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ORIGINAL PAPER

Application of compost and clay under water-stressed conditions influences functional diversity of rhizosphere bacteria

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Abstract Applications of compost and clay to ameliorate soil constraints such as water stress are potential management strategies for sandy agricultural soils. Water repellent sandy soils in rain-fed agricultural systems limit production and have negative environmental effects associated with leaching and soil erosion. The aim was to determine whether compost and clay amendments in a sandy agricultural soil influenced the rhizosphere microbiome of *Trifolium subterraneum* under differing water regimes. Soil was amended with compost (2% *w*/*w*), clay (5% *w*/*w*) and a combination of both, in a glasshouse experiment with well-watered and water-stressed (70 and 35% field capacity) treatments. Ion Torrent 16S rRNA sequencing and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analysis of functional gene prediction were used to characterise the rhizosphere bacterial community and its functional component involved in nitrogen (N) cycling and soil carbon (C) degradation. Compost soil treatments increased the relative abundance of copiotrophic bacteria, decreased labile C and increased the abundance of recalcitrant C degrading genes. Predicted N cycling genes increased with the addition of clay (N_2 fixation, nitrification, denitrification) and compost + clay (N_2 fixation, denitrification) and decreased with compost (for denitrification) amendment. Water stress did not alter the relative abundance of phylum level taxa in the presence of compost, although copiotrophic *Actinobacteria* increased in relative abundance with addition of clay and with compost + clay. A significant role of compost and clay under water stress in influencing the composition of rhizosphere bacteria and their implications for N cycling and C degradation was demonstrated.

Keywords Rhizosphere bacterial gene frequency · PICRUSt · Water stress · Arbuscular mycorrhiza

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Introduction

Semiarid regions are widely used for agricultural production and support one fifth of the world's population (Galbally et al. 2008). As these areas are likely to expand with climate change, they will play an increasingly important role in sustaining agricultural productivity and global food security for a growing population (IPCC 2007). However, semiarid soils are generally of low soil quality, characterised by poor soil structure and low soil organic matter (SOM), making them susceptible to external stresses, particularly soil erosion and water stress (Hoyle et al. 2013). Furthermore, many agricultural practices (e.g. stubble burning) exacerbate soil degradation, resulting in reduced SOM content (Sánchez-García et al. 2016) and decreased soil aggregate stability, both of which

further reduce soil quality (Six et al. 2002). Soil structure is important for nutrient retention and plant water availability through pathways associated with aggregation of soil particles (Tisdall and Oades 1982), which in turn influences plant performance (Duong et al. 2012). Therefore, management practices that improve soil structure and SOM content are vital to support crop production under a changing climate. Organically amended soils generally have higher SOM, physical structure and biological fertility (Stockdale et al. 2013). The application of composted urban or agricultural waste products to soil is one strategy to increase SOM and improve soil structure (Duong et al. 2012; Hernández et al. 2015), soil water relations (Ng et al. 2015) and nutrient retention.

Productivity on semiarid soils is further compounded by water repellence, a condition that results from waxy organic compounds coating soil particles (Müller and Deurer 2011). This is particularly true for the ancient, highly weathered agricultural soils of southwestern Australia (Anand and Paine 2002; Jenkins et al. 2016), where more than 5 million hectares are water repellent (Roper 2004). These soils are sometimes amended with clay as a management strategy to mitigate water repellence (Nulsen 1993). Claying can reduce nutrient leaching as well as soil water repellence (Betti et al. 2015) and has the additional benefits of improving soil water holding capacity, increasing soil microbial biomass, increasing soil pH and increasing soil CEC (Müller and Deurer 2011). However, under some circumstances, claying practices could be detrimental to soil structure if insufficient organic matter is available, leading to hard setting (Djajadi and Hinz 2012); in other instances, copper immobilisation can be induced by claying (Müller and Deurer 2011). Thus, carefully selected amendments of compost and clay to soil have the potential to alleviate water repellence and deficiency in SOM, together with other plant microbial benefits in semiarid sandy soils.

Synergistic interactions between clay and SOM play a fundamental role in developing and maintaining soil structure by influencing soil biological and physical processes (Djajadi and Hinz 2012). However, the microbial communities that mediate these soil processes, including SOM turnover and water repellence, are often overlooked (Saison et al. 2006; Lavecchia et al. 2015). A better understanding of the mechanisms and controls involved in these microbial processes will enable improved management to balance clay and compost amendments, as this is necessary for effective structural improvement of sandy soils (Djajadi and Hinz 2012).

Soil microbial communities are intricately coupled to ecosystem functions due to their roles in cycling of carbon (C), nitrogen (N) and phosphorus (P) and in symbiont and pathogen associations with plants (van der Heijden et al. 2008; Pii et al. 2015; Trivedi et al. 2016). Application of compost to soil has been widely reported to influence soil bacterial composition (e.g. Paranychianakis et al. 2013; Lavecchia et al. 2015), and a number of these microbial changes have been related to functional genes associated with N cycling (e.g. Paranychianakis et al. 2013; Sánchez-García et al. 2016) and C transformations (Bowles et al. 2014; Ng et al. 2014). Indeed, the rhizosphere microbial community may stimulate or inhibit N and C cycling thereby influencing SOM and nutrient availability to the plant (Zhu et al. 2014). Thus, agricultural management practices such as application of clay and compost to sandy soils can have ramifications for biogeochemical cycling at the ecosystem scale.

Predicting changes to soil microbial composition following application of compost is troublesome due to the inherit heterogeneity of composts and soil. However, consideration of a broader understanding of the dominant features of similar soil microbes and ecological theories may be useful in predicting changes in community composition following amendment (DeVries and Shade 2013). For example, gram-negative bacteria are often described as *r*-strategist and characterised as fast-growing with low substrate affinity when compared to gram-positive bacteria (Prescott et al. 1996). Long-term application of compost has been shown to increase the abundance of gram-negative soil bacteria (Proteobacteria, Bacteroidetes, Gemmatimonadetes and most Chloroflexi) when compared to a fallow treatment based on sequencing approaches (Chaudhry et al. 2012). Increases in gram-negative bacteria associated with composts have also been reported using fatty acid methyl ester and phospholipid fatty acid analysis (Peacock et al. 2001; Islam et al. 2009). However, other studies found that the relative abundance of gram-positive rhizosphere bacteria (Actinobacteria) increased following compost addition (Lavecchia et al. 2015). This is especially true under water-stressed conditions, where the slow-growing gram-positive bacteria (*K*-strategists) are widely claimed to outcompete their gram-negative counterparts when resources are scarce (Manzoni et al. 2012). Thus, examining ecological strategies of soil bacteria based on *r*-/*K*- selection theory (Chen et al. 2016a, b) can be a useful framework for predicting functional changes associated with soil bacteria following disturbance (DeVries and Shade 2013).

Microbial *r*-strategists (copiotrophs) require higher concentrations of nutrients, prefer labile C (weak substrate affinity) and have rapid reproduction capacity (Fierer et al. 2007). In contrast, *K*-strategists (oligotrophs) utilise lower concentrations of nutrients more efficiently, have a greater substrate affinity and can outcompete *r*-strategists under nutrient-limited conditions (Fierer et al. 2007; Fuchslueger et al. 2014; Chen et al. 2016a, b). Indeed, microbial *K*-strategists are more efficient in assimilation of C into biomass and have a greater potential for C sequestration in soil (Chen et al. 2016a, b).

No studies have coupled a clay-compost interaction with an exogenous disturbance of water stress to contribute to understanding the microbial community resilience in agricultural environments exposed to continuing reductions in rainfall.

To this end, the impact of compost and clay amendments on rhizosphere bacterial community composition and function in a sandy semiarid agricultural soil under water-stressed conditions was investigated. Changes in community composition were assessed using tag sequencing approaches whilst the effects on N cycling and C mineralisation functional genes were predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Langille et al. 2013).

The hypotheses were (i) soil amendments consisting of compost, clay and a combination of compost + clay will increase the relative abundance of gram-negative bacteria such as Proteobacteria, Bacteroidetes, Gemmatimonadetes and many Chloroflexi, because *r*-strategists are expected to utilise resources faster than gram-positive bacteria, and (ii) soil water stress will alter the relative abundance of rhizosphere bacteria, increasing *K*-strategy-type bacteria belonging to gram-positive phyla.

Materials and methods

Experimental design

A pot experiment was used to investigate the influence of clay and compost on rhizosphere bacteria and any correlation with plant growth under well-watered and water-stressed conditions. The experimental design consisted of two factors: four soil treatments (compost 2% *w*/*w*, clay 5% *w*/*w*, the combination of compost 2% *w*/*w* with clay 5% *w*/*w* and unamended) and two water treatments (well-watered 70% field capacity and water-stressed 35% field capacity) in a randomised block design with four replicates. Soil chemical and physical characteristics were altered by soil amendments prior to the plants being sown (Table 1). Watering of individual pots occurred twice per day in non-draining sealed pots. The amount of each soil amendment was selected based on that which was previously reported to stimulate microbial activity and influence soil strength and aggregation (Djajadi and Hinz 2012). *Trifolium subterraneum* L. cv Dalkeith was grown in a water repellent agricultural soil, with homogenously applied clay, compost or the combination of both, under well-watered and water-stressed conditions in a glasshouse experiment.

Soil collection, compost, clay and growth conditions

Field soil (0–10 cm) was collected on 20 September 2013 from The University of Western Australia's farm *Ridgefield* at Pingelly, Western Australia (−32.534, 117.086). Soil was sieved (< 4 mm) immediately after collection then stored in the cool room at 4 °C until potting and sowing commenced. The agricultural soil had been managed as an annual pasture (subterranean clover/ryegrass) and included naturally occurring inocula of rhizobia and arbuscular mycorrhizal (AM) fungi. The soil amendments (compost and clay) were homogenously mixed into the soil using a cement mixer for 30 min at 40 rpm. The clay used was kaolin as this is a common clay type for this region (Djajadi and Hinz 2012), and the compost had a high P and N content (Table S9).

Establishment, sampling and harvesting procedures

Subterranean clover was chosen because it is an important pasture species in this region. Seeds were graded to 1–1.5 mm in diameter to ensure uniformity, then soaked in 50% ethanol:50% H_2O (Milli-Q filtered) overnight. The seeds were then arranged on a Whatman filter paper on a Petri dish soaked with deionised water for 2 days until the free radicle had emerged. Germinated seeds were planted at 10 mm depth, arranged in seven pairs of equal spacing. Plants were thinned to seven per pot after emergence. Pots were maintained under glasshouse conditions and watered accordingly to maintain either well-watered (70% FC) or water-stressed (35% FC) soil conditions. Plants were harvested 60 days after emergence (DAE). Shoot and root biomass was measured at harvest after oven-drying at 60 °C for 72 h. A soil water retention curve was used to calculate the field capacity (FC) of the soil, where 100% field capacity was calculated at −10 KPa and permanent wilting point was −1500 KPa. Soil control and soil treatments were incorporated with compost, clay and compost + clay (Mickan et al. 2016).

AM fungal root colonisation assessment

Freshly washed roots (0.5 g fresh weight) were sampled at the harvest time to assess mycorrhizal colonisation. These roots were cut into ~ 1 cm pieces and cleared in 10% KOH, acidified and stained with Trypan blue (0.05%) in lactoglycerol (1:1:1 lactic acid/glycerol/water) (Abbott and Robson 1981). Percentage root length colonised by AM fungi (%RLC) was assessed by using the gridline intercept method scoring more than 100 intercepts under a microscope at ×100 magnification (Giovannetti and Mosse 1980).

Soil analysis

The EC and pH were measured (1:5 soil/water ratio) using a probe inserted into water or 0.01 M $CaCl_2$ mixtures, respectively. Calcium, magnesium, sodium, sulphur, aluminium, copper, zinc and iron contents of each soil were measured by inductively coupled plasma emission spectroscopy (PerkinElmer Optima 5300DV ICP-OES). Total P, S and K were measured using standard methods (McDonald et al. 1998). Total C and N were measured by combustion using an Elementar Analyser (Vario Macro CNS; Elementar, Germany). Dissolved organic C was extracted using 0.5 M

Table 1 Chemical and physical composition of soil and soil amendments: clay (kaolin) added at 5% *w/w*, compost added at 2% *w/w* and the combination of clay 5% *w/w* and compost 2% *w/w*

	Control		Compost		Clay		Compost + clay	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Al (mg/kg)	1444.7a	16.0	1455.6a	20.0	1495.6a	21.0	2991.2b	23.0
Ca (mg/kg)	1134.3a	33.8	1201.9a	22.0	1134.3a	34.0	1144.3a	28.0
Cu (mg/kg)	0.5a	0.0	0.5a	0.0	0.5a	0.0	0.5a	0.0
Fe (mg/kg)	49.7a	1.4	53.0a	0.9	48.5a	1.2	48.7a	0.8
K (mg/kg)	261.8a	5.2	265.8a	8.8	258.9a	10.1	261.8a	10.0
Mg (mg/kg)	139.2a	3.7	141.8a	5.1	141.0a	5.1	139.2a	5.0
Mn (mg/kg)	11.9a	0.3	12.9a	0.1	10.8a	0.9	11.9a	1.0
Na (mg/kg)	82.0a	3.5	82.0a	5.1	83.2a	4.2	82.0a	3.4
P (mg/kg)	77.7a	0.4	84.3b	1.1	73.7c	0.4	77.9a	1.0
S (mg/kg)	41.8a	2.9	53.6b	0.1	41.2a	0.1	41.6a	0.8
Zn (mg/kg)	0.8	0.0	1.4	0.0	0.8	0.0	0.8	0.0
%Clay	5.3	0.3	4.7	0.1	10.2	0.3	10.4	0.4
%Silt	11.3	0.6	12.9	0.2	11.2	0.7	11.8	0.6
%Sand	82.4	0.8	83.4	0.3	78.4	1.0	77.9	1.0
pH in H_20	5.9	0.0	5.8	0.0	7.1	0.0	7.1	0.0
pH in $CaCl_2$	5.2	0.0	5.3	0.0	6.9	0.0	7.1	0.0
EC (μS/cm)	157.3	1.5	168.9	3.8	482.7	22.5	502.1	32.1

Standard errors (SE) are based on the mean of each soil treatment ($n = 4$), letters denote significant differences ($P<0.05$)

K_2SO_4 (20 g soil to 80 mL extract) and analysed using an OI Analytical Aurora 1030 Wet Oxidation TOC Analyzer (College Station, TX, USA). Soil mineral N (NO_3^- and exchangeable NH_4^+) were measured by extracting 20 g with 80 mL 0.5 M K_2SO_4 and analysing the extracts colorimetrically for exchangeable NH_4^+ using the salicylate–nitroprusside method (Searle 1984) and NO_3^- concentration using the hydrazine reduction method (Kempers and Luft 1988) on an automated flow injection Skalar AutoAnalyser (San plus, Skalar Analytical, The Netherlands).

N and P analysis of soil and plant

Total N and P from soil and plant tissue were determined using a Kjeldahl digest (Bremner and Mulvaney, 1982). Total N was determined using an ammonium N in green method Na Nitroprusside, and total P was calculated using molybdenum blue colorimetry (Blakemore et al. 1972).

DNA extraction

Rhizosphere soil was sampled at 60 DAE, after removing the bulk soil by gently shaking off roots using a fine sterile brush and stored at −20 °C. Genomic DNA was extracted from 0.5 g of rhizosphere soil using MoBio PowerSoil® DNA Isolation Kit (Geneworks, Australia) utilising bead beating and column purification, according to the manufacturer's guidelines. Extracted DNA was quantified and checked for purity at A260/280 nm (Nanodrop, Thermo Fisher Scientific, USA) prior to storage at −20 °C. Universal core bacterial primers 515F and 806 R (Caporaso et al. 2010; Mori et al. 2013), modified with Golay barcodes (Caporaso et al. 2012) fused to Ion Torrent adapters, were used to amplify fragments of the bacterial 16S rRNA gene from the DNA sample using amplification conditions described previously (Gleeson et al. 2016; Weerasekara et al. 2016). Following amplification, all PCR products were checked for size and specificity by electrophoresis on 1.5% *w/v* agarose gel and the target-sized amplicon (~ 300 bp) excised for purification with MoBio UltraClean™ GelSpin™ DNA Extraction Kit (Geneworks, Australia). PCR products were gel purified, quantified (Qubit; Thermo Fisher Scientific, Australia) and adjusted to 10 ng/μL using molecular grade water and then pooled equally for subsequent sequencing. Sequencing was performed on the Ion Torrent Personal Genome Machine (Life technologies, USA) using 400 base pair chemistry as described in Whiteley et al. (2012). Following sequencing, individual sequence reads were filtered using PGM software to remove low quality and polyclonal sequences. All the PGM quality-filtered data were exported as FastQ file and split into *.fasta and *.qual files before analysis using the Mothur pipeline.

Bioinformatics

Raw sequence data were processed using Mothur version 1.35.1 (Schloss et al. 2009), using a modified standard operating procedure (Schloss et al. 2011). Sequences were culled

based on quality control: barcode miss match = 1, primer mismatch = 2, ambiguous = 0, $Q > 20$, maximum homopolymers = 8 and minimum length = 150. After quality control, a total of sequences were retained and pre-clustered to remove any PCR-based bias, and unique sequences were aligned against the Silva 106 database (Pruesse et al. 2007). Chimeric sequences were identified using uchime (chimera.uchime; Edgar et al. 2011) and removed in Mothur. Rare sequences ($n = 1$) were removed, and the remaining sequences were subsampled to 16,358 per sample based on the sample with the lowest sequence count to allow fair comparisons (Gihring et al. 2011). Diversity indices were calculated within Mothur, as were Operational Taxonomic Unit (OTU) richness (S) per sample was calculated by the sum of the number of OTUs. The Simpson's diversity index ($1 - D = 1 - \Sigma_{pi}^2$) was calculated for each sample where $_{pi}$ is the frequency that each OTU occurred in each sample. Evenness was calculated [ED = (1 / D) / S)], where S is the OTU richness in each sample and D is Simpson's diversity index.

Predictive functional profile of the microbial communities

To investigate molecular-level functional traits, a PICRUSt (https://picrust.github.com) was performed (Langille et al. 2013). This uses 16S rRNA bacterial community data to infer metagenomic profiling. A closed reference OTU picking against the 13.5 Greengenes database was done within Mothur using the same quality control procedures as for bioinformatics above. A PICRUSt formatted *biom file was created inside Mothur (make.biom) and analysed via PICRUSt according to the instructions provided by the developers. The accuracy of metagenome predictions was tested using the Nearest Sequenced Taxon Index. The accuracy of prediction is related to the presence of closely related representative bacterial genome where lower values reveal a closer match (Langille et al. 2013).

Statistics

To evaluate the effect of soil amendments and water stress on plant and mycorrhizal parameters, bacterial community composition, bacterial relative abundance, OTU richness, Good's coverage, Simpson's diversity and evenness were analysed using linear regression and two-way analysis of variance (ANOVA), with significant results further analysed with post hoc subsequent pairwise comparisons (Tukey's HSD). All statistical analyses were tested in the R package (Ihaka and Gentleman 1996). These data were transformed where necessary to meet assumptions of normality by square root or log, and results were corrected using the Bonferroni correction method for multiple comparisons. To assess the similarities of bacterial communities, the OTU count data was ordinated using a Nonmetric Multidimensional Scaling (NMDS) multivariate analysis using a (Bray–Curtis) dissimilarity matrix. A correspondence correlation analysis was used to explore the relationship between taxa the phylum level and environmental parameters. The significance of water stress and soil treatments driving bacteria community composition was assessed with PERMANOVA using distance matrices (adonis function) and square root-transformed OTU relative abundance data. Finally, all OTU relative abundance data were compared using the 'metastats' function to determine which OTUs differed significantly between soil amendments and water treatments (White et al. 2009). All statistical and modelling analyses were performed using the Vegan library in the R statistical package (Ihaka and Gentleman 1996).

Results

Plant growth and AM fungal colonisation

Soil amendments and water stress treatments significantly affected P uptake, shoot and root mass and AM fungal colonisation and there was an interaction between them for shoot mass and AM fungal colonisation (Supplementary Table S1).

For well-watered conditions, plant growth and AM fungal colonisation were altered by soil amendments as follows: shoot mass was increased with application of compost by 44.1 ± 5.7% ($P = 0.013$) (Fig. 1a) whilst root mass was decreased by application of clay by 134.4 ± 1.7% ($P = 0.012$) and compost + clay by 17.7 ± 1.6% ($P = 0.002$) (Fig. 1b). AM fungal colonisation (%RLC) was reduced by application of compost by 29.8 ± 1.7% ($P = 0.001$) (Fig. 1c), and P uptake was decreased by application of clay by 46.87 ± 3.2% ($P = 0.032$) and compost + clay by 37.5 + 2.9% ($P < 0.001$) (Fig. 1d).

Water-stressed conditions altered plant growth depending upon soil amendments relative to the well-watered conditions (Fig. 1a, b). The shoot mass was reduced in all water-stressed treatments compared to the well-watered treatments: by 60.17 ± 3.7% ($P < 0.001$) following application of clay, by 61.7 ± 4.4% ($P < 0.001$) following application of compost, by 69.0 ± 3.7% ($P < 0.001$) following application of compost + clay and by 74.6 ± 3.2% ($P < 0.001$) in the unamended soil. Root mass was also reduced under water-stressed conditions for the following treatments: by 51.4 ± 3.1% ($P = 0.004$) with application of clay, by 48.3 ± 1.8% ($P < 0.001$) with application of compost + clay and by 36.5 ± 2.5% ($P = 0.005$) in the unamended soil.

Changes in AM fungal colonisation (%RLC) varied with soil amendment under water stress (Fig. 1c). For soil amended with clay, there was a slight reduction in %RLC of 14.3 ± 0.8% ($P = 0.002$), whereas there was a decrease in %RLC of 22.8 ± 1.2% ($P = 0.002$) in the unamended soil. P uptake decreased under water stress for all the treatments

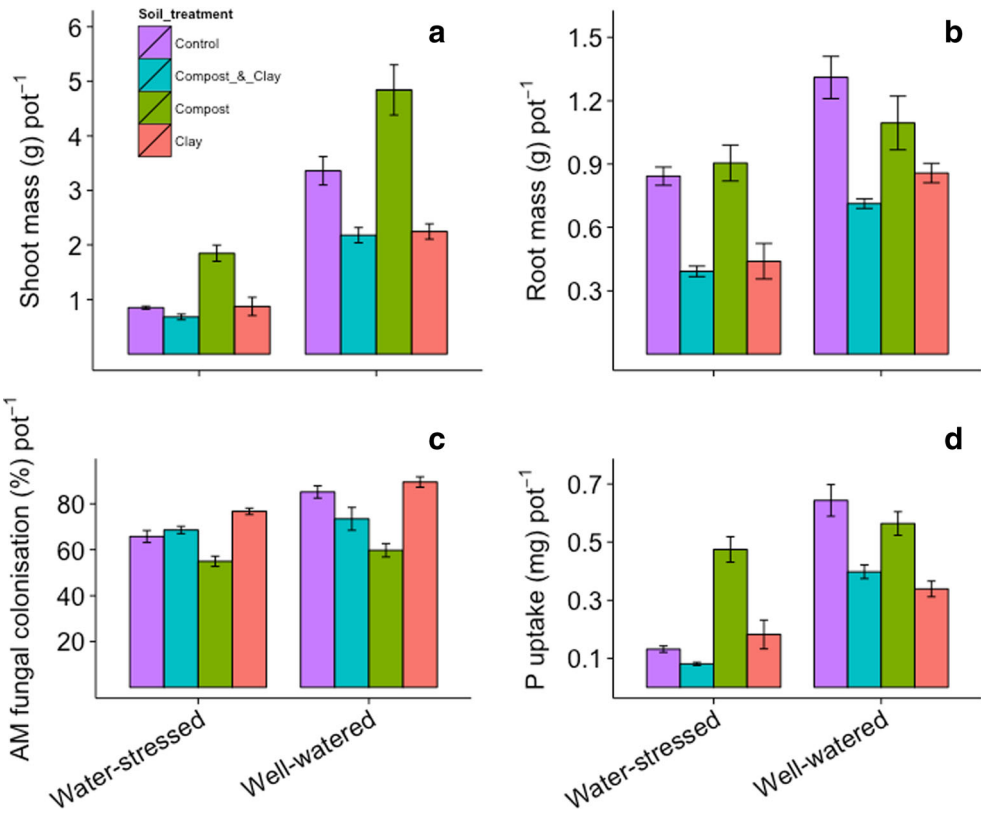

Fig. 1 Dry shoot (**a**) and root (**b**) mass of *Trifolium subterraneum*, AM fungal root length colonisation (%RLC) (**c**) and P uptake (**d**) in response to soil amendments of compost 2% *w/w*, clay 5% *w/w* and the combination of compost 2% *w/w* + clay 5% *w/w*. The control was no addition of compost or clay. Water treatments were well-watered (75% FC) and water-stressed (35% FC). Bars represent standard error ($n = 4$)

except the compost only treatment (Fig. 1d). Under water-stressed conditions, there was a reduction in P uptake of 46.1 ± 2.9% ($P = 0.032$), 79.6 3.8 ± % ($P < 0.001$) and 79.5 ± 4.2% ($P < 0.001$) when the soil was amended with clay, compost + clay and unamended, respectively.

Bacterial community composition and diversity

The bacterial community composition in soils amended with clay, compost and clay + compost consisted of 1,776,970 bacterial 16S rRNA sequences. After quality filtering for poor quality reads (see 'Materials and methods'), chimeras (54,134 chimeras) and short read length were removed, 1,057,260 sequences were retained, averaging 31,347 sequences per sample. After clustering at 97% similarity, there were 88,248 non-singleton OTUs and 23,766 singleton OTUs. The singletons were removed from further data analysis, and sequences were sampled to an equal depth to the lowest sample of 12,847 sequences per sample (see Gihring et al. 2011). The objective of subsampling the reads was to remove the effects of sequence depth.

Rarefaction analysis (Supplementary Fig. S1) indicated that the sampling procedure did not reach saturation. Nevertheless, a high proportion of the bacterial population was sampled as inferred by the Good's coverage estimator score reaching 82 to 84% coverage (Table 2), and this was consistent across all soil and water treatments. Overall, all the soil amendment treatments had a significant effect on the OTU richness ($P = 0.002$), whereas the water treatments alone or interactions between the soil amendment and water treatments did not. In contrast, the alpha diversity inverse Simpson index was significantly impacted by both soil amendment ($P < 0.001$) and water ($P < 0.001$) treatments. There was also an interaction between the soil amendment and water treatments ($P < 0.001$).

Alpha diversity indices for rhizosphere bacterial communities in well-watered soil receiving different amendments had distinct patterns. The application of compost increased the OTU richness (12.9%, $P = 0.017$), and this effect was greater with the compost + clay treatment (21.1%, $P < 0.001$). The clay-only treatment had no significant effect on OTU richness (Table 2). The inverse Simpson diversity index for bacterial community in the well-watered soil increased for all treatments relative to the control but was most pronounced in the compost + clay treatment (161.2%, $P < 0.001$).

The water stress treatment did not affect OTU richness for the soil amendment treatments. The inverse Simpson diversity index showed water stress had no effect for compost-alone or

Table 2 OTU alpha diversity indices based on 97% similarity of rhizosphere bacteria impact following soil amendment with compost 2% *w/w*, clay 5% *w/w* and the combination of compost 2% *w/w* and clay 5% *w/w* (the control had no addition of either compost or clay)

Water treatment	Soil	Goods coverage		OTU richness		Inverse Simpson	
		Mean	SE	Mean	SE	Mean	SE
Water-stressed	Clay	0.84	0.02	3280.74	318.42	63.42	21.91
	Compost	0.82	0.01	3691.32	79.60	90.56	5.24
	Compost + Clay	0.84	0.00	3467.19	58.28	73.86	7.30
	Control	0.85	0.00	3251.19	46.51	62.56	2.24
Well-watered	Clay	0.85	0.00	3235.20	73.46	101.52	5.73
	Compost	0.83	0.00	3572.06	52.30	101.97	7.21
	Compost + Clay	0.82	0.01	3830.25	126.60	158.81	4.60
	Control	0.85	0.00	3163.24	45.90	60.79	1.39

The two 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 ($n = 4$)

clay-alone amendment, but for compost + clay application under water stress, the inverse Simpson diversity index significantly decreased (−53.49%, $P < 0.001$) (Table 2).

Relative abundance of bacterial phyla

The overall rhizosphere bacterial composition (OTUs based on 97% similarity) was dominated by Proteobacteria (10,943 OTUs) and in order of decreasing abundance other phyla were Actinobacteria (3827 OTUs), Bacteroidetes (2009 OTUs), Acidobacteria (1950 OTUs), Gemmatimonadetes (1262 OTUs), Firmicutes (1255 OTUs) and Verrucomicrobia (749 OTUs) (Supplementary Fig. S2). Phyla representing less than 2% of the total bacterial community grouped together under 'Other' classification included Cyanobacteria (84 OTUs) and Fibrobacteres (18 OTUs).

A two-way ANOVA revealed that different soil amendments significantly altered the composition of the bacterial community and all the phyla were affected (Supplementary Table S2). To a lesser extent, water-stressed soil resulted in a significant shift in the relative abundance of Proteobacteria, Actinobacteria, Acidobacteria, Verrucomicrobia, Chloroflexi, Gemmatimonadetes and Firmicutes. Finally, the relative abundance of Proteobacteria, Actinobacteria and Acidobacteria displayed a significant two-way interaction with both soil amendment and water treatments.

Changes in the relative abundance of phyla varied with soil amendments within the well-watered treatment (Fig. 2a). At the phylum level, the largest positive increases in relative abundance were associated with gram-negative bacteria for all treatments. For compost-amended soil, they were Chloroflexi (219.4 ± 14.1%) and Proteobacteria (38.6 ± 2.7%); for clay amended soil, they were Bacteroidetes (258.6 ± 16.9%), Gemmatimonadetes (74.6 ± 9.9%) and Proteobacteria (70.8 ± 1.6%); and for compost + clay amended soil, they were Chloroflexi (536.8 ± 48.8%), Bacteroidetes (309.6 ± 17.0%) and Proteobacteria (92.7 ± 1.4%) (Fig. 2a). In contrast, a reduction in the relative abundance of Verrucomicrobia, Acidobacteria, Planctomycetes, Firmicutes, Firmicutes and Actinobacteria occurred with most treatments in the well-watered soil including (i) compost-amended soil (Verrucomicrobia (−52.5 ± 0.9%), Acidobacteria (−44.3 ± 0.51%), Planctomycetes (−21.1 ± 2.5%) and Firmicutes (−19.6 ± 5.9%)); (ii) clay amended soil (Acidobacteria (−81.1 ± 0.9%), Planctomycetes (−55.9 ± 0.9%), Firmicutes (−45.6 ± 2.2%), Actinobacteria (33.8 ± 4.8%) and Verrucomicrobia (−30.0 ± 0.3%)); and (iii) compost + clay amended soil (Acidobacteria (−82.3 ± 0.1%), Verrucomicrobia (−56.2 ± 0.7%), Planctomycetes (−51.4 ± 1.5%), Firmicutes (−44.0 ± 2.0%) and Actinobacteria (−41.0 ± 3.5%)). The two most abundant phyla, Proteobacteria and Actinobacteria, contributed to 48.5% of the total community, and changes in their relative abundance were associated with both the soil amendments and the water treatments. There was also a significant interaction between the soil amendment and water treatments and the relative abundance of these bacterial groups (Supplementary Table S2 and Supplementary Fig. S2).

Overall, the relative abundance of bacteria for all soil treatments responded differently under water-stressed compared to the well-watered condition (Fig. 2b). Water-stress increased the relative abundance of Actinobacteria for all treatments except compost application by 55.1 ± 8.9% for clay application, 68.3 ± 5.1% for compost + clay application, and 27.3 ± 1.6% in the unamended control. However, a decrease in the relative abundance of Proteobacteria (−13.8 ± 4.1%) was observed when soil was amended with clay. Application of compost + clay decreased the relative abundance of Proteobacteria (−17.9 ± 2.0%) under water-stressed

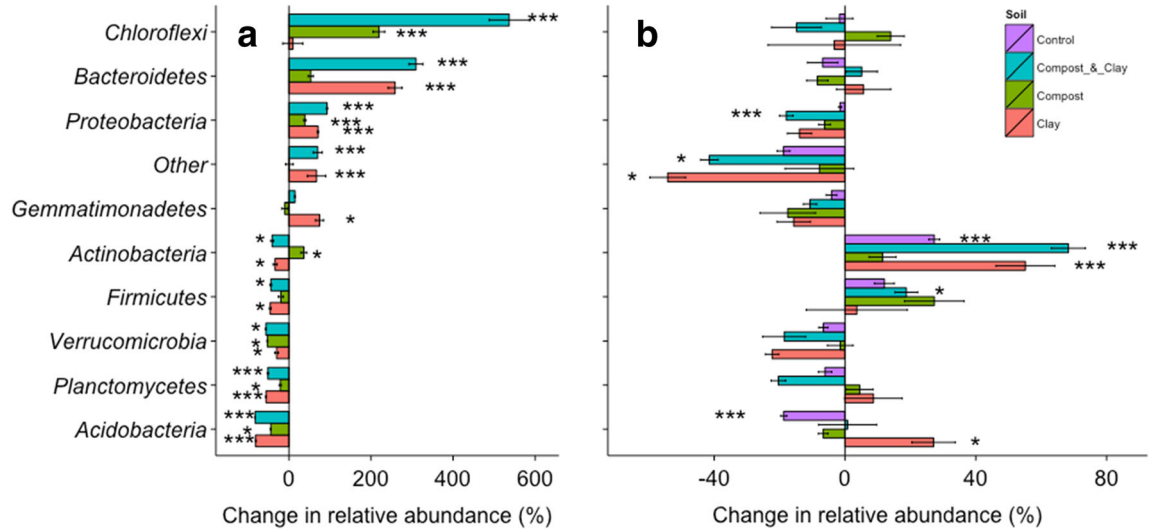

Fig. 2 Variation in rhizosphere bacterial relative abundance from soil treatments compared to unamended soil control at the phylum level for (**a**) within the well-watered treatment (70% FC) and (**b**) water stress alterations (35% FC) in relative abundance of bacteria within each soil (soil control inclusive) as compared to the well-watered control (70% FC). Soil treatments consisted of amending soil with compost 2% *w/w*, clay 5% *w/w* and the combination of compost 2% *w/w* and clay 5% *w/w*. Post hoc Tukey HSD significant *P* values are indicated by *single asterisk* and *three asterisks* corresponding to $P < 0.05$ and < 0.001, respectively. Bars represent the mean value for each treatment and error bars are the standard error of the mean ($n = 4$)

conditions (Fig. 2b). For the remaining treatments, there was no significant change in relative abundance at the phylum level.

Distinct bacterial communities from soil amendments and water treatments

To explore the effects of water stress and soil amendment on the bacterial community composition, a canonical correspondence analysis (CCA) was performed to determine which significantly correlated environmental variables (P, AM fungi (AMF), colonisation, NO_3^- (NO_3) and dissolved organic C (DOC)) best explained changes in bacterial community composition (Fig. 3). Further analysis of community composition by PERMANOVA indicated significant community separation due to water treatment ($P < 0.001$), soil amendments ($P < 0.001$) and the interaction of soil amendment × water ($P = 0.007$) (Supplementary Table S3). The largest separation of soil samples occurred along axis 1 (Fig. 3) showed a distinct clustering for the control samples that clearly separated from the compost + clay and clay-only amended soils. There appears to be a distinct treatment effect driving the bacterial community composition, since the communities from for the control and compost soil samples clearly separate from the compost + clay and clay-only amended soils along axis 1 whilst the well-watered treatments separate from the water-stressed treatments along axis 2 except for the compost only treatment (Fig. 3).

The influence of environmental factors on the relative abundance of phyla within the bacterial community for each soil sample was also explored by CCA analysis (Fig. 3). The abundance of Bacteriodetes, Proteobacteria and Gemmatimonadetes was highly correlated with DOC and NO_3^- concentration and therefore were most abundant in clay- and clay + compost-treated soil. In contrast, Acidobacteria, Planctomycetes and Firmicutes were associated with lower DOC and NO_3^- concentrations, as in the unamended soil. There was a higher relative abundance of Actinobacteria within the compost only treatment soils that were associated with a higher P content whilst Chloroflexi and Chlorobi were more abundant in the clay +compost treatment. Finally, Fibrobacteres and Cyanobacteria were most abundant in the well-watered clay amended soil and with an increase in AMF colonisation.

Analysis of OTU frequency change between treated soils

Based on distinct clustering for community composition at both the OTU and phylum level for soil amendments and water treatment, highly abundant taxa and those identified to be most likely influencing community change were further investigated. Analysis of the top 25 core OTUs (Supplementary Table S4), which contributed to 66.8% of total sequences (singletons removed), revealed dynamic responses for both soil and water treatments. A 'metastats' function analysis showed the response (change in relative abundance) of the top 25 most abundant OTUs to the soil amendment treatments (compost, clay and compost + clay) relative to the control treatment under both well-watered (Supplementary Table S5) and water-stressed conditions (Supplementary Table S6).

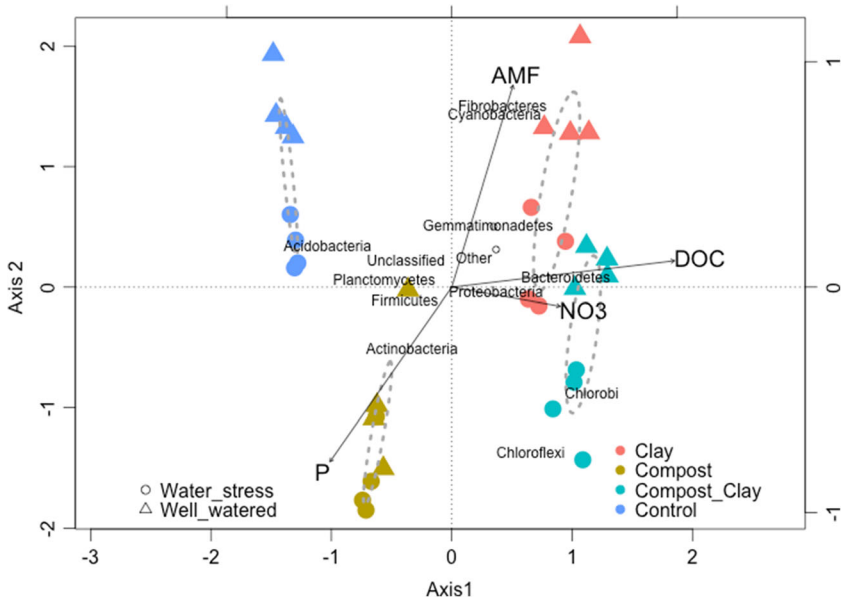

Fig. 3 Canonical correspondence analysis (CCA) comparing the rhizosphere bacterial communities from well-watered treatment (70% FC, triangles) and water-stressed (35% FC, circles) treatment. Soil treatments consisted of amending soil with compost 2% *w/w*, clay 5% *w/w* and the combination of compost 2% *w/w* and clay 5% *w/w* and environmental variables (arrows, Colwell P (P), nitrate (NO_3^-), dissolved organic C (DOC)). Dispersion ellipses were calculated based on soil treatment independent of water treatment at the 95% confidence interval. Environmental variables selected were based on significance calculated from individual CCA results and variance inflation factors calculated during CCA. Each data point represents an individual sample

Soil treatments altered the frequency of the top 25 OTUs in at least one of the soil treatments. As a proportion of the top 25 OTUs, soil treatments increased individual OTU frequency as follows: compost-amended soil by 24%, clay amended soil by 36% and compost + clay amended soil by 36% (see Supplementary Table S5). There was also a 28, 48 and 48% decrease in the relative abundance for some the top 25 OTUs associated with compost, clay and compost + clay amended soil, respectively. The increase in the most abundant OTU001 *Bacillaceae* (66,840 sequences) was associated with both clay and compost + clay amendments but not compost. In contrast, soil amendment with clay and compost + clay resulted in a decrease in the relative abundance of *Gemmatimonadaceae* sp., *Actinomycetales* sp., *Actinomycetales* sp. and *Chitinophagaceae* sp. For all soil amendments (compost, clay and compost + clay), the relative abundance of *Bradyrhizobiaceae* sp. and *Nitrosomonadaceae* sp. increased in frequency whilst the relative abundance of *Cytophagaceae* sp., *Acidobacteriacea*, *Actinomycetales* and *Gemmatimonadaceae* sp. decreased in frequency relative to the unamended control soil (Supplementary Table S5) even though all the OTUs in the phylum Bacterioidetes increased (Fig. 2a).

Water stress also altered the relative abundance of OTUs, with 68% of the top 25 OTUs changing in OTU frequency in response to at least one soil amendment treatment; however, there were no observed OTUs that shared a uniform directional change in frequency across all soil treatments. As a proportion of the top 25 OTUs, water stress increased individual OTUs frequency by 8, 20 and 24% for the soil amended with compost, clay and compost + clay amended soil, respectively, and in the water-stressed control (non-amended soil), there was an increase of 12% compared to the un-stressed control (see Supplementary Table S6). A decrease in the frequency for some of OTUs occurred in the 'compost' by −0.4%, 'clay' by −12%, 'compost + clay' by −12% and the control by −12%. Soils amended with compost under water-stressed conditions resulted in the smallest change in abundance, with only two OTUs increasing in frequency (*Gemmatimonadaceae* sp. and *Actinomycetales* sp.), whereas for the clay and compost + clay, amendments resulted in a 40% change in OTU frequency (Supplementary Table S5).

Rhizosphere bacterial metabolic profile prediction

Using 16S rRNA gene profiling information, PICRUSt (Langille et al. 2013) predicted the abundance of C and N functional genes and found significant differences between the soil amendments and water conditions (Supplementary Table S7). The accuracy of PICRUSt decreases with increases in the average Nearest Sequenced Taxon Index (NSTI) scores. A mean NSTI score of ~ 0.17 was obtained for the dataset used in this study, which is greater than the mean NSTI score of 0.129 ± 0.001, suggesting an acceptable level of confidence. Additionally, there was low variability among all samples (Supplementary Table S8) and these data were comparable to other soil experiments (Metcalf et al. 2016; Pii et al. 2016).

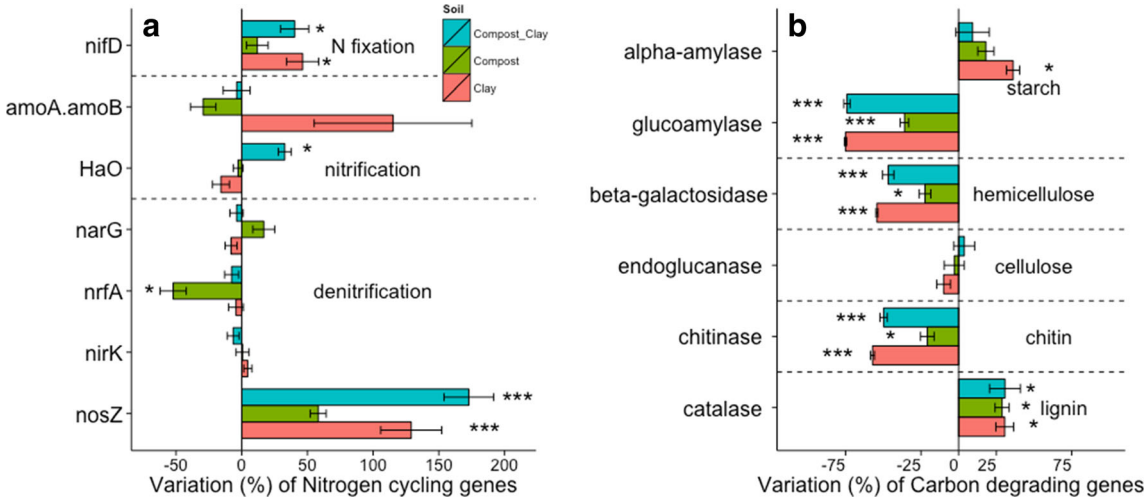

Fig. 4 The percentage variation of genes for soil treatments as compared to the unamended control soil within well-watered conditions (70% FC) of the most abundant PICRUSt predicted N cycling genes (**a**) and C degrading genes (in order of complexity from labile to recalcitrant) (**b**). Soil treatments consisted of amending soil with compost 2% *w/w*, clay 5% *w/w* and the combination of compost 2% *w/w* and clay 5% *w/w*. Post hoc Tukey HSD significant *P* values are indicated by *single asterisk* and *three asterisks* corresponding to $P < 0.05$ and < 0.001, respectively. Bars represent the mean value for each treatment and error bars are the standard error of the mean ($n = 4$)

Analysis of the most abundant N metabolism pathway genes in well-watered soil revealed that there was an increase in the abundance of N_2 fixing functional gene *nifD* (nitrogenase molybdenum-iron protein alpha chain [EC 1.18.6.1]) associated with application of clay (46.3 ± 12.2%) and compost + clay (42.2 ± 10.7%) (Fig. 4a). The nitrification functional gene *HaO* (hydroxylamine dehydrogenase [EC 1.7.2.6]) increased with the application of compost + clay (42.2 ± 4.8%). The relative abundance of functional genes involved in denitrification both decreased for *nrfA* nitrite reductase (cytochrome c-552) [EC 1.7.2.2] with the addition of compost (−52.1 ± 9.9%) and increased for *nosZ* (nitrous oxide reductase [EC 1.7.2.4]) with the application of clay (128.9 ± 23.2%) and compost + clay (172.8 ± 18.8%). Actual sequence counts for predicted nitrogen cycling genes ranged from ~ 100 gene counts for *amoB* (control soil) to ~ 5000 for *nifD* (control soil) (Supplementary Fig. S3).

Alterations in the relative abundance of predicted carbon degrading functional genes highlighted that labile C degrading starch compounds were most affected under well-watered conditions, with an increase in alpha-amylase [EC 3.2.1.1] following application of clay (36.1 ± 4.3%) and decreases in glucoamylase [EC 3.2.1.3] associated with application of compost (−35.9 ± 2.8%), clay (−74.0 ± 2.0%) and compost + clay (75.0 ± 0.7%) (Fig. 4b). Decreases in the hemicellulose degrading genes beta-galactosidase [EC 3.2.1.23] were observed following the application of compost (−22.3 ± 2.2%), clay (−46.7 ± 3.4%) and compost + clay (−54.21 ± 0.7%). Cellulose degrading genes were unaffected by soil amendment treatments. There was a decrease in the relative abundance of chitinase [EC 3.2.1.14] degrading genes for all soil treatments: compost (−20.78 ± 4.5%), clay (−49.7 ± 2.4%) and compost + clay (−57.1 ± 1.3%). In contrast, there was an increase in lignin degrading catalase [EC 1.11.1.6] genes for all soil amendments including compost (by 28.8 ± 4.7%), clay (by 30.7 ± 10.2%) and compost + clay (by 30.5 ± 5.8%). Actual sequence counts for predicted C degrading genes ranged from ~ 1300 gene counts for alpha-amylase (control soil) to ~ 30,000 for glucoamylase (control soil) (Supplementary Fig. S4).

There was no significant difference in the relative abundance of predicted N cycling genes between different soil amendments with the exception of the denitrifying *nrfA* gene which decreased following the application of clay by 27.2 ± 9.5% (Fig. 5a).

There was no significant difference in the relative abundance of predicted C cycling genes between different soil amendments with the exception of the starch degrading glucoamylase gene which decreased by 12.5 ± 3.2% within the control soil (Fig. 5b).

Discussion

Plant growth

In accordance with the hypothesis, compost increased shoot mass for both water treatments due to its high N and P content, although root mass was unaffected. Composts may contain high concentrations of major nutrients including N, P and K, as well as most micronutrients (Bar-Tal et al. 2004; Quilty and Cattle 2011). Composts can improve soil structure, nutrient cycling, soil health and fertiliser use efficiency (Barzegar

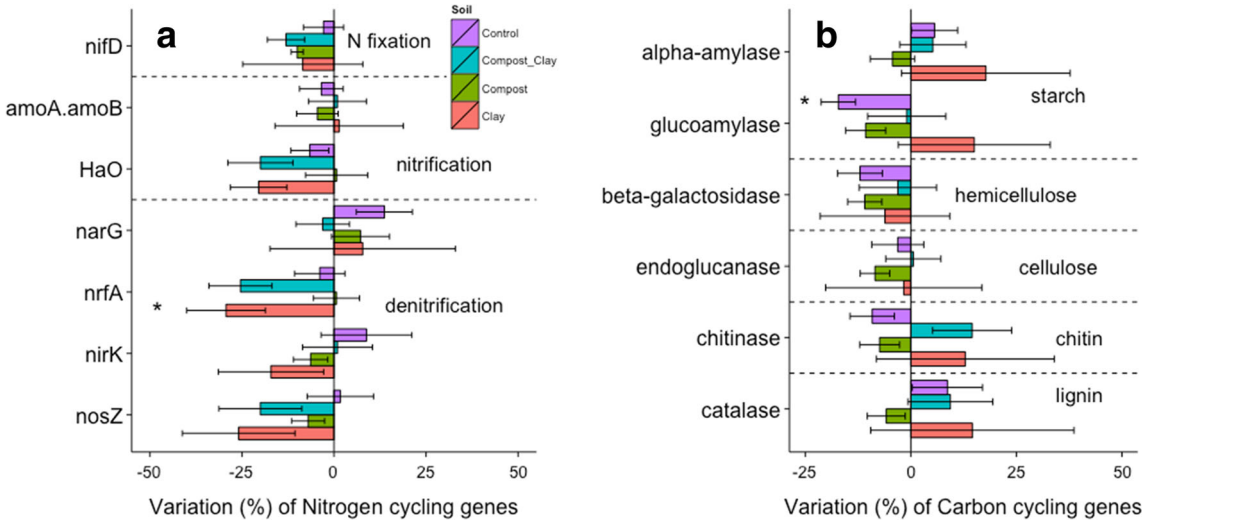

Fig. 5 The effect of water stress as compared to the well-watered control percentage variation of the most abundant PICRUSt predicated N cycling genes (**a**) and C degrading genes (in order of complexity from labile to recalcitrant) (**b**). Soil treatments consisted of amending soil with compost 2% *w/w*, clay 5% *w/w* and the combination of compost 2% *w/w* and clay 5% *w/w*. Post hoc Tukey HSD significant *P* values are indicated by *single asterisk* and *three asterisks* corresponding to $P < 0.05$ and < 0.001, respectively. Bars represent the mean value for each treatment and error bars are the standard error of the mean ($n = 4$)

et al. 2002; Bar-Tal et al. 2004; Quilty and Cattle 2011) and this can also result in higher yields (Drury et al. 2014).

Addition of clay, either singly or in combination with compost, had a negative plant growth response with a marked reduction in shoot and root mass relative to the control. One possible explanation for this anomaly is that kaolinite clay used in this study can have some nutrient sorption properties making soil nutrients less available to plants (Hall et al. 2010). Another consideration is that clay additions to soil can lead to a reduction in mineralization and the bioavailability of nutrients due to interactions with SOM (Baldock and Skjemstad 2000; Wang et al. 2003). In particular, the interactions of clay and SOM within the soil matrix can lead to the formation of strong chemical bonds and closed environments through aggregate formation or physical–chemical stabilisation creating a barrier for biological decomposition (Oades 1988; Bronick and Lal 2005; Lützow et al. 2006). It is likely that clay-induced physical or chemical protection of SOM restricted the bioavailability of nutrients in both the clay and compost + clay amended soils based on two lines of evidence. First, total dissolved organic C was higher when clay was applied either singly or combination with compost under both water-stressed and well-watered conditions inferring reduced biological decomposition of labile C (Khalil et al. 2005). Second, there was a significantly higher amount of inorganic N in soil when clay was applied either alone or combined with compost, implying the plants were unable to access this N resource. It is also likely that the thorough homogenisation of the clay within the soil during the experimental set-up further reduced the microbial decomposition and bioavailability of plant-available nutrients to due to encrusting of fine clay particles (Baldock and Skjemstad 2000; Krull et al. 2003).

AM fungal colonisation

Compost additions to soil can have negative, neutral or positive effects on colonisation of roots by AM fungi, and this is highly dependent on compost maturation and parent material (Cavagnaro 2014). However, AM fungal responses, including those observed here, are complex and measurements of AM fungal colonisation at one point in time may be inadequate to fully explain the relationships (Mickan et al. 2016). In this experiment, the addition of clay alone to soil under well-watered conditions increased the AM fungi colonisation (assessed as %RLC). For this study, clay increased AM fungal colonisation to the detriment of root growth and thus plant performance. These findings are in agreement with Koller et al. (2013) who found that a decrease in % AM fungal colonisation was inversely correlated with an increase in the total root length colonised by AM fungi and associated with greater N uptake. Thus, while %RLC is a valuable indicator for quantifying AM fungi, it needs also to be considered in relation to total root growth (Carvalho et al. 2015).

Rhizosphere bacteria and predicted functional gene

Previous studies have observed shifts in soil bacterial composition following short-term (Saison et al. 2006) and long-term (Chaudhry et al. 2012) soil amendment with compost. However, in our study, we investigated the combined influence of clay and water stress on bacterial composition in soil amended with compost. Overall, there was greater bacterial community richness and diversity in the rhizosphere of the sandy soil following the addition of compost and clay, as found previously (Pera et al. 1983; Zhen et al. 2014). This

can occur due to an increase in population size and activities of heterotrophic bacteria as labile C compounds enter the soil (Foutaine et al. 2003; Blagodatskaya et al. 2007; Fierer et al. 2007). However, some of these changes could be attributed to an allochthonous bacterial community introduced from the soil amendments. Given the duration of the experiment, the dominant bacterial populations under each treatment combination are most likely to be indigenous and physiologically adapted to the prevailing conditions (Schimel et al. 2007).

Under well-watered conditions, the compost, clay and compost + clay amendments significantly altered the rhizosphere bacterial community in this sandy agricultural soil. As hypothesised, there was an increase in the relative abundance of gram-negative phyla (Proteobacteria, Chloroflexi, Bacteroidetes, Gemmatimonadetes) for all amendments relative to the control (Fig. 1a). These findings are consistent with other studies that found an increase in the relative abundance of Proteobacteria and Bacteroidetes following compost and clay amendment (Pera et al. 1983; Peacock et al. 2001; Islam et al. 2009; Chaudrey et al. 2012). However, Proteobacteria and Bacteroidetes were most abundant when clay was added either alone or in combination with compost. Based on a CCA analysis, the proliferation of these phyla was linked to increases in DOC and NO_3^- associated with the clay amendments. Perhaps, the clay creates a physical niche in which bacteria can proliferate, especially as the clay provides a high surface to area ratio compared to the compost.

Competition for resources typically results in dominance of one or a few populations with the highest growth rates (Fontaine et al. 2003). Members of these fast-growing phyla are very versatile and capable of degrading a variety of organic compounds (Fierer et al. 2007) thereby increasing the availability of essential micro- or macronutrients to plants (Lesaulnier et al. 2008). Consequently, these taxa are particularly adept at responding to a variety of labile C compounds entering soils and have been described as opportunists, *r*-strategists or copiotrophs (Fierer et al. 2007; Langenheder and Prosser, 2008; Jenkins et al. 2010).

Dominance of Acidobacteria, Planctomycetes, Verrucomicrobia, Firmicutes and Actinobacteria occurred in the unamended soil; their relative abundance decreased in the amended soils. This is as expected, because many of these taxa are slow-growing, oligotrophic K-strategists that play a major role in SOM turnover (Fierer et al. 2007). Actinobacteria and Acidobacteria in particular are known for their ability to degrade recalcitrant materials (Goodfellow and Williams 1983) and complex substrates such as hemicellulose, cellulose and chitin (Ward et al. 2009). A decrease in the relative abundance of these phyla usually accompanies the addition of organic amendment (Fontaine et al. 2003). Previous studies have shown that additions of labile C substrates to soil increase the microbial activity due to a substrate-induced succession of microorganisms from *r*- to K-strategist (Fontaine et al. 2003; Blagodatskaya et al. 2007). According to Fontaine et al. (2003), fast-growing *r*-strategists initially dominate after organic amendment because they rapidly outcompete the K-strategist for labile C substrates. Later, once the readily degradable component of the resource has been exhausted, the *r*-strategists are superseded by slow-growing K-strategists that thrive on degrading recalcitrant organic compounds or low available substrates (Blagodatskaya et al. 2014). Interestingly, increases in relative abundance of gram-positive K-strategist Actinobacteria (Fig. 1a) as well as *r*-strategists were observed in the well-watered soils amended with compost alone. This probably reflects the transition from *r* to K strategy during compost degradation.

Water stress had a profound effect on the rhizosphere community structure, with a distinct shift in the relative abundance of gram-positive bacteria (Actinobacteria, Firmicutes). This corresponds with observations of rhizosphere bacterial composition in other semiarid to arid environments (Chowdhury et al. 2009) and is consistent with numerous studies suggesting gram-positive bacteria are generally more resistant to water stress and drought than are gram-negative bacteria (Schimel et al. 2007; Manzoni et al. 2012; Fuchslueger et al. 2014; Lavecchia et al. 2015). Gram-positive bacteria are broadly classified as K-strategists or oligotrophs and are often metabolic specialists (Cozzolino et al. 2016). They are characterised by higher substrate affinities and slower specific growth rates thereby outcompeting *r*-strategists under harsher environmental conditions when resources are restricted (Fierer et al., 2007; Jenkins et al. 2010; Chen et al. 2016a, b). Indeed, many Actinobacteria taxa are desiccation-tolerant and capable of persisting under dry soil conditions (Jenkins et al. 2009), which probably explains their prevalence in arid and semiarid environments (Manzoni et al. 2012).

In contrast to the effects of water stress on the gram-positive bacteria Actinobacteria and Firmicutes, water stress decreased abundance of gram-negative bacteria, especially phyla Proteobacteria, Verrucomicrobia and Gemmatimonadetes. These taxa may be more drought-intolerant and perhaps water stress affects their ecophysiology through starvation, induced osmotic stress and resource competition (Jenkins et al. 2009). Gram-negative bacteria with their thin peptidoglycan cell layer are largely putative *r*-strategists or copiotrophs in soil (Chen et al. 2016a, b). They have evolved survival strategies including high metabolic diversity and weaker substrate affinity that enable them to use labile C and N rapidly when resources are rich (Jenkins et al. 2010; Chen et al. 2016a, b). Additionally, as gram-negative (*r*-strategists) bacteria have higher growth rates than gram-positive bacteria, they can increase in abundance more rapidly and outcompete the gram-positive bacteria under unstressed conditions (deVries and Shade 2013).

Interestingly, soil amendment had a less pronounced effect on the rhizosphere community in water-stressed compared to

well-watered soil. Nevertheless, there was a marked increase in the abundance of Actinobacteria in the unamended, clay and compost + clay treatments under water stress and to a lesser extent for soil amended with compost only. In contrast, only the compost treatments in the well-watered soils lead to an increase in the abundance of Actinobacteria relative to the unamended soil. Based on a CCA analysis, Actinobacteria were associated with soil P levels and associated with the compost samples (Fig. 2). A possible explanation for this is that when compost was applied singly it provides a porous micro-habitat than the clay amended soil (Parkin, 1987). Under water stress, this property allows a competitive advantage for the fast-growing Chloroflexi (as inferred by a slight increase their relative abundance in the compost treatment). Actinobacteria as gram-positive bacteria are more resilient to stress (Guldimann et al. 2016) and thrive due to a lack of competition from fast-growing *r*-strategists (Jenkins et al. 2009, 2010).

Overall, the application of compost to soil in our experiment did not affect the microbial community composition with water stress; only two of the top 25 most abundant OTUs (*Gemmatimonadaceae* and *Actinomycetales*) increased with compost application. Hence, the application of compost reduced the influence of water stress on the rhizosphere bacterial community and this has beneficial implications for water-limited agricultural agroecosystems.

Application of compost decreased the abundance of the *nrf*A gene within the rhizosphere of plants grown in well-watered soil. Nitrite-ammonifying bacteria carry the nitrite reductase gene *nrf*A gene that catalyses the reduction of nitrite to ammonia a key step in the dissimilatory nitrate reduction to ammonium (DNRA) pathway that promotes the retention of N in soils (Welsh et al. 2014). In contrast, soils receiving clay either singly or in combination with compost had a higher abundance of the denitrifying nitrous oxide reductase gene (nosZ) that catalyses the reduction of nitrous oxide to dinitrogen (Giles et al. 2012). Denitrification and respiratory ammonification pathways both compete for nitrate and nitrite. Yoon et al. (2015) found that denitrification tended to dominate in soils with low C/N ratios, whereas DNRA was the predominant pathway in soils with high C/N ratios. Interestingly, the opposite trend was observed in this study as DOC was actually higher in the soils receiving clay. This disparity could be due to clay-induced differences in soil structure, water availability, inorganic N content, organic C and aeration (Parkin 1987; Gu et al. 2013). As water-filled pore space is unlikely to reach > 70% where it is needed to induce denitrification in semiarid soils (Barton et al. 2013), it is more likely that oxygen depletion during periods of increased microbial activity following clay amendment led to formation of anaerobic microsites in the sandy soil that promoted denitrification (Parkin 1987). Further support for the dominance of the DNRA over denitrification pathway in the composted only soil was observed at the harvest (after 60 days) when soil nitrate had diminished in the compost-amended soil compared to soil amended with clay. As denitrification leads to N loss whilst respiratory ammonification retains N, manipulating these pathways via soil amendment (e.g. clay and compost) has implications for N retention, plant productivity and climate change (Yoon et al. 2015).

The addition of clay, either with or without compost, also increased the abundance of the nitrogenase gene (*nif*D) that catalyses the process N_2 fixation (Levy-Booth et al. 2014). This is consistent with previous studies that found N-fixing composition were more abundant and diverse in the clay soils compared to sandy soils due to better soil aggregation (Gupta and Roper, 2010; Pereira e Silva et al. 2011). One has to exercise caution when extrapolating from the recovery of a 16S rRNA target to a putative ecophysiology of an organism because fixed N may have been held in clay matrix (Pera et al. 1983) and therefore would have little impact on plant growth. N-fixing bacilli are often isolated from rhizosphere soil (Achouak et al. 1999) and *Bacillaceae* spp. (OTU001) was the most abundant OTU whenever clay was applied to soil.

The addition of compost, clay or compost + clay to soil resulted in an increase in predicted lignin and starch degrading functional genes (e.g. catalase and alpha-amylase, respectively) and a decrease in the chitin and more labile C (glucoamylase, beta-galactosidase) degrading genes. Soil amendment with compost has been shown to enhance hydrolytic activities enzyme activities involved in C cycling previously (Sonia et al. 2011). The decrease in labile degrading C genes was more pronounced under well-watered conditions whenever clay was applied to the soil. An explanation for the significant finding is related to the interaction of clay and SOM within the soil matrix and aggregate formation leading to a reduction in the mineralization and bioavailability of nutrients (Baldock and Skjemstad 2000; Wang et al. 2003). As mentioned earlier, the physical–chemical stabilisation of the soil and properties of the clay create a barrier for biological decomposition due to soil aggregation processes protecting organic matter (Oades 1988; Bronick and Lal 2005; Lützow et al. 2006). Further research is required to quantify microbial activity, including gene function, in close proximity to organic matter inside aggregates which are formed when clay is added to sandy agricultural soil such as the soil used here.

Conclusion

Three important trends related to the combination of soil amendments and water treatments were highlighted in this study. First, clay and compost (either alone or in combination) significantly altered plant growth, AM fungal colonisation, rhizosphere bacterial community composition in both well-watered and water-stressed soils. Second, water stress

impacted on the rhizosphere bacterial community in all soil treatments except the compost only amended soil, indicating that compost increased the resistance of rhizosphere bacterial community to water stress. In contrast, there were distinct responses in community composition to water stress within the unamended soil and for soil amendment with both clay and compost. Finally, changes in the physiochemical properties of soil after compost and clay application such as available soil P, NO_3^- and DOC are likely to be the main factors that contributed to changes in these bacterial populations. Further research is required to verify changes in microbial gene function in relation to the bacterial and fungal predicted gene abundance.

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