (55 °C–95 °C with a fluorescence measurement every 0.5 °C) and PCR products were visually inspected on an agarose gel (1.5%).

3. Results

3.1. Characterisation of hydrogels

Polymeric hydrogels were produced at two scales and provided for model bacterial studies and in-field wheat studies. The synthesis of PAA was carried out as previously described utilizing free radical polymerisation (Pham et al., 2017), while the previous technique for grafting mannan polysaccharide functionality onto the crosslinked polymer chains (Pham et al., 2017) was extended to a lignocellulose component. Here, free radicals of the polymer chains reacted with hydroxyls of the lignocellulose fibrils, as illustrated in Fig. 1. Cryo-SEM imaging (Fig. 2a and b) indicates that the macroporous cellular structure of PAA and PAA-L hydrogel and its accessibility is maintained in the hydrous state. The incorporation of high surface area lignocellulose nanofibrils within the PAA structure also maintained its osmotic swelling properties under the compressive pressure of overlying soil in the root zone (Fig. 2c and d). A further description of nanofibers within PAA-L, having 50–100 nm width, is given in Fig. S1.

3.2. Model ingress studies

The lignocellulose component provided nanofibrils which enabled preferential attachment of bacterial cells (Fig. S-2) resulting in significantly enhanced microbial ingress and colonisation compared to PAA in both pure culture studies (Fig. 3) and in the field soil microcosm studies (Fig. S-3). As shown microscopically in both systems, microbial cells were detected throughout the hydrogel albeit at higher concentrations near the surface, suggesting a distribution of aqueous nutrients and a diffusion gradient. Such a gradient would allow a continuous recharge and maintenance of contact with the soil matrix through uninterrupted water films. Additionally, extraction of the hydrated PAA-L from subsequent wheat field soil system also clearly showed the hydrogel in close contact with the developed root system (Fig. S-4) providing continuous wetting films and suggesting that PAA-L also acts as an extension to the rhizosphere. It is noteworthy that of the three model organisms, *J. lividum*, a representative of the *Oxalobacteraceae*, colonised the largest area throughout PAA-L (Fig. 3) suggesting enhanced plant beneficial functionality.

3.3. Wheat field studies

Crop production in the semi-arid lands of the southern Australian grain growing regions is water and nutrient-limited, additionally the region is experiencing a drying climate (State of The Environment Committee, 2011) and particularly a decline in early season rainfall during seedling germination and establishment (Whetton, 2015). Such current climate change projections for this region anticipates an overall decrease in wheat production of about 13% by 2050 (Sudmeyer et al., 2016). Field studies were carried out during the 2013 (Pingelly) and 2014 wheat seasons (Dandaragan, Badgingarra; Fig. S-5) which spanned average regional wheat yields while including one soil that had a significant degree of water repellency. Seasonal rainfall during field studies reflected the drying climate e.g. the Dandaragan region had 450 mm cumulative rainfall where only 35 mm (8%) occurred during the grain

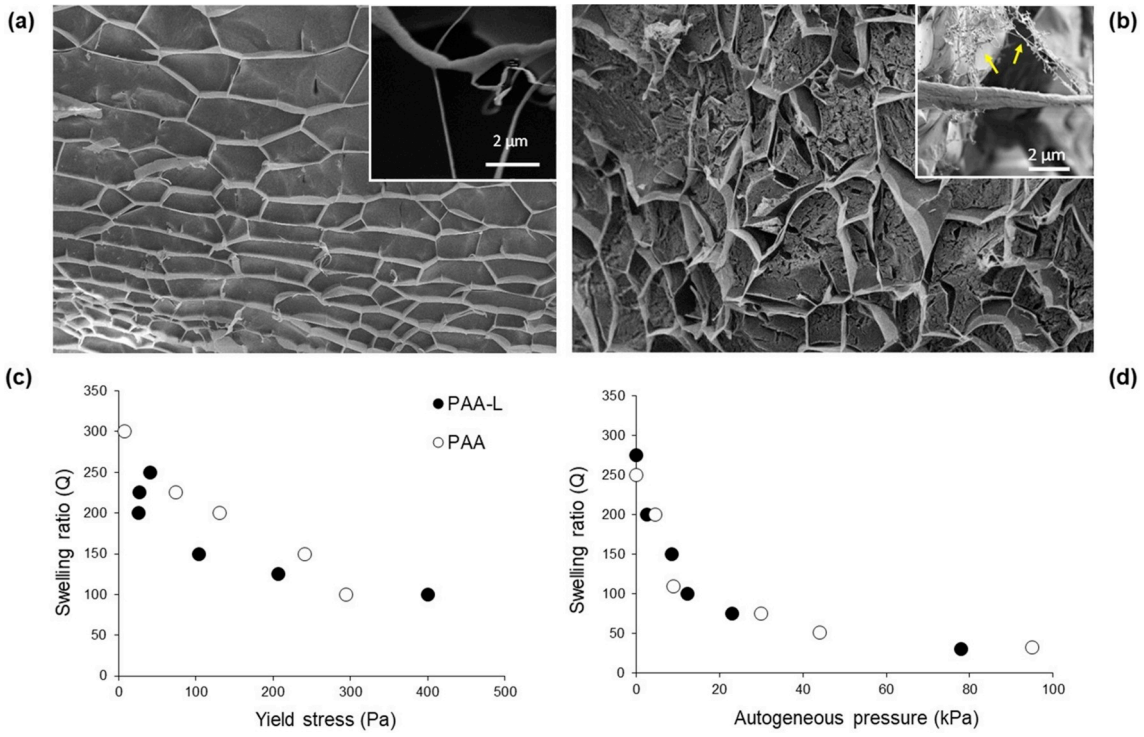

Fig. 2. Characterisation of hydrated hydrogels by cryo-scanning electron microscopy of (a) PAA and (b) PAA-L indicating the swollen macroporous wall polymer structure of the PAAs together with incorporated lignocellulose nanofibrils (arrows). The influence of incorporation on the (c) micromechanical (yield stress) of the hydrogels and (d) swelling behaviour (Q) under imposed compressive loads of soil.

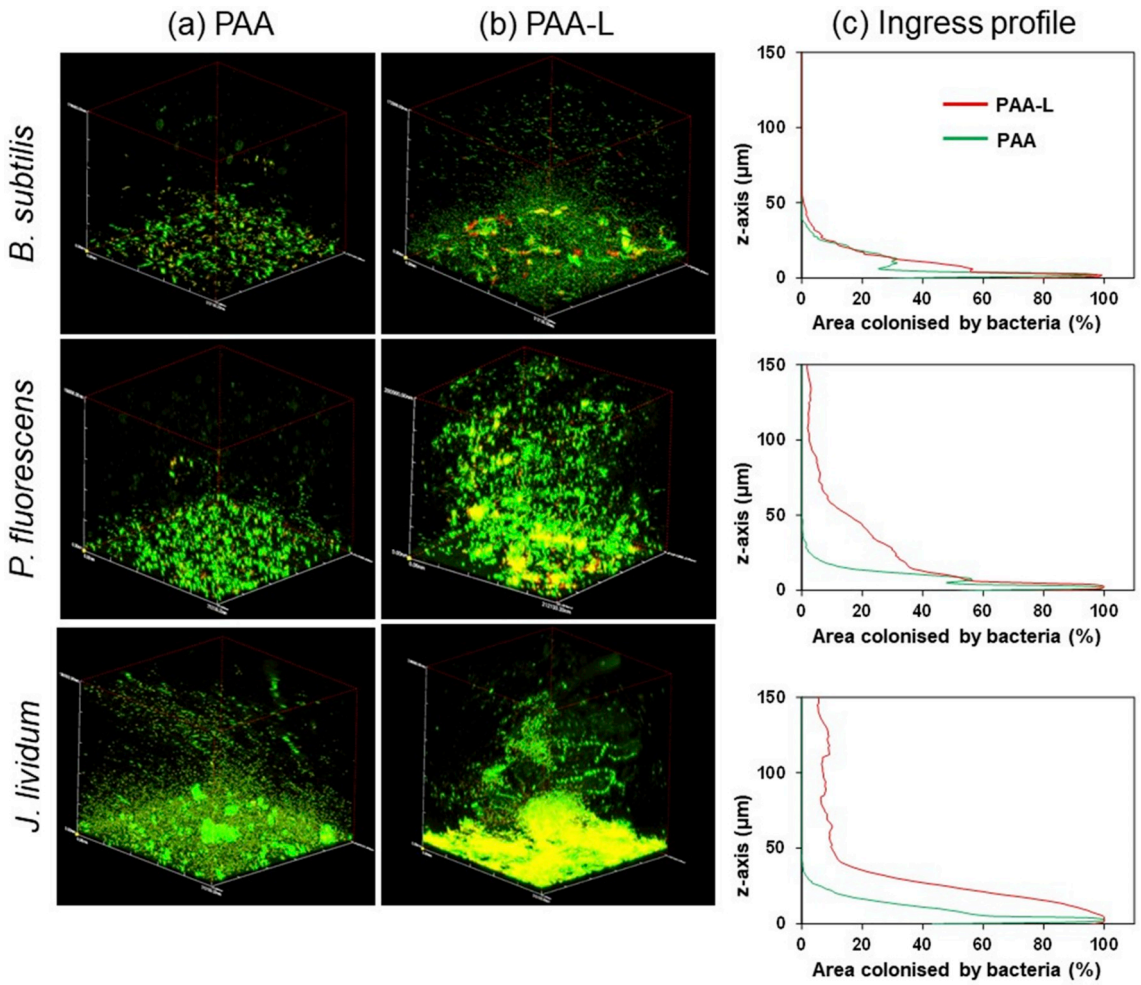

Fig. 3. Ingress of *B. subtilis*, *P. fluorescens* and *J. lividum* into PAA and PAA-L. (a and b). Confocal laser scanning microscopic images demonstrating the bacterial ingress into PAA and PAA-L respectively. (c) Quantitative ingress profile showing bacteria-colonised areas into PAA and PAA-L. Presence of lignocellulose enhances the colonisation of all three species of bacteria at the hydrogel interface with soil as well as initiating ingress into the swollen gel.

filling stage (Fig. S-6).

The impact on microbial populations and taxa selectivity was examined in these three low-fertility wheat field soils both with and without hydrogel addition over two periods of time (4 and 12 weeks) as given in Fig. S-8. Within these soils, three regions were examined (soil, rhizosphere soil and PAA-L hydrogel). Here, the hydrogel microbiomes were more similar to the rhizosphere microbiome (41.6% similarity) than to those of soil (39.5% similarity) as seen in Fig. 4b. Over time, the similarity of PAA-L and rhizosphere microbiomes increased slightly from 44.2% to 47.3% (Fig. 4b), though they remained significantly different after 12 weeks ($P = 0.001$). Time also affected the rhizosphere microbiomes but not the bulk soil bacterial communities ($P = 0.001$ and $P = 0.114$, respectively). PAA-L amendment did not significantly affect the microbiomes of bulk soil nor the wheat rhizosphere compared to the unamended controls ($P = 0.954$ and $P = 0.748$, respectively) suggesting that the PAA-L enriches rhizobacteria in localized focal points without affecting the surrounding microbial habitats. Bacterial abundance, determined by qPCR of the 16S rRNA gene (Fig. 4a and combined for the three sites (Badgingarra, Dandaragan and Pingelly), was only moderately increased in the rhizosphere soil and PAA-L compared to bulk soil at 4 weeks. After 12 weeks this was further enhanced such that the bacterial abundance was significantly higher in PAA-L hydrogel compared to bulk soil ($P = 0.002$) but not rhizosphere soil ($P = 0.666$). Together, these findings suggest that PAA-L amendments consistently select their own distinct microbiome and represent nodes (hotspots) of microbial abundance within soils.

Combining sequencing data for the three wheat soils, the most prominent and considerably upregulated prokaryotes associated with PAA-L were members of the *Oxalobacteraceae* (Fig. 5) that are known to rapidly proliferate under high resource conditions despite being competition-sensitive (Ofek et al., 2012). After four weeks of field incubation, PAA-L contained a higher abundance of *Oxalobacteraceae* (11.2%) than both bulk and rhizosphere soils (0.7% and 1.3%, respectively) (Fig. 5). This family has been frequently detected in plant rhizospheres (Baldani et al., 2014) including wheat (Donn et al., 2015) and certain genera have been linked with increased biomass productivity of this crop (Anderson and Habiger, 2012). After 12 weeks, the *Oxalobacteraceae* were outcompeted by other taxa *Burkholderiaceae*, *Xanthomonadaceae* and *Sphingobacteriaceae*) (Fig. 5) which have also been linked to plant-growth promotion (Kuklinsky-Sobral et al., 2004) as well as disease suppression (Postma et al., 2008). Alpha diversity measures of the Chao Index (Fig. S-7) of the soil, rhizosphere and PAA-L hydrogel in field incubation showed significantly lower prokaryotic diversity present in the hydrogel compared to the corresponding soil and rhizosphere ($P < 0.05$).

The relative composition of the bacterial communities in close contact to the plant root (within the rhizosphere or PAA-L hydrogel) having potentially beneficial functionality are shown in Fig. S-8 in terms of the taxa abundance that contribute to microbiome similarity and dissimilarity between sample types. Under field conditions, the prokaryotic community composition of both the rhizosphere and the PAA-L was significantly different from the bulk soil and wheat rhizosphere

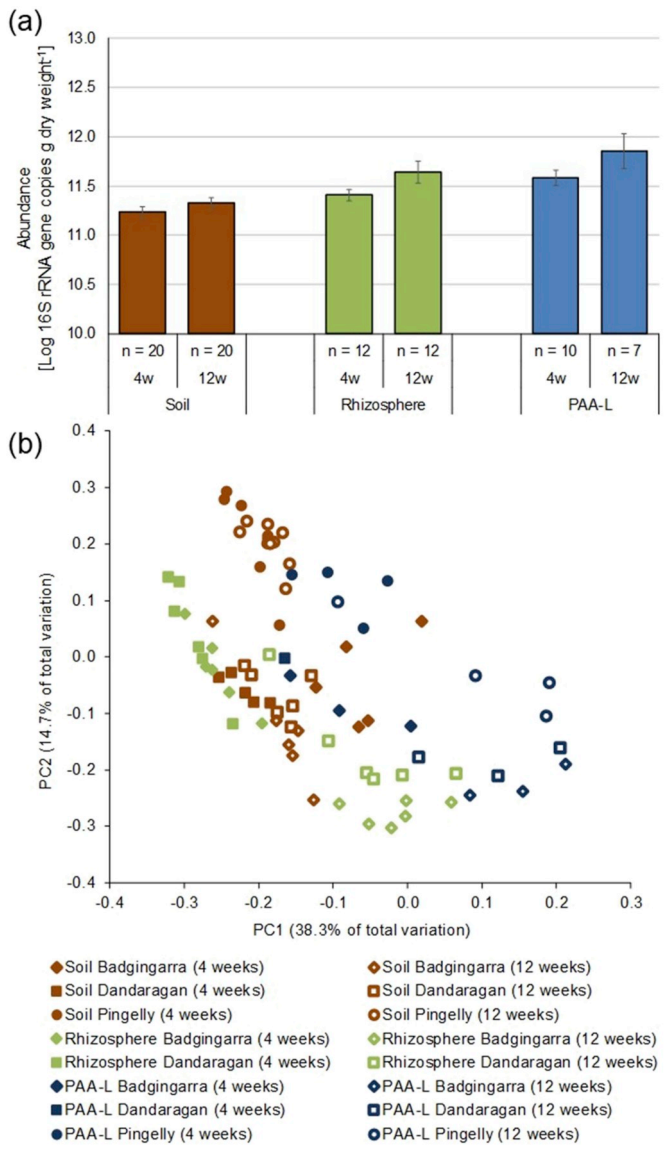

Fig. 4. (a) Bacterial abundance determined by qPCR of the 16S rRNA gene (in situ soil regions). Error bars represent standard errors of the mean; (b) PCoA plot based on weighted UniFrac distances showing that community structures of PAA-L, rhizosphere and field soil differ and the former two converge over time.

communities (Fig. 4b and Fig. S-8, P = 0.001). Within the PAA-L community some potential plant-growth promoting taxa increased in realtive abundance compared to the other two microbial habitats i.e. bulk and rhizosphere soil.

Predicted plant-growth promoting functions were significantly upregulated in PAA-L extracted from the wheat root zones of field soils (Fig. 6) compared to both bulk soil and wheat rhizosphere soil despite a changing microbial community composition over time. This suggests a functional redundancy between different rhizobacterial taxa while ensuring a continuous potential plant-growth promotion. For example, the upregulation of 3-phytase (Fig. 6a), which releases phosphate (Idriss et al., 2002) from phytic acid (myo-inositol hexakisphosphate), also suggests a potential role for PAA-L in supporting rhizobacteria to enhance plant phosphorus (P) availability. The rhizosphere is particularly important for P nutrition as rapid reactions between phosphate and mineral particles impede the mobility of P resulting in little access to P outside of the rhizosphere. Interestingly, potentially functional PAA-L may provide a route for phosphate transfer between microbial cells and plant root cells while minimizing contact with P-fixing soil minerals.

No observable differences occurred between the controls (soil and rhizosphere soil) and PAA-L amended field treatments during the early stages of plant growth. Additionally, while predicted microbial genes were upregulated for root growth and branching (Fig. 6c and d), our studies showed no structural changes in roots that penetrated the hydrogel. However, rainfall patterns for the semi-arid Western Australian sites indicate considerable water stress during the grain development period (Fig. S-6). This coincided with a predicted upregulation in 1-aminocyclopropane-1-carboxylate deaminase (*acdS*) activity (Fig. 6b) which influences the stress response of plants (Glick, 2014) while being a widespread trait in *Burkholderia* (Smalla et al., 2001) which were also upregulated (Fig. 5).

3.4. Impacts on wheat yields

Fig. 7 provides the wheat yields from the Badgingarra, Dandaragan and Pingelly test sites obtained with PAA-L compared to the control (no hydrogel) during the respective wheat seasons. Both Dandaragan and Pingelly showed an increase of more than 20% in grain yield with added PAA-L compared to the control (Fig. 7) during a season where most of the rainfall (415 mm or 92% of total rainfall) occurred during May–October resulting in only 35 mm or 8% during the grain filling stage indicative of significant water stress, as detailed in Fig S-6 (Bureau of Meteorology, 2018). In general, under these conditions, control plots gave 2.6 t/ha yields (Fig. 7) compared to the calculated water-limiting yield potential of 4.2 t/ha (Hochman et al., 2016) or 62% of the waterlimited potential wheat yield for these conditions (Fig. S-9). In comparison, PAA-L amended plots provided wheat yields ranging from 3.1 to 3.3 t/ha (Fig. 7) i.e. 74%–79% of the waterlimiting yield potential, thereby representing an increase of 12%–17%. In contrast, the significantly more hydrophobic Badgingarra test site (MED 2.4) gave no increase in wheat yield on addition of PAA-L to the root zone (Fig. 7). The influence of greater run-off losses due to significantly lower water infiltration rates, as shown in Fig. S-10, could not be overcome by the small quantities of hydrogel added to the root zone.

4. Discussion

Potential changes in global precipitation patterns associated with climate change are now reflected in drying climates in many grain producing areas. Additionally, extremes in precipitation patterns may impact negatively on soil microbial communities affecting their functional capacity within the plant rhizosphere of many food production crops (Taylor et al., 2004; Rousk et al., 2013). Previously we have shown that polymeric hydrogels, particularly those that exploit specific interactions with soil microbial communities enhance the dynamics and selectivity of ingress of plant beneficial bacteria at model and soil microcosm scales (Pham et al., 2017).

Here we show that a polymeric hydrogel (PAA-L) of polyacrylic acid and lignocellulose (nanofibrils) provides enhanced microbial ingress and colonisation in soil microcosm studies, in comparison to PAA and bulk soil. When extracted from a wheat plant root system in the field, the PAA-L hydrogel showed close proximity with the developing root providing continuous moist microbial pathways (Long and Or, 2005). Under moisture stress conditions, the osmotic potential of the hydrogels maintains moisture in preference to surrounding bulk soil (Pham et al., 2017) allowing the viability of rhizobacterial communities to be maintained in close proximity to both soil particles and the developing root system.

Sequencing studies, carried out on the semi-arid wheat production soils undergoing a season of low average rainfall clearly showed microbial population increases and taxa selectivity in the rhizosphere and PAA-L hydrogel regions of the root zone compared to the bulk soil. Additionally, the population similarity of PAA-L and rhizosphere increased with time while the bulk soil did not. Thus, these hydrogels enrich rhizobacteria close to the developing root and consistently select

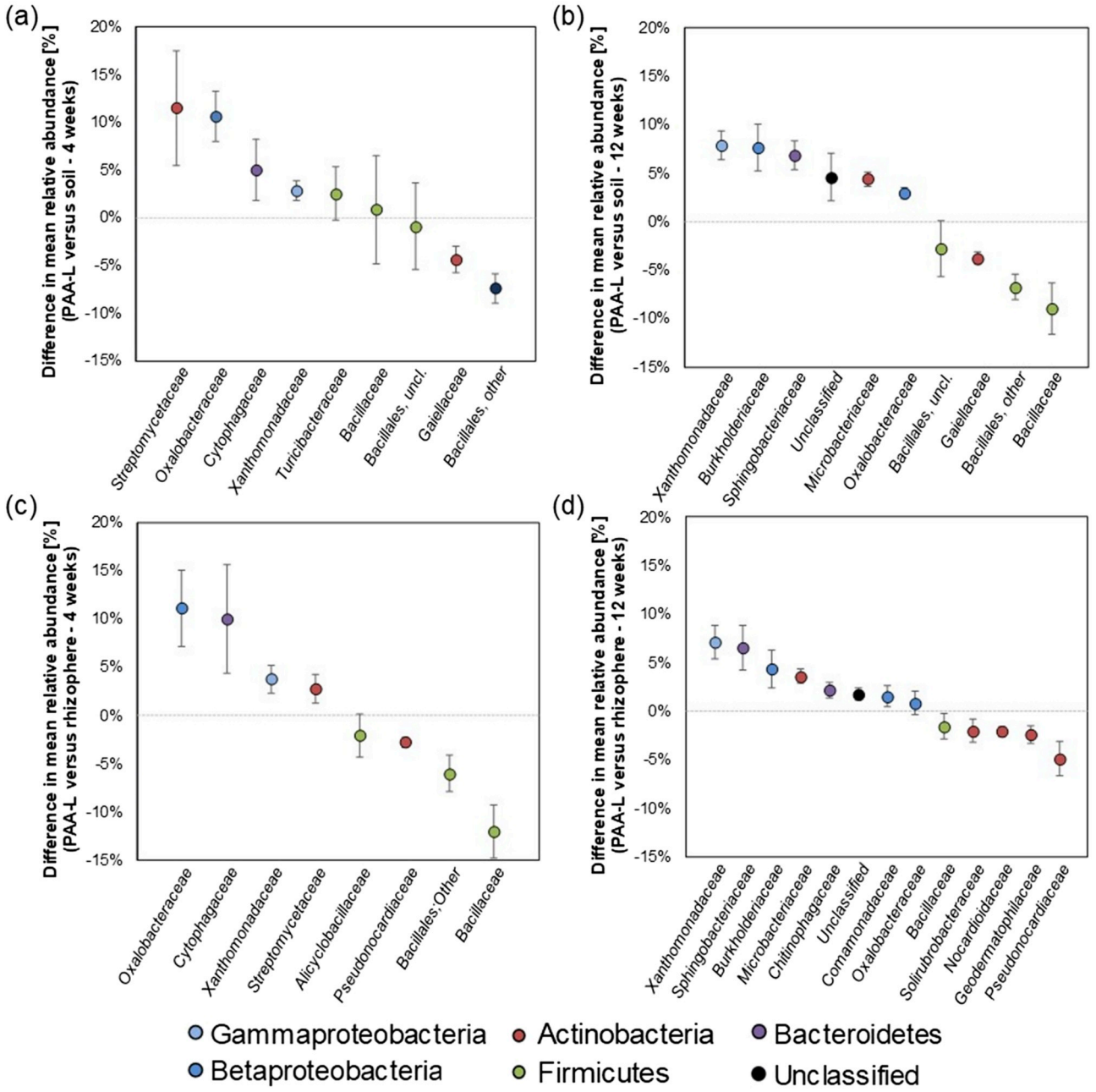

Fig. 5. PAA-L preferentially attracts rhizobacterial taxa. Differences in relative abundance of taxa that cumulatively explain more than 50% dissimilarity between the two sample groups (SIMPER analysis). (a) PAA-L versus soil after 4 weeks (n = 15). (b) PAA-L versus soil after 12 weeks (n = 20). (c) PAA-L versus rhizosphere after four weeks (n = 10). (d) PAA-L versus rhizosphere after 12 weeks (n = 12).

their own distinct microbiome, behaving as nodes or hotspots of microbial abundance within unaffected soils, and thereby suggesting they act as an extension to the rhizosphere itself.

Sequencing PAA-L populations revelaed increased abundance of taxa associated with increased wheat biomass (Anderson and Habiger, 2012) and disease suppression (Postma et al., 2008). Additionally, alpha diversity was lower in PAA-L compared to both bulk soil and rhizosphere indicating a clear selectivity towards plant beneficial communities over time.. Importantly, the predicted upregulation for genes specifically associated with moisture stress responses of plants, coincided with the moisture stress resulting from the rainfall patterns during these field trial periods.

PCoA measures of the impact of the polymeric hydrogel on microbial populations and taxa selectivity in three regions of the wheat field soils examined showed that the bulk soil remained largely unchanged throughout the season, while the rhizosphere (soil) and the PAA-L hydrogel changed with time, and importantly the rhizosphere and PAA-L hydrogel converged over time.

In-field plots during wheat seasons having significant water stress, particularly during grain filling, showed an increase in yield of more than 20% with PAA-L addition compared to the control (no hydrogel) when soils were not severely non-wetting. Hydrogel amendment, by addition at a rate of 10–20 kg ha^{-1} at sowing representing about one hydrogel granule per seed, increased the wheat yields by about 15% of their water-limited yield potential, compared to no hydrogel amendment. In areas of severe soil-water repellence, hydrogel amendment did not overcome the constraint of rainwater losses during the season.

In summary, our results demonstrate that small quantities of mannose functionalised hydrogels specifically situated in close proximity to the developing root zone effectively create extensions to its

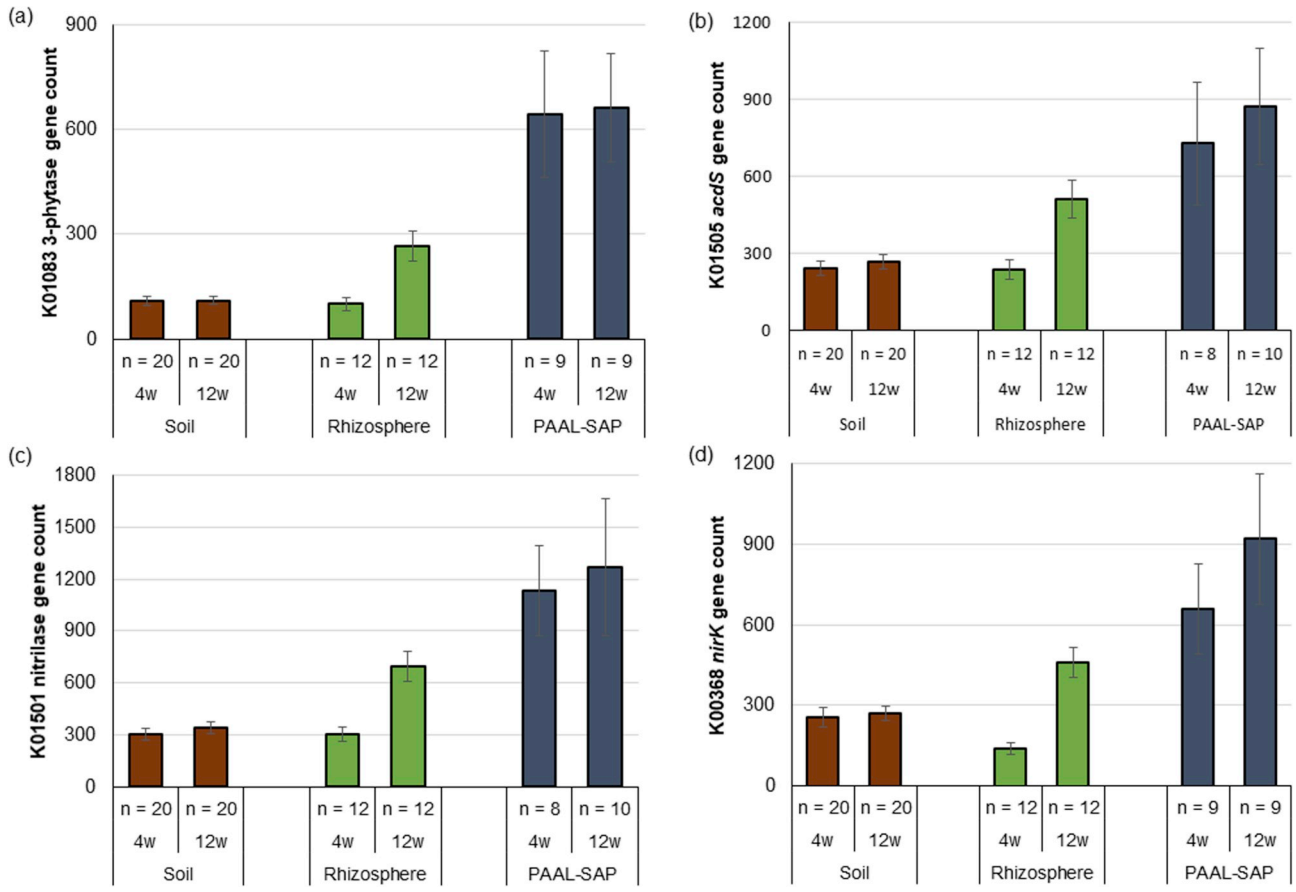

Fig. 6. Predicted genes implicated in plant-growth promotion upregulated in PAA-L compared to bulk and rhizosphere soil regions after 4 and 12 weeks of field incubation in soils planted to wheat (*Triticum aestivum* cv. Mace). (a) 3-phytase catalyses the release of phosphate from phytate. (b) 1-aminocyclopropane-1-carboxylate deaminase (*acdS*) influences the stress response of plants by decreasing the level of 1-aminocyclopropane-1-carboxylate, a precursor to ethylene high concentrations of which can inhibit plant growth or cause death. (c) Nitrilase catalyses the production of the plant hormone indole-3-acetic acid (IAA) via the indole-3-acetonitrile pathway. IAA is produced by a variety of rhizobacteria and stimulates root growth. (d) *nirK*, produces nitric oxide that in interplay with IAA or on its own can stimulate root branching.

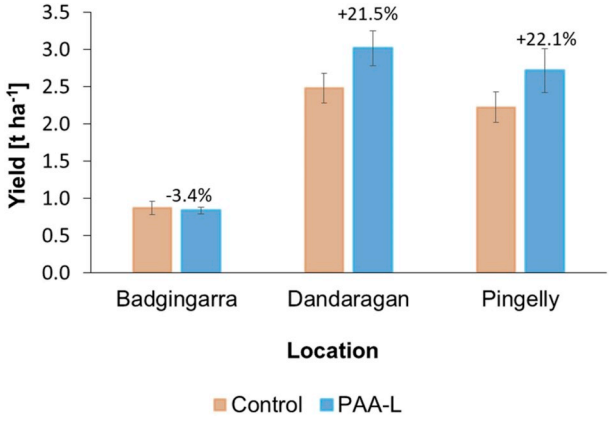

Fig. 7. Wheat grain yield increases due to PAA-L addition to the root zone. Badgingarra test site gave no increase in wheat yield beyond the control being a non-wetting soil type (Molarity of Ethanol 2.4; King, 1981). Error bars represent standard errors of the mean.

naturally occurring counterpart - the rhizosphere. The high specific surface area and aspect ratio of these nanofibrils within the osmotic hydrogel environment provided a high affinity for bacterial adhesin – fibril interaction, preferential attachment and subsequent microcolony formation. Next generation sequencing of the 16S rRNA genes showed a significant selectivity in the population distribution of PAA-L microbial community towards potential plant-growth promoting taxa exceeding both the bulk and rhizosphere soil habitats. This considerable upregulation of these PAA-L prokaryotes, linked to plant growth and disease suppression were able to sustain significant beneficial microbial activity during periods of moisture stress. Potentially, such osmotic hydrogels suggest the ability to modulate effects of drying and hydrophobicity in rhizosphere environments during periods of increasing moisture stress. These data clearly demonstrate that these hydrogel-nanofibril materials may open new approaches to engineering the plant rhizosphere and new opportunities to maintain grain yields during periods of drying climate.

Author contributions

DVM, DEM and AHW designed the study. FM, PM, JB, and VKT performed the experiments. PM and MS synthesized and provided materials. FM, VTHP and PM performed data analyses. DEM, FM and DVM wrote the manuscript with input from all authors.

Declaration of competing interest

Authors, A. H. Wissemeier and M. Seufert, were employed by company BASF SE (Germany). All other authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.107715.

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