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ORIGINAL PAPER

Soil disturbance and water stress interact to influence arbuscular mycorrhizal fungi, rhizosphere bacteria and potential for N and C cycling in an agricultural soil

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Abstract

The objective of this study was to determine how soil disturbance and soil water deficit alter colonisation of roots by naturally occurring arbuscular mycorrhizal (AM) fungi and rhizosphere bacteria. Soil cores were collected at the end of summer from a cropped paddock with a 5-year history of no-tillage in south-western Australia which has a Mediterranean climate. Well-watered and water-stressed treatments were maintained at 70 and 35% field capacity, respectively. AM fungal colonisation was determined using microscopy, rhizosphere bacterial community composition was assessed using barcoded PCR-amplified bacterial 16S rRNA genes, and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analysis of functional gene prediction was used to characterise the rhizosphere functionality for some nitrogen (N) cycling and soil carbon (C) degradation. Mycorrhizal colonisation and plant growth were reduced by disturbance under water stress but not for well-watered soil cores. The rhizosphere bacterial community composition shifted with both soil disturbance and soil moisture, and there was an interaction between them. Disturbance decreased the relative abundance of *Proteobacteria* and *Acidobacteria* and increased the relative abundance of *Actinobacteria* and *Firmicutes* in water-stressed soil. The predicted abundance of some genes involved in N reactions was negatively influenced by both disturbance and soil moisture, but the predicted abundance of C degrading predicted genes was only marginally affected. This study highlighted how soil disturbance prior to seeding can alter soil biological processes that influence plant growth and that this could be most pronounced when water is limiting.

Keywords Soil microbiology · PICRUSt · Abiotic stress · Next generation sequencing

Introduction

Decreasing precipitation is characteristic of arid and semi-arid regions around the world (Diffenbaugh and Giorgi 2012). Arid, semi-arid and Mediterranean-type regions currently support agricultural production for close to a third of the world's population (Barton et al. 2013). The semi-arid agricultural soils of south-western Australia produce around 40% of the total cereal production in Australia (Knopke et al. 2000). These soils are characterised by poor structure, low organic matter and carbon (C) content, and low levels of microbial activity which makes them susceptible to external stresses associated with climate change and management practices such as tillage (Hoyle and Murphy 2006; Fisk et al. 2015). Rain-fed agricultural production systems in these regions are particularly vulnerable to changes in precipitation because they are climate-dependent (Moriondo et al. 2011). Thus,

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Mediterranean regions are projected to experience greater soil moisture deficits during the growing season and longer drought spells (Dubrovský et al. 2014). They are particularly responsive to climate change and are global "hot spots" of future environmental change (Giorgi 2006). However, the mechanisms involved in maintaining the functional stability of these soils are largely unknown.

Microorganisms within both the rhizosphere and the mycorhizosphere influence plant physiology in a number of ways, including processes associated with the formation of mycorrhizas (Smith and Read 2008) and activities of free living and symbiotic N fixing bacteria (Glick 1995; Jones et al. 2009). Plants can also benefit from complex fungal and bacterial communities for accessing nutrients and water and for protecting against pathogens (Bulgarelli et al. 2013; Bálint et al. 2015). As such, rhizosphere communities are so important for plant growth they are considered to be the host plant's 'second genome' (Grice and Segre 2012).

Decreasing precipitation and associated reduced water availability can impact soil biological processes relating to soil function in a relative short time frame of days to months (Kwon et al. 2013; Mickan et al. 2017). They alter the rate and extent of microbial mediated biogeochemical processes in soil by changing the quantity of organic matter and its decomposition by microorganisms (Fuchslueger et al. 2014). Sustained soil water deficiency alters soil structure by reducing water-filled pore space, and consequently increases the volume of air-filled soil pores (Moyano et al. 2013). Sustained soil water deficiency also decreases nutrient mobility and increases nutrient concentrations in the residual water (Schimel et al. 2007; Fuchslueger et al. 2014).

Whilst soil microorganisms in semi-arid regions are commonly subject to seasonal water stress due to precipitation dynamics at the ecosystem scale, some groups of organisms are better able to adapt to soil water deficits than others by activating strategies such as dormancy (Manzoni et al. 2012). Nevertheless, there is a physiological cost to microbial communities associated with survival in water-stressed soil which corresponds to changes at the ecosystem level for C and nutrient flows (Schimel et al. 2007). As soil moisture decreases, conditions become less favourable for soil fauna and bacteria (Manzoni et al. 2012; Barnard et al. 2013) but fungi are generally more tolerant of water stress (Fuchslueger et al. 2014; Frank et al. 2015). Consequently, fungal-dominated soil ecosystems have been reported to be more resistant to drought than bacteria-dominated soil ecosystems (DeVries et al. 2012). However, evidence for effects of soil moisture on the bacterial community is either lacking or equivocal (Manzoni et al. 2012). The hyphal network of AM fungi can dominate soil fungal communities around roots and help plants tolerate water stress by enabling plants to scavenge water and nutrients beyond the root depletion zone (Ruth et al. 2011; Ruiz-Lozano et al. 2012; Mickan 2014).

Rain-fed agricultural systems in the Mediterranean region of south-western Australia use adaptive management practices to maintain production under reduced winter precipitation (Fisk et al. 2015). No-till and reduced-till practices are common in this region, with approximately 90% of farmers using at least some form of no-till (Llewellyn et al. 2012). There are many studies of physical and chemical effects of reduced tillage at field scales that illustrate improvement in soil water-holding capacity, decreased erosion and increased nutrient retention (So et al. 2009). Whilst recent studies have investigated no-till effects on soil bacteria (Chávez-Romero et al. 2016) and AM fungal colonisation (Verzeaux et al. 2016), there is a gap pertaining to whether soil disturbance influences rhizosphere bacteria-associated N and C cycling in relation to AM fungal colonisation in soils with a water deficit.

The impact of disturbance of agricultural soil on microbial communities is of particular interest because of the roles of these microorganisms in nutrient cycling (Stevenson et al. 2014). Whilst there is a large body of literature on the effect of tillage on soil fungi and bacteria, these studies are typically comparisons of soils which have been either exposed or not exposed to conservation tillage for several years (Helgason et al. 2010; Stevenson et al. 2014). There are fewer studies reporting the immediate effect of soil disturbance on AM fungi (van der Heyde et al. 2017) and the interaction with rhizosphere bacterial communities. Previous studies have demonstrated soil disturbance can alter mycorrhizal colonisation through dilution of, or damage to, infective propagules (Jasper et al. 1991; Mickan et al. 2017).

The formation of mycorrhizal associations with host plants has potential to alleviate plant water stress directly through increased water scavenging capability and nutrient acquisition, especially for P (Ruth et al. 2011). Previous studies have reported soil disturbance can affect the viability of AM fungi in natural forest ecosystems, although this may have a lesser effect for agricultural soil under pasture where there is a higher microbial abundance (Jasper et al. 1991). To further complicate matters, AM fungi from different genera can differ in their ability to form mycorrhizal associations when soil is disturbed, having both the potential to either increase or decrease following physical disturbance (Boddington and Dodd 2000). Fungi are susceptible to disruption of the soil environment caused by tillage because physical disturbance breaks up their mycelia (Schimel et al. 2007; DeVries et al. 2012).

In this experiment, physical disruption of field-collected soil cores was investigated to determine its effect on AM fungal colonisation under well-watered and water-stressed conditions (Mickan et al. 2016) in association with subterranean clover (*Trifolium subterraneum*) as the test plant (Jasper et al. 1989). AM fungi were quantified as both root length colonised and the proportion of root colonised (Giovannetti and Mosse 1980) to estimate the extent of colonisation of roots by these symbiotic fungi. For the rhizobacterial

community, 16S rRNA sequencing and PICRUSt were used to determine the disturbance effects on taxonomic and putative functional attributes involved in N and C cycling in relation to water stress. The starting hypotheses were (1) soil disturbance at the time of seeding will decrease colonisation of roots by AM fungi in *Trifolium subterraneum*, especially under water stress. (2) Soil disturbance at the time of seeding when under water stress will alter the composition rhizosphere bacterial community in favour of an increase in the relative abundance of spore-forming *Firmicutes* and *Actinobacteria.* (3) Soil disturbance at the time of seeding will increase the potential activity of enzymes associated with degradation of C fractions and N transformations.

Materials and methods

Experimental design and disturbance regime

A pot experiment was used to investigate the influence of soil disturbance on plant growth, AM fungal colonisation and the rhizosphere bacterial community under both well-watered and water-stressed soil conditions. Adjacent pairs of field-collected soil cores were sampled from a paddock after a wheat crop with a history of 5 years without tillage were used. The experimental design consisted of two factors: soil disturbance (undisturbed and disturbed) and water treatment (well-watered 70% field capacity and water-stressed 35% field capacity). The experiment consisted of four replications in a randomised block design and was destructively harvested at 60 days after emergence (DAE).

Zero soil disturbance was achieved using a custom designed metal cage soil corer that internally housed a PVC pipe, 100 mm in diameter by 150 mm depth. The metal cage with a cutting edge of exactly the same internal diameter as the PVC pipe containing the core was driven into field soil to the required depth, then dug out so that the metal cage was removed leaving the PVC housing. The bottom of the PVC housing was capped carefully to create an undisturbed soil core. The additional soil core was collected in the same way next to each undisturbed soil core to make a total of 16 soil cores of which 8 were undisturbed and 8 were disturbed. For the soil disturbance treatment, the soil in this core was disturbed after transferring to a cylinder 100 mm wide by 1000 mm long, capped at each end, and inverted 5 times at a speed of 1 invert per 10 s to mimic physical disturbance then returned to the PVC housing and packed to its original bulk density ready for planting.

Soil collection and analysis

The cores (0–15 cm) were collected from field dry soil, during fallow at the end of summer from a paddock with a grain cropping history near Moora, Western Australia (30°38′16.8″S, 116°0′36″E). Separate soil cores were sampled for each experimental unit (pot). The site receives an average annual rainfall of 461 mm (60 rainy days > 1 mm), and temperature ranging from winter 7–18 °C to summer 17–34 °C (http://www.bom.gov.au/climate/averages/tables/cw_008091.shtml).

A total of 16 soil cores were collected and the bulk soil collected adjacent to the twin (disturbed and undisturbed) soil cores was sieved (< 4 mm) prior to characterisation and analysis. Bulk density, moisture content, volumetric water content (VWC), pH and particle size analysis were determined for each soil type as described previously (Rayment and Lyons 2011). Soil pH was measured (1:5 soil/water ratio) using a probe inserted into 0.01 M $CaCl_2$ mixture of soil and water which calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), manganese (Mn), aluminium (Al), sulphur (S), copper (Cu) and iron (Fe) were characterised using air-dried soils (sieved < 2 mm), and were shaken for 5 min using the Mehlich-III extraction (Mehlich 1984), and each soil were measured by inductively coupled plasma emission spectroscopy (Perkin Elmer Optima 5300DV ICP-OES). Total C and nitrogen (N) were measured by combustion using an Elementar Analyser (Vario Macro CNS; Elementar, Germany). All measurements were completed in triplicate. Soils were classified as a Tenosol using the Australian Soil classification series (Isbell 2002) and described as 'sand' based on the Australian Field Texture Grade (McDonald et al. 1998). The soil was composed of 1% silt, 4.2% clay, and 94.8% sand (Table 1). The light soil disturbance treatment had no effect on the soil physical or chemical properties assessed in this agricultural soil.

Table 1 Initial soil properties of an agricultural soil collected near Moora, Western Australia. There was no effect of soil disturbance treatment on these soil properties

	Mean	SE
Al mg/kg	318.73	23.97
Ca mg/kg	633.99	89.02
Cu mg/kg	0.55	0.07
Fe mg/kg	97.92	7.69
K mg/kg	33.60	4.97
Mg mg/kg	47.94	5.57
Mn mg/kg	5.13	1.18
P mg/kg	91.23	16.93
S mg/kg	11.66	1.98
Zn mg/kg	3.01	0.32
N (%)	0.064	0.01
C (%)	0.67	0.16
pH ($CaCl_2$)	6.13	0.11

Soil water retention curve

A soil water retention curve using a pressure plate apparatus was used to calculate field capacity of the soil, where 100% field capacity was calculated at − 10 kPa and permanent wilting point was − 1500 kPa (Mickan et al. 2016). Soil was packed to a bulk density corresponding to the field conditions into a core 54 mm diameter by 10 mm high, saturated with water at atmospheric pressure, and gravimetric water content was determined. Triplicate batches of replicated, saturated samples were transferred to porous ceramic plates, placed in pressure chambers, and subsequently equilibrated at matric potentials of − 10, − 100, and − 1500 kPa. Gravimetric water contents were measured after equilibration and then adjusted to volumetric values using bulk density.

Sowing, sampling and harvest procedures

Subterranean clover (*Trifolium subterraneum* L. var. 'Dalkieth') seeds were obtained from the Department of Primary Industries and Regional Development (https://dpird.wa.gov.au/) and seeds were graded to 2.00 to 2.25 mm in diameter to ensure uniform seed size. Seeds were imbibed and aerated in deionised water for 3 h, then planted into soil cores for germination with soil moisture maintained at 12% for 6 days for all treatments. Seven germinated seeds were planted evenly in each pot at 10 mm depth. Plants were thinned to five per pot after emergence to ensure an equal number and even distribution of seedlings. Pots were placed under glasshouse conditions and the water regimes for both water stressed were kept the same at 70% field capacity, and water stresses were initiated 14 days after seedling emergence for the 35% field capacity treatments. Pots were harvested destructively 60 days after emergence (DAE). Shoot and root biomass were recorded before and after oven-drying at 60 °C for 72 h.

DNA extraction, PCR amplification and agarose gel electrophoresis

Rhizosphere soil (~ 2 to 5 g) was carefully collected from each pot ($n = 4$) with a sterile brush at DAE 60, after bulk soil was gently extracted off roots (Mickan et al. 2017). Rhizosphere soil was transferred to − 20 °C freezer for subsequent DNA extraction. DNA was extracted from soil using a PowerSoil® DNA Isolation Kit (MoBio, Carlsbad, CA USA) following the instructions of the manufacturer, and blanks where no soil was added to DNA extraction as negative controls were used to identify both DNA contamination and PCR contamination (Vestergaard et al. 2017). DNA was quantified (Qubit, Life Technologies, Australia) and adjusted to 1 ng/μL using molecular grade water (SIGMA) prior to being stored at − 20 °C until further analysis. Bacterial 16S rRNA genes were amplified from DNA samples using universal core bacterial primers 515F and 806 R (Caporaso et al. 2010; Mori et al. 2014) modified with Golay barcodes (Caporaso et al. 2012) following Mickan et al. (2017). Sequencing was performed on the Ion Torrent Personal Genome Machine Sequencer (PGM; Life Technologies, Carlsbad, CA, USA) using Ion 318 chips and Ion PGM Sequencing 400 kit as described previously (Whiteley et al. 2012; Gleeson et al. 2016). PGM semiconductor sequencing was performed at the Lottery West State Biomedical Facility Genomics Node in Perth, Western Australia.

Ion torrent sequencing output

Ion Torrent semiconductor sequencing yielded a total of 2,074,019 barcoded sequences for the 16 samples. The range was from 45,361 to 94,531 sequences per sample, with an average of 71,517 sequences per sample, and a mean amplicon length of 274 base pairs (bp) before quality filtering. After the removal of 29,313 chimeric sequences, the total number of unique sequences (sequences identified as being identical to the reference sequence) was determined to be 348,241 of the total remaining sequences (1,888,913). These high-quality reads were clustered de novo into 147,002 species-level OTUs (97% sequence identity) in Mothur. Rarefaction to 20,000 sequences was decided as this was the lowest sequence count for one of the samples and therefore retains an equal number of experimental replications (see Gihring et al. 2011).

AM fungal root colonisation

After rhizosphere soil was collected, roots were washed free of soil and coarse organic matter, and fresh and dry weights of roots were recorded. Root subsamples (0.5 g fresh weight) were selected at random (from segments cut into approximately 1 cm and dispersed in water) to assess mycorrhizal colonisation and the remainder was oven-dried at 60 °C for 72 h. Total root dry weight per pot was determined for the AM fungal colonisation subsample using percentage moisture of fresh root mass. The root subsamples were cleared in 10% KOH, acidified, and stained with Trypan blue (0.05%) in lactoglycerol (1:1:1 lactic acid/glycerol/water) and destained in lactoglycerol (Abbott and Robson 1981). Percentage root length colonised by AM fungi (%RLC) was calculated using the gridline intercept method, scoring more than 100 intercepts under a microscope at × 100 magnification (Giovannetti and Mosse 1980). Direct assessment of the proportion of root length colonised by AM fungi was measured. The length of root colonised by AM fungi was calculated using total root length (Newman 1966), and the proportion of root length that was mycorrhizal (Giovannetti and Mosse 1980).

Bioinformatics

Raw sequence data were processed using Mothur version 1.35.1 (Schloss et al. 2009), with a slightly modified standard operating procedure (Schloss et al. 2011). Sequences were removed based on if they did not meet the following quality control criteria: barcode miss match = 1, primer mismatch = 2, ambiguous base calls = 0, minimum quality score Q > 20, maximum homopolymers length = 8 and minimum/maximum length = 150/350. After quality control, the retained sequences were pre-clustered to remove any PCR-based bias and singletons. Chimeric sequences were also identified and removed using uchime (chimera.uchime; Edgar et al. 2011) in Mothur. Unique sequences were aligned against the Silva 106 database (Pruesse et al. 2007) and assigned operational taxonomic unit (OTU-based taxonomic analysis) based on a 97% similarity cutoff. Alpha rarefaction was performed and the sequence data were subsampled to 10,000 sequences per sample to ensure fair comparisons between the samples (Gihring et al. 2011; Schöler et al. 2017). Diversity indices were calculated within Mothur, and OTU richness per sample was calculated following Mickan et al. (2017).

The OTUs that were not present in all of the treatments were discarded from the analysis. The top 50 most abundant OTUs as determined by sequence count were analysed, representing 32.7% of all sequences (Supplementary Table S2). The taxonomic assignment was restricted to the family level, as there were large numbers of unclassified affiliations at deeper taxonomic resolution.

Predictive functional profile of the microbial communities

A Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (https://picrust.github.com) was performed (Langille et al. 2013). The genes characterised were those identified in C and N cycling (Mickan et al. 2017).

Statistics

All data analyses were conducted in the R statistical environment (R core team 2015). Two-way analysis of variance (ANOVA) was applied to test for the effects of 'water' and 'disturbance' treatments on bacterial, abundance, OTU richness, plant growth and AM fungal colonisation. *Post-hoc* analysis was performed using a Tukey HSD test on the disturbance treatments in each water treatment only if the ANOVA analysis yielded significant results. If response variables displayed homogeneous error in the variance of residuals, a logarithmic or square root transformation was performed. The observed richness was calculated based on the number of OTUs detected in a sample and the coverage was calculated using Good's coverage estimator (Good 1953). A non-metric multidimensional scaling plot of soil bacterial communities and putative functional genes was used to display responses to water treatments (well-watered 70% FC, water-stressed 35% FC) and disturbance (undisturbed, disturbed), using OTU-based (97% similarity) Bray-Curtis dissimilarities distances.

A permutational multivariate analysis of variance (PERMANOVA) was used to test the significant difference between taxonomic bacterial (OTU level) and functional gene community data (beta diversity) and treatments (water and disturbance) in the vegan package (Oksanen et al. 2013) based on Bray-Curtis distances calculated from relative abundances of OTUs at 97% similarity. Effects of the water treatments and soil disturbance on relative abundances of bacterial phyla and AM fungal colonisation were assessed using linear mixed models (LMMs) in the nlme package (Pinheiro et al. 2017). Bacterial sequence data at the genus resolution of the OTUs were differentially and separately represented (in terms of relative abundance) for the water and disturbance treatments ($p < 0.05$; White et al. 2009).

To gain a greater understanding of how individual taxa (OTUs) responded to water and disturbance treatments, a statistical analysis was conducted using Metastats pairwise comparison (White et al. 2009; Mickan et al. 2017), to identify taxa that most differed between each treatment. This approach identified the top 50 OTUs (Supplementary Table S3) whose relative abundance was either increased or decreased in association with water and disturbance treatments (Supplementary Table S4). OTUs that were not present in all of the treatments were discarded from the analysis. The top 50 most abundant OTUs as determined by sequence count were analysed, and this represented 32.7% of all sequences (Supplementary Table S3).

Results

Plant dry weight

Dry shoot biomass was significantly altered by both water ($P < 0.001$) and disturbance ($P = 0.017$) treatments. Soil disturbance had a negative effect on shoot mass in the water stress treatment (−48.1%; $P < 0.001$) but not in the well-watered treatment ($P = 0.549$) (Fig. 1a). To a lesser extent, dry root mass was significantly reduced under water stress ($P < 0.001$) but was unaffected by soil disturbance ($P = 0.068$) and there was no interaction between the water and disturbance treatments ($P = 0.146$). There was no change in root mass under either water-stressed ($P = 0.288$) or well-watered soil conditions ($P = 0.133$) in association with soil disturbance (Fig. 1b).

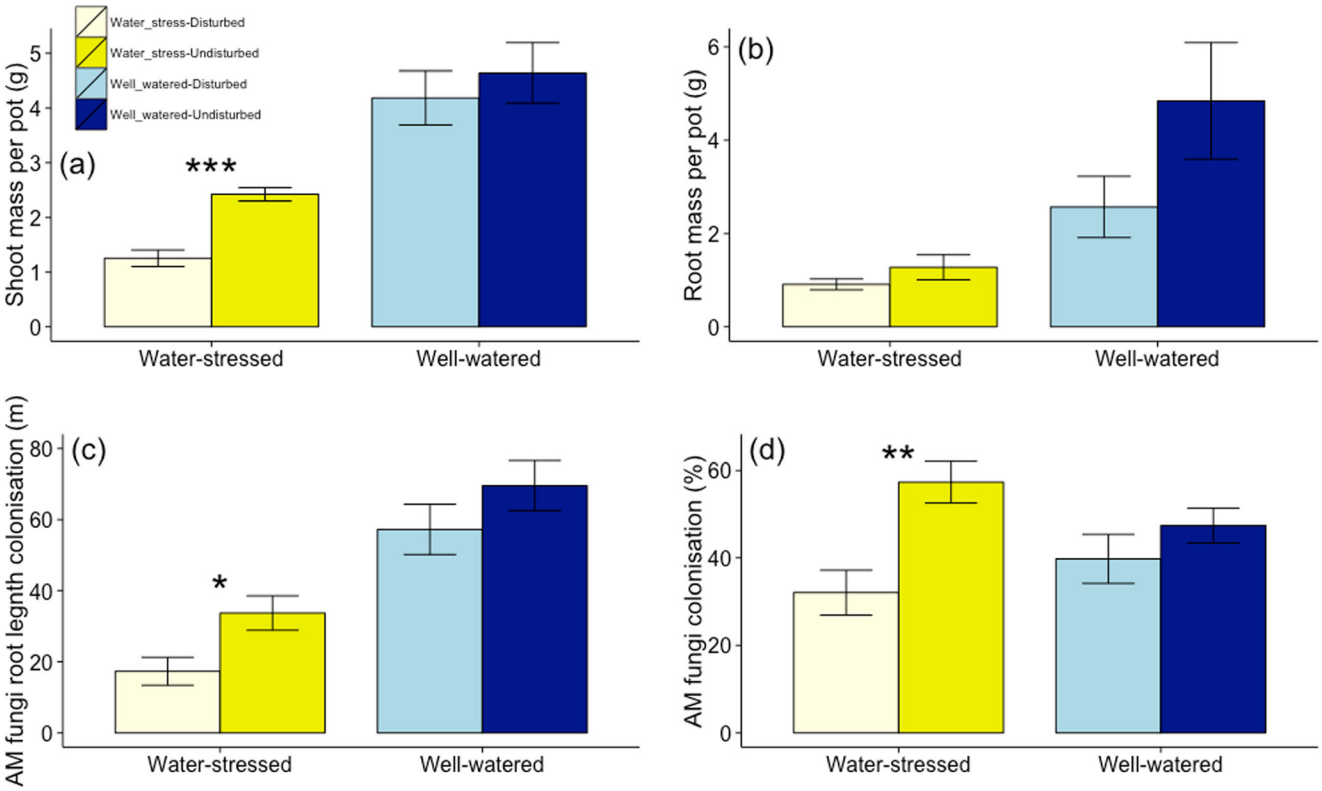

Fig. 1 Plant shoot (**a**), root (**b**), AM fungal colonisation as root length colonised (RLC) (**c**) and percentage root length colonised (%RLC). **d** Response to soil disturbance (disturbed, undisturbed) and water (water-stressed 35% FC, well-watered 70% FC) treatments. Error bars are the standard error of the mean ($n = 4$)

AM fungal colonisation

Root length colonised by AM fungi responded to soil disturbance ($P = 0.014$) and soil moisture conditions ($P = 0.001$) in a similar way to shoot mass (Fig. 1c). The % root length colonised (Fig. 1d) was significantly affected by soil disturbance ($P = 0.001$) but not by the variation in soil moisture ($P = 0.825$). Soil disturbance only had a negative effect on AM fungal colonisation under water-stressed conditions, where there was a decrease of 48.7% ($P = 0.018$) in root length colonised and a decrease of 44% ($P = 0.002$) in % root length colonised (Fig. 1c, d). For well-watered soil conditions, soil disturbance did not affect AM fungal colonisation assessed as either root length colonised ($P = 0.240$), or % root length colonised ($P = 0.289$) (Fig. 1c, d). There was no significant two-way interaction for the water and disturbance treatments for either root length colonised ($P = 0.717$) or % root length colonised ($P = 0.089$).

Bacterial community assemblages

Alpha diversity

There was no significant difference in the alpha diversity indices quantified (Good's coverage, OTU richness or inverse Simpson) for the water treatments, the disturbance treatments or the interaction between water and disturbance treatments (Table 2, Supplementary Table S1).

Relative abundance

Overall, the rhizosphere bacterial community was dominated by *Actinobacteria* (38%), *Proteobacteria* (34%) and *Bacteroidetes* (12%) (Fig. 2). Soil disturbance influenced the composition of rhizosphere bacteria at the phylum level with significant changes in relative abundance of *Proteobacteria* ($P = 0.01$), *Acidobacteria* ($P = 0.04$), *Gemmatimonadetes* ($P < 0.001$) and *Verrucomicrobia* ($P = 0.00$) (Table 3). For example, soil disturbance decreased the relative abundance of *Proteobacteria* under both well-watered and water-stressed conditions and of *Acidobacteria* only under water-stressed conditions (Fig. 2). In contrast, the relative abundance of *Gemmatimonadetes* and *Verrucomicrobia* increased in response to soil disturbance under both well-watered and water-stressed conditions whilst that of *Actinobacteria* and *Bacteroidetes* ($P = 0.05$) only increased under water-stressed conditions. Abundances of *Proteobacteria* ($P = 0.03$), *Actinobacteria* ($P = 0.01$), *Firmicutes* ($P = 0.00$) and *Chloroflexi* ($P = 0.00$) were also significantly affected by soil moisture (Table 3). The relative abundance of *Proteobacteria*

Table 2 Alpha diversity indices based at the OTU resolution (similarity = 97%) of soil bacteria following disturbance treatment (disturbed, undisturbed). The water treatments were sustained well-watered (70% FC) and water-stressed (35% FC). Values are the mean for each treatment and standard error (SE) of the mean ($n = 4$)

		Water-stressed		Well-watered	
		Disturbed	Undisturbed	Disturbed	Undisturbed
Good's coverage	Mean	0.750	0.755	0.739	0.771
	SE	0.014	0.013	0.019	0.027
OTU richness	Mean	5650.36	5573.87	5928.18	5158.51
	SE	306.67	329.69	423.53	593.49
Inverse Simpson	Mean	93.00	125.76	101.44	103.49
	SE	17.08	20.32	14.05	23.54
Shannon	Mean	6.52	6.57	6.68	6.29
	SE	0.14	0.16	0.17	0.39

decreased in response to water stress whereas it increased for the phyla *Actinobacteria*, *Firmicutes* and *Chloroflexi.* There was no interaction between soil disturbance and soil moisture on the relative abundance of phylum resolution.

Impact of soil disturbance and soil moisture on bacterial OTUs

In order to visualise the overall distribution of OTUs at the 97% similarity level, a non-metric multidimensional scaling (NMDS) plot showed that the bacterial community composition was influenced by water and physical disturbance treatments (Fig. 3). Soil disturbance was highly correlated with axis 1 with distinct clustering of samples along this axis from undisturbed to disturbed. To a lesser extent, the bacterial communities cluster along axis 2 according to soil moisture, particularly in undisturbed soil cores where there was a clear separation in the NMDS. The separation between the well-watered and water-stressed treatments and with soil disturbance indicates that the bacterial communities are dissimilar under these conditions. Subsequent community analysis by PERMANOVA revealed that both soil water ($P = 0.012$) and soil disturbance ($P < 0.001$) as well as the interaction between them ($P = 0.024$) had a significant impact on bacterial community composition (Table 4).

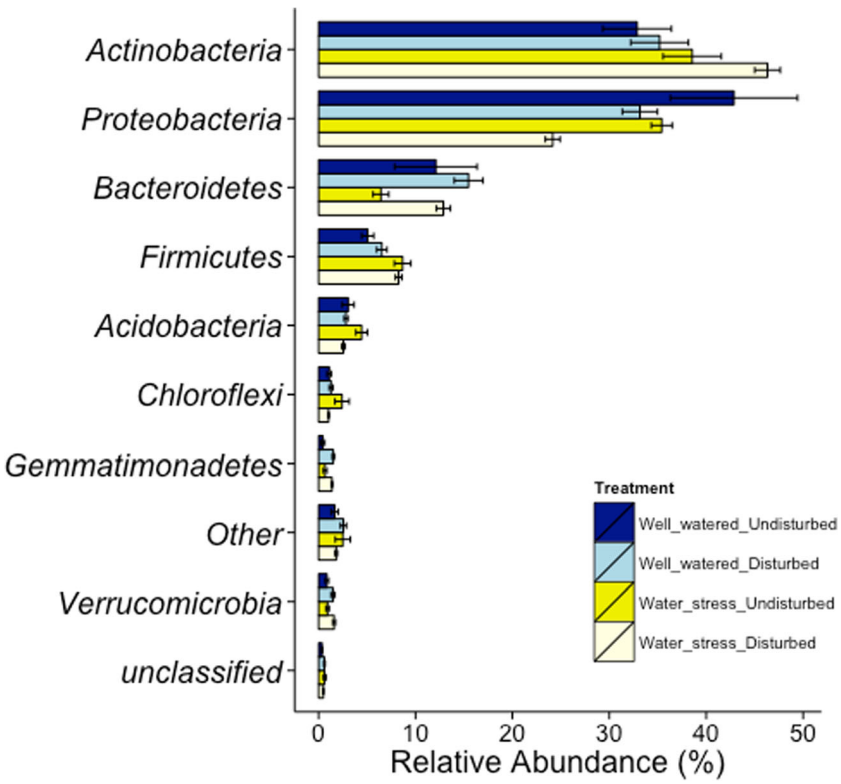

Fig. 2 Rhizosphere bacterial relative abundance for water (35%FC, 70%FC) and disturbance (disturbed, undisturbed) treatments as observed at the Phylum level. Error bars are the standard error of the mean ($n = 4$)

Table 3 Two-way ANOVA results showing *P* values fixed at Phylum resolution of relative abundance. Treatments were 'disturbance' (disturbed, undisturbed) and 'water' (well-watered 70% FC, water-stressed treatment 35% FC). Significant *P* values indicated in italics

	Disturbance		Water		Disturbance*water	
	F	*P*	*F*	*P*	*F*	*P*
Proteobacteria	3.161	*0.011*	8.780	*0.035*	0.923	0.826
Actinobacteria	3.161	0.101	8.780	*0.012*	0.923	0.356
Acidobacteria	5.524	*0.037*	1.559	0.236	3.432	0.089
Bacteroidetes	4.498	0.055	3.185	0.100	0.442	0.519
Gemmatimonadetes	33.857	*< 0.001*	0.078	0.785	1.379	0.263
Firmicutes	0.729	0.410	18.840	*< 0.001*	2.300	0.155
Chloroflexi	0.729	0.410	18.840	*< 0.001*	2.300	0.155
Verrucomicrobia	12.995	*0.004*	0.267	0.615	0.024	0.879
Other	0.055	0.819	0.011	0.917	2.903	0.114
Unclassified	0.437	0.521	0.790	0.391	4.870	*0.048*

Response of individual OTUs to water and disturbance treatments

Using 'metastats' the top 50 OTUs (Supplementary Table S2) for which the relative abundance either increased (56%), decreased (8%) or had no influence (36%) in association with soil moisture and soil disturbance (Supplementary Table S3).

Under well-watered conditions, soil disturbance increased the relative abundance of eight OTUs (Supplementary Table S3) including those of families *Cytophagaceae* ($P < 0.001$), *Chitinophagaceae* (two OTUs: $P = 0.03$, $P = 0.05$ respectively) and *Bacteroidetes* ($P < 0.001$). An increase in relative abundance was also observed for OTUs of Verrucomicrobia (*Opitutaceae*; $P = 0.03$), Firmicutes (*Paenibacillaceae*; $P = 0.03$), Proteobacteria (*Rickettsiales*; $P = 0.01$) and Gemmatimonadetes OTU 33 (Unclassified; $P < 0.001$).

Under water stress, soil disturbance increased the relative abundance of the same taxa as occurred under well-watered conditions, including two *Bacteroidetes* [*Cytophagaceae* ($P = 0.03$), *Chitinophagaceae* (two OTUs, $P = 0.02$, $P = 0.01$, respectively)], *Verrucomicrobia* (*Opitutaceae;* $P = 0.01$), *Firmicutes* (*Paenibacillaceae;* $P = 0.03$) and *Gemmatimonadetes* (unclassified; $P = 0.01$). In addition, there was an increase in the relative abundance of *Actinobacteria* (*Streptomycetaceae*; $P = 0.00$) in the water-stressed soils. In contrast to the well-watered conditions, soil disturbance decreased the relative abundance of *Proteobacteria* (*Oxalobacteraceae*; $P = 0.00$), *Acidobacteria* (*Koribacterace*; $P = 0.03$) and *Bacteroidetes* (*Sphingobacteriaceae*; $P = 0.04$).

Under water stress, only four OTUs showed a directional change from that which occurred under water stress in comparison to well-watered soil that was disturbed. There was a

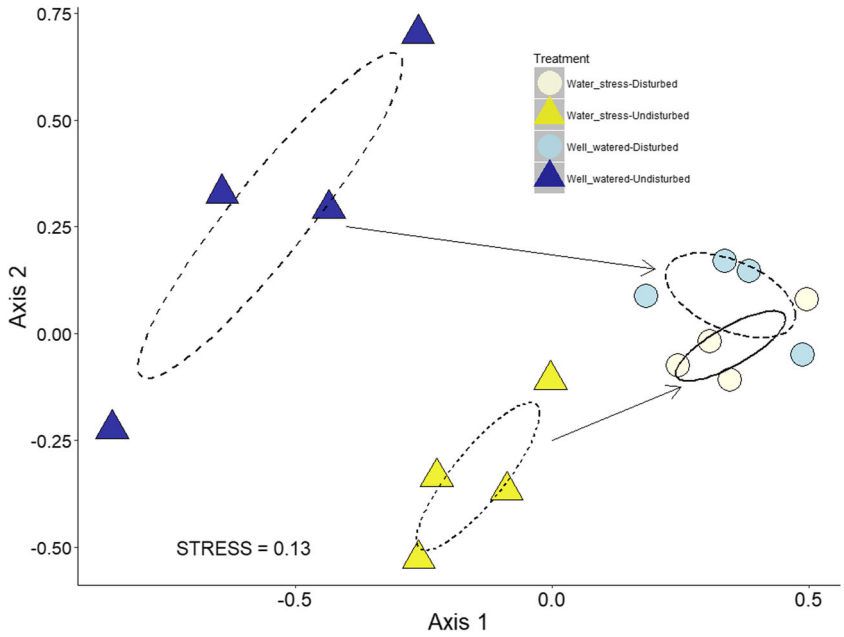

Fig. 3 Non-metric multidimensional scaling plot of soil bacterial communities in two water treatments (well-watered 70% FC, water-stressed 35% FC) and at two disturbances (undisturbed, disturbed) using OTU-based (97% similarity) Bray-Curtis dissimilarities distance

Table 4 OTU community assemblage analysis by PERMANOVA results based on 97% similarity OTU abundance data (square root transformed), using 999 permutations. Treatments consisted of water (water-stressed 35% FC, well-watered 70%FC) and disturbance (disturbed, undisturbed). Significant *P* values indicated in italics

	Degrees of freedom	Sum of squares	Mean squares	F. model	R^2	Pr(>F)
Disturbance	1	0.616	0.616	2.509	0.140	*0.001*
Water	1	0.439	0.439	1.787	0.100	*0.012*
Disturbance * water	1	0.397	0.397	1.615	0.090	*0.024*
Residuals	12	2.947	0.246	0.670		
Total	15	4.399	1.000			

marked increase in the relative abundance of three OTUs of *Actinobacteria* affiliated with *Streptomycetaceae* ($P < 0.00$, $P = 0.03$, $P = 0.02$ respectively) and one *Firmicutes* affiliated with *Paenibacillaceae* ($P = 0.01$).

Water stress also increased the relative abundance of two other *Firmicutes* belonging to *Bacillaceae* ($P = 0.00$, $P < 0.00$), two *Proteobacteria* affiliated with *Oxalobacteraceae* ($P = 0.01$, $P = 0.01$ respectively), two *Actinobacteria* affiliated with *Streptomycetaceae* ($P = 0.01$, $P = 0.03$ respectively), one *Gemmatimonadetes* ($P = 0.01$) and one *Bacteroidetes* ($P = 0.01$). Interestingly, there was a decrease in the relative abundance of one *Bacteroidetes* OTU affiliated with *Chitinophagaceae* ($P = 0.03$) (Supplementary Table S4).

16S rRNA gene profiling information PICRUSt (Langille et al. 2013) was used to predict the abundance of C and N functional genes (Mickan et al. 2017) between the soil disturbance and water treatments (Supplementary Fig. S1). Analysis by a two-way ANOVA revealed soil disturbance altered the following nitrification genes: amoA (70%, $P = 0.00$), HaO (27%, $P = 0.04$) and denitrification genes narG (−35%, $P = 0.03$), narfA (50%, $P < 0.00$), narH (−32%, $P = 0.04$), narI (−34%, $P = 0.03$) and norB (−35%, $P = 0.05$) (Table 5). Water stress altered the abundance of denitrification genes nosZ (−48%, $P = 0.01$) and norB (−35%, $P = 0.01$). A two-way interaction (disturbance*water treatments) was detected for napA ($P = 0.02$) and napB ($P = 0.03$). There was no detected alteration in the abundance of C-predicted genes for soil disturbance, although water stress altered the abundance of starch degradation genes alpha amylase (32%, $P = 0.02$), beta galactosidase (25%, $P = 0.01$) and chitin degradation genes for chitinase (15%, $P = 0.01$).

An NMDS plot was used to visualise the overall distribution of predicted N and C reactions in association with soil moisture and soil disturbance (Fig. 4). Community analysis by PERMANOVA showed N was influenced mostly by disturbance ($P = 0.001$), but also by water ($P = 0.00$) with distinct clustering (Fig. 4a; Table 6). To a lesser extent, communities assessed by C cycling reactions showed the soil moisture had a significant ($P = 0.04$) though marginal influence, but soil disturbance had no influence ($P = 0.37$) (Fig. 4b; Table 6).

Discussion

Water stress influenced plant growth, AM fungal colonisation, composition of rhizosphere bacteria communities and the N and C functional attributes when exposed to soil disturbance. Specifically, under water stress, shoot mass and mycorrhizal colonisation decreased following soil disturbance but this did not occur in the well-watered soil. Whilst other authors have reported AM fungal responses to soil disturbance (Jasper et al. 1989; Hart et al. 2016; van der Heyde et al. 2017) and water stress (see review Augé 2001), they have not previously been investigated in combination under controlled conditions. Overall, AM fungi and bacteria in this soil were less responsive to soil disturbance when soil was maintained under well-watered compared to water-stressed conditions, and indeed, the interaction with disturbance and water stress brought the bacterial communities closer together.

The most likely mechanism for reduced mycorrhizal colonisation in disturbed soil under water-stressed conditions is that the AM fungal hyphae were damaged (Jasper et al. 1989; Evans and Miller 1990). Consequent decreases in root colonisation could reduce both the nutrient and water acquisition by the plant, particularly under water-stressed conditions (Mickan 2014). Interestingly, soil disturbance prior to seeding negatively influenced mycorrhizal colonisation of roots under water stress, and this was associated with decreased plant growth. A possible explanation for this response is that drought reduces the growth of extra-radical hyphae and overall fungal fitness (Compant et al. 2010; Neumann et al. 2010) making them more susceptible to damage by disturbance. Therefore, it is likely the disturbance damaged the AM fungal propagules at a level sufficient to reduce the capacity for reproductive success.

The relative abundance of rhizosphere bacterial phyla was also primarily affected by soil disturbance under water-stressed conditions. Soil disturbance is likely to have exacerbated drought conditions by increasing the soil surface area available for evaporation and further water loss (Silva-Olaya et al. 2013). This corresponds with previous studies where soil tillage altered the bacteria communities and increased the microbial activity, decomposition and the rate of C

Table 5 Two-way ANOVA results showing *P* values of PICRUSt predicted nitrogen and carbon genes. Treatments were disturbance (disturbed, undisturbed) and water (well-watered 70% FC, water-stressed treatment 35% FC). Significant *P* values indicated in italics

			Disturbance		Water		Disturbance * water	
		Predicted gene	*F*	*P*	*F*	*P*	*F*	*P*
Nitrogen cycling genes	N fixation	nifd	0.001	0.972	2.202	0.151	0.680	0.418
	Nitrification	amoA.amoB	13.290	*< 0.001*	0.245	0.625	0.003	0.960
		Hao	4.804	*0.038*	1.150	0.294	0.058	0.812
	Denitrification	narG	5.593	*0.026*	2.212	0.150	0.698	0.412
		nrfA	16.511	*< 0.001*	0.217	0.645	0.156	0.696
		nirK	1.625	0.215	2.316	0.141	0.852	0.365
		nosZ	0.352	0.559	8.885	*0.006*	3.593	0.070
		narH	4.805	*0.038*	2.357	0.138	0.665	0.423
		narI	5.148	*0.032*	2.416	0.133	0.723	0.404
		napA	1.529	0.228	2.149	0.156	5.967	*0.022*
		napB	2.110	0.159	2.049	0.165	5.518	*0.027*
		norB	4.276	*0.049*	7.727	*0.010*	3.093	0.091
Carbon-degrading genes	Starch	Glucoamylase	1.726	0.201	0.089	0.768	3.955	0.058
		Alpha amylase	0.456	0.506	6.216	*0.019*	0.187	0.669
	Hemicellulose	Beta galactosidase	1.519	0.230	8.726	*0.006*	0.018	0.893
	Cellulose	Endoglucanase	0.148	0.704	0.321	0.577	0.546	0.467
	Chitin	Chitinase	0.001	0.982	7.298	*0.012*	0.481	0.495
	Lignin	Catalase	1.322	0.262	0.040	0.844	2.927	0.100

mineralisation rate (assessed by CO_2 production) (Silva-Olaya et al. 2013; Dong et al. 2017). Shifts in bacteria abundance in response to soil disturbance and soil moisture may be explained by release of labile C from the breakdown of macroaggregates (Cambardella and Elliott 1992; Al-Kaisi and Yin 2005) promoting growth of bacterial copiotrophs over oligotrophs (Fierer et al. 2007).

In this study, soil disturbance was associated with an increase in the relative abundance of *Actinobacteria* and *Bacteroidetes* and a decrease in the relative abundance of *Proteobacteria* and *Acidobacteria*. *Actinobacteria*, *Bacteroidetes* and *Firmicutes* have been previously described as fast-growing copiotrophs (r-strategists) that thrive in environments with high C availability (Cleveland et al. 2007;

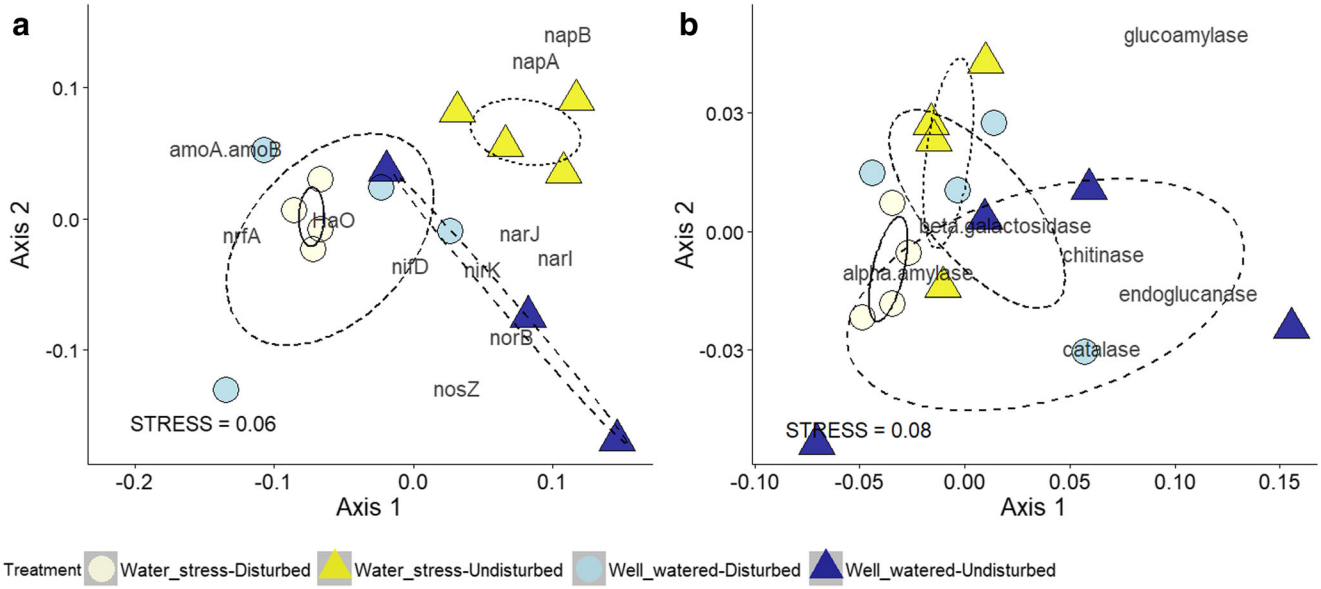

Fig. 4 Non-metric multidimensional scaling plot of PICRUSt predicted N (**a**) and C (**b**) genes in two water treatments (well-watered 70% FC, water-stressed 35% FC) and at two disturbances (undisturbed, disturbed) using OTU-based (97% similarity) Bray-Curtis dissimilarities distance

Table 6 PICRUSt predicted C and N functional gene assemblage analysis by PERMANOVA results based on 97% similarity OTU abundance data (square root transformed), using 999 permutations. Treatments consisted of water (water-stressed 35% FC, well-watered 70%FC) and disturbance (disturbed, undisturbed). Significant *P* values indicated in italics

		Degrees of freedom	Sum of squares	Mean squares	F. model	R^2	*P*
Nitrogen	Disturbance	1	0.073	0.073	7.149	0.287	*< 0.001*
	Water	1	0.047	0.047	4.623	0.186	*0.004*
	Disturbance * water	1	0.022	0.022	2.132	0.086	0.121
	Residuals	11	0.113	0.010	0.442		
	Total	14	0.256	1.000			
Carbon	Disturbance	1	0.003	0.003	1.072	0.063	0.375
	Water	1	0.007	0.007	2.789	0.165	*0.039*
	Disturbance * water	1	0.003	0.003	1.050	0.062	0.374
	Residuals	12	0.032	0.003	0.710		
	Total	15	0.045	1.000			

Jenkins et al. 2010), although they can respond differently to C sources (Pascault et al. 2013). In contrast, *Acidobacteria* tend to dominate in oligotrophic environments (Cleveland et al. 2007; Fierer et al. 2007; Jenkins et al. 2010) and could be outcompeted by copiotrophic bacteria following disturbance. Therefore, this study supports that of Fierer et al. (2007) showing bacterial phyla classified in terms of ecophysiology in relation to C availability. Some bacterial phyla were clearly influenced by water availability because there was a marked increase in the relative abundance of *Actinobacteria* and *Firmicutes* under water-stressed conditions. This could be explained by the spore-forming capability among the *Actinobacteria* and *Firmicutes* making them more resistant to both exogenous disturbance and desiccation (Holmes et al. 2000; Taketani et al. 2016). Soil water acts selectively on *Actinobacteria* communities, possibly favouring desiccation-tolerant taxa during drier months (Zenova et al. 2007). There was a marked increase in the relative abundance of *Streptomyces* in the disturbed soil under water stress, and this has been identified as a drought-tolerant taxon previously (Jenkins et al. 2009).

The combination of disturbance and water stress are stress multipliers, and it is possible that the decrease in mycorrhizal colonisation demonstrated here is associated with the decrease in relative abundance of *Proteobacteria*. *Proteobacteria* are known to have plant growth-promoting rhizobacterial representatives (Artursson et al. 2006) which can in turn influence plant growth. The increase in the relative abundance of *Oxalobacteraceae* (*Proteobacteria*) and *Bacillus* (*Firmicutes*) in undisturbed soil cores under water stress was correlated with increases in both mycorrhizal colonisation and shoot mass. Previous studies have reported similar interactions and showed *Oxalobacteraceae* were common on hyphae of AM fungi (Scheublin et al. 2010). *Oxalobacteraceae* and *Bacillus* are frequently recovered from germinating seeds where they are recognised as plant growth-promoting rhizobacteria (Green et al. 2006, 2007; Ofek et al. 2012; Fu et al. 2016). Thus, a reduction in mycorrhizal colonisation has species-dependent responses on influencing hyphal biomass (Abbott and Robson 1985) and if hyphal mass is decreased, this in turn could reduce the relative abundance of *Oxalobacterace*. This could contribute to reduced plant growth.

Using the PICRUSt package which predicts phylogenetic function, we assessed the potential to alter N (Ashworth et al. 2017) and C metabolism with soil disturbance (Mickan et al. 2017). The abundance of N cycling genes is likely to be both directly and indirectly influenced by soil and environmental conditions (Hayden et al. 2010). Soil disturbance and water stress both altered the abundance of predicted nitrification and denitrification N cycling genes in the rhizosphere soil. Water stress generally decreased the abundance of denitrification genes which is likely to be because denitrification in soil is driven by anaerobic microsites created by high soil moisture (Keil et al. 2015). Soil disturbance resulted in an increase in nitrification (*amoA* and *HaO*) genes, and this is likely to occur when soil aggregates are disrupted to expose organic matter to microbial degradation. Water stress corresponded with increases in the abundance of C cycling enzymes across the spectrum from labile to recalcitrant (starch, alpha amylase, hemi-cellulose, beta galactosidase, chitin and chitinase).

Conclusion

Mechanical disturbance of dry soil at the time of planting decreased above and below ground plant biomass in a glasshouse experiment for plants grown in cores of agricultural soil with a history of cropping with zero-tillage. A likely biological mechanism responsible for this could be linked to both decreased mycorrhizal colonisation and altered composition of the soil bacterial community. The impact of soil disturbance was most pronounced under water-stressed conditions. Reduced tillage in water-limited environments has direct plant growth benefits including the increased abundance of beneficial microbes such as AM fungal and those involved with N cycling. Future research to determine influence on higher

disturbance events has such a deep ripping on soil microbial communities, and the effect to plant productivity through microbial processes is recommended.

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