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Responses of the soil microbial community to nitrogen fertilizer regimes and historical exposure to extreme weather events: flooding or prolonged-drought

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Abstract

Extreme weather events, including flooding and prolonged-drought, may establish long-lasting effects on soil biotic and abiotic properties, thus influencing ecosystem functions including primary productivity in subsequent years. Nitrogen (N) fertilizer addition often improves soil fertility, thereby potentially alleviating legacy effects on soil function and plant productivity. The soil microbial community plays a central role in mediating soil functioning; however, little is known about the legacy impacts of extreme weather events and N fertilizer addition on soil bacterial communities and the key processes involved in carbon (C) cycling. Here, the potential legacy effects of waterlogging, prolonged-drought and N fertilizer addition (0, 100, 200 and 300 kg N/ha) on soil bacteria and microbial respiration were investigated. The abundance, diversity and composition of the bacterial community, and basal and induced-respiration rates, in a farming soil system were examined, using quantitative PCR, high-throughput DNA sequencing, and MicroResp^{TM}. Soils *previously* exposed to short-term waterlogging events and prolonged-drought (by air-drying for 4 months) were used in our study. Prolonged drought, but not waterlogging, had a strong legacy effect on the soil bacterial community and microbial respiration. The addition of N fertilizer up to 300 kg N/ha could not fully counteract the legacy effects of prolonged-drought on soil bacteria. However, N addition did increase bacterial abundance and diversity, and inhibited soil microbial respiration. Significant correlations between microbial respiration and bacterial community structure were observed, but N addition weakened these relationships. Our results suggest that the resilience (rate of recovery) of soil bacterial communities and functions to prolonged-drought is limited in farming systems, and therefore, may take a long time to recover completely. Subsequently, this should be explicitly considered when developing adaptation strategies to alleviate the impacts of extreme weather events.

Keywords: Waterlogging; prolonged-drought; legacy impacts; soil bacterial community; microbial respiration; N fertilizer addition.

1. Introduction

The frequency and intensity of extreme weather events are projected to increase under future climatic conditions which can significantly impact ecosystem functions, including biogeochemical cycling and productivity of farming systems (IPCC 2007). Extreme drought and waterlogging can impact ecosystems directly via altered water supply to plant and microbial communities and indirectly via changes in soil physico-chemical properties. For example, soil nutrient availability may be affected by a change in soil structure and pH which can alter the rate of soil processes catalysed by soil microbial communities (Ponnamperuma, 1984; Yang et al., 2016). Similarly, prolonged drought and water deficit stress can limit substrate diffusion to such an extent that microbial access and activities are reduced (Stark and Firestone, 1995; Voroney et al., 2007; Brunner et al., 2015). Soil microbes exposed to drought periods may alter their rates of function due to physiological stresses, potentially changing the rate and pathways of C and N transformation (Schimel et al., 2007). Microbes survive by accumulating solutes such as amino acids when moisture is limiting, to decrease their internal water potential and avoid dehydration and death (Harris, 1981). However, the cost of accumulating solutes is energetically expensive (Schimel et al., 2007).

Our understanding of the direct effects of environmental variables (e.g. temperature, water) on microbial communities and functions has improved recently (Rousk et al., 2013; Liu et al., 2017). For example, changes in soil moisture due to water stress are well known to affect microbial abundance (Gordon et al., 2008), structure (Placella et al., 2012) and process rates (Placella et al., 2012; Goransson et al., 2013). In addition, microbial responses to environmental disturbances may relate to their historical conditions (Schimel et al., 2007; Evan and Wallenstein, 2011). However, we have limited knowledge whether extreme weather events have legacy impacts on the resilience (rate of recovery) of the microbial

78 community (abundance, composition and diversity) and their contribution to ecosystem
79 functioning (Rousk et al., 2013; de Vries et al., 2012), but this information is critical to fully
80 understand their role in natural and agricultural systems (Martiny et al., 2017). The responses
81 of microbial community and functions to environmental disturbances may vary (Allison and
82 Martiny, 2008). For example, (1) microbial community composition can be resistant or
83 resilient to disturbances (Bowen et al., 2011; Shade et al., 2011); (2) shifts in microbial
84 community composition due to stress exposure do not affect ecosystem functions (Wertz et
85 al., 2007); and (3) microbial function, but not community composition, respond to
86 disturbance (Agrawal, 2001). If the resilience of the microbial community is low (i.e. slow
87 rate of recovery), it is important to identify the consequences for ecosystem functioning
88 including C cycling, which could affect C fluxes to the atmosphere and accelerate climate
89 change (Martiny et al., 2017; Treseder et al., 2012; Trivedi et al., 2016). For agricultural
90 systems, any legacy impact will have important consequences for farm productivity via the
91 impact on the rate of nutrient cycling.
92
93 Soil processes involved in C cycling may be altered in response to extreme weather events
94 (Baldwin et al., 2015; Sanchez-Andrez et al., 2010; Mesiner et al., 2015; Liu et al., 2017).
95 Shifts in microbial communities and the links to soil C processes upon exposure to extreme
96 weather events have been observed in previous studies (Liu et al., 2017). Although extreme
97 weather events may initiate legacy effects on soil processes, biota and plant growth (Meisner
98 et al., 2013a; Cavagnaro, 2016; Banerjee et al., 2016), less is known about the legacy effects
99 on soil processes involved in C cycling. Extreme weather events, including flooding and
100 prolonged-drought, alter soil moisture conditions which is a key factor influencing C and
101 nutrient cycling in soils (Martins et al., 2016; Liu et al., 2017). Soil microbial communities
102 are the main drivers of ecosystem functioning (Delgado-Baquerizo et al., 2016a), including

processes directly involved in C cycling (Singh et al., 2010; Trivedi et al., 2016) and nutrient cycling, and exposure to extreme weather events may have long-lasting effects on these processes in subsequent years (Meisner et al., 2013a). Previous studies have suggested different vulnerabilities of different soil microbiota to drought and flooding stresses (Graff and Conrad, 2005; Schimel et al., 2007; Chodak et al., 2015); however, little is known about the taxonomic structure and diversity of soil bacterial communities in response to the legacy effects of extreme weather events.

N fertilizer supply is used globally to enhance soil fertility and hence improves plant productivity during conventional farming practices (Lu et al., 2015; Nkebiwea et al., 2016). An increased rate of N fertilizer may be used to mitigate negative effects of extreme weather events on soil fertility. However, evidence for the response of soil bacterial communities to N fertilizer addition remains contradictory (Marschner et al., 2003; Ogilvie et al., 2008; Lupwayi et al., 2011; Roberts et al., 2011). For example, microbial community composition was reported to be unresponsive to N addition in crop soils (Roberts et al., 2011), whereas others have observed a shift in community structure and a decrease in bacterial diversity in grassland soils (Zeng et al., 2016). Ramirez et al., (2012) found consistent responses of soil biota, particularly shifts in bacterial composition, to N amendment across a wide range of ecosystems. Additionally, whether legacy impacts of extreme weather events on microbial communities may be moderated by N fertilization remains largely unknown, but remains critical for understanding C and nutrient cycling in agricultural systems.

Carbon cycling in soils play a critical role in maintaining soil nutrients which are directly related to crop productivity (Gougoulias et al., 2014). Thus, it is imperative to examine the response of C cycling processes to the legacy effects of extreme weather events and

128 determine whether this can be modulated by different rates of N fertilizer. Soil microbial
129 respiration (a proxy for soil organic C decomposition) in response to altered precipitation
130 may be highly variable and dependent on ecosystem type (Borken et al., 2006; Cleveland et
131 al., 2010; Van Straaten et al., 2010). This process is an important flux in the soil C cycle and
132 linked to soil organic C pools (Gougoulias et al., 2014). In some studies, N fertilizer addition
133 often inhibits soil microbial respiration rates in natural and agricultural systems (Kowalenko
134 et al., 1978; Bowden, 2004; Treseder, 2008; Gagnon et al., 2016), thereby potentially
135 increasing C sequestration rates (Ramirez et al., 2012). Above findings support the nutrient
136 mining theory (i.e. when N is limiting, the microbial community "mines" soil organic matter
137 (SOM) to secure their N requirement, potentially leading to loss of soil C via increased
138 microbial respiration) (Moorhead and Sinsabaugh, 2006). However, we have limited
139 knowledge regarding potential modification of this relationship via the legacy impacts of
140 extreme weather events. This knowledge is particularly important if the response of microbial
141 community composition to extreme weather events (flooding vs drought) is divergent. This
142 divergence in community composition will have consequences for total metabolic activities,
143 including the rate of soil respiration (Singh et al., 2010), which is currently not well-known.
144 Additionally, the underlying mechanism of microbial respiration response to extreme weather
145 events under different rates of N fertilizer remains relatively unclear (Ramirez et al., 2010),
146 but important for formulating environmentally sustainable farming.
147
148 In this study, we examined the response of the soil bacterial community and microbial
149 respiration to the legacy effects of extreme weather events and different rates of N fertilizer
150 addition in agroecosystems, using cotton as a model system. Given differential response of
151 microbial communities to flooding and drought, we hypothesized that (1) soil bacterial
152 communities will have lower resilience to historical prolonged-drought than to waterlogging

exposure; and (2) N fertilization will modulate the response of the microbial community and respiration to historical extreme weather events through altered soil physicochemical properties. Our hypotheses are based on previous findings, which reported a consistent impact of drought on soil microbial communities and activities, and some have even reported strong legacy impacts of drought on plant-microbial interactions (Meisner et al., 2013a; 2013b; de Vries, 2012). The impact of waterlogging on microbial communities and activities are known, but impacts seem to be transient (Bossio and Scow, 1995; Unger et al., 2009) or less pronounced than other factors such as land-use types (Drenovsky et al., 2010). Additionally, in dryland farming, microbial diversity, abundance, and activities are limited by water availability (Martins et al., 2015; Maestre et al., 2015) and further loss of soil water under drought treatment can generate a stronger legacy impacts.

2. Materials and methods

2.1 Glasshouse experimental setup and soil sampling

A glasshouse experiment was conducted at Western Sydney University (WSU), Australia for approximately 7 months, using soils collected from a cotton field which had been exposed to waterlogging events in 2013-2014, simulated by running furrow irrigation for 120 hrs at an early and late flowering stage of cotton crop at the Australian Cotton Research Institute (ACRI) in Narrabri (30.31^{o}S, 149.78^{o}E), New South Wales (NSW), Australia. This region represents a semi-arid ecosystem and experiences hot summers with maximum and minimum daily temperature of 35^{o}C and 18^{o}C, respectively. Mean annual precipitation is 644 mm, of which approximately one-third falls during the summer months (Bureau of Meteorology, NSW). Annual rainfall in 2013-2014 in the field area is approximately 410.9 mm (Bureau of Meteorology, NSW). The soil is cracking grey clay soil (vertosols) with an alkaline pH of

178 7.5-8.0; soils are designated as Ug 5.25 under the Northcote classification system (Northcote
179 et al., 1975). Crops were grown following a typical regime of commercial management
180 practices, as described in Hearn and Fitt (1992), including high resource inputs, irrigation and
181 tillage.
182
183 Here, we define our flooding treatment as extreme using the following logic. Any flooding in
184 the Narrabri region (our experimental site) is considered an extreme event. This region
185 experienced flooding events in 1955, 1964, 1971, 1974, 1998, 2004, 2008, 2011 and 2012,
186 indicating flooding has become more common in the past 15 years. In addition, our
187 experimental flooding events were repeated within the growing season, thereby assuring that
188 flooding was an extreme event. Our flooding treatment was similar to the flood in 1995
189 which was considered to be a once-in-a-century flood. In this study, no rain occurred during
190 periods of the waterlogging treatments in the field. The length of the simulated prolonged-
191 drought in this study was similar to the historical drought stress in the Narrabri region during
192 early 2000's, which is known as Millennial Drought (Murphy and Timbal, 2007). Our
193 drought approach was also similar to the study of Meisner et al., 2013, which defined their
194 drought as an extreme event.
195
196 Bulk soils consisted of top soil (0-10 cm) and sub-soil (10-20 cm) and were collected from
197 waterlogged and control areas (non-waterlogged) at the end of the experiment (July 2014),
198 and then immediately transferred to WSU. Half of the soils collected from the control area
199 were spread on a tarp in a shed for air-drying to simulate prolonged-drought conditions for 4
200 months (from beginning of February to the end of May 2015), according to an established
201 method for drought impacts (de Vries et al., 2012; Meisner et al., 2013a). Soil moisture was
202 checked regularly and it dropped to 6.3% after 2 months, thereafter remaining at that level.

In the first week of June 2015, soils were placed into plastic pots (25 x 23 x 19 cm), and moved to glasshouse bays (~50 m^3 each) with controlled temperature (28^oC/17^oC (day/night) to mimic annual mean climatic conditions in the field in Narrabri. Pots were assigned to a treatment and randomized within a glasshouse compartment. Treatments included three water conditions: (1) Control; (2) Post-waterlogging (Post-WL); and (3) Post-prolonged drought (Post-PD) x four urea fertilizer (46% of N) levels (0, 100, 200 and 300 kg N/ha applied once before planting), to follow local farming practices in Narrabri, NSW. There were four replicates per treatment, giving a total of 48 pots. Other nutrients, such as P, K and trace elements, were applied to all pots in the same amount to mimic commonly applied nutrients for irrigated cotton in the field (Braunack et al., 2013). Cotton CSIRO cultivar Sicot 71BRF seeds were used for sowing. All pots were watered every 2-3 days to bring them back to field capacity. LED lights were installed in the bays to supplement natural light, and maintain photoperiods at 12 hours, to support cotton growth and development. The LED lights operated daily from 6:00 am to 6:00 pm.

Soil samples were collected four times: (1) pre-sowing (11th June 2015 prior to N fertilizer application); (2) early squaring (first square emergence, 74 days after planting (DAP) for control and Post-WL pots; and 89 DAP for Post-PD pots); (3) early flowering (95 DAP for control and Post-WL pots; and 108 DAP for Post-PD pots); and (4) harvest (172 DAP for control and Post-WL pots; and 186 DAP for Post-PD pots). Two soil cores of 3-cm diameter and 10-cm depth were taken from each pot at each sampling time. All collected soil samples were immediately sieved through a 4 mm-mesh sieve to remove plant residue before analyses.

228 *2.2 Soil physicochemical analyses*

229 The soil moisture content was determined by oven-drying samples at 105^{o}C for 24 hr. For
230 soil pH, a suspension of fresh soil and milli-Q water (in a ratio of 1:5) was shaken for 1 hr,
231 prior to measurement with a pH meter (Seve-nEasy pH, Metler, Toledo, Switzerland). Soil
232 NH_4^+ and NO_3^- were extracted using 2M KCl and analysed by a SEAL AQ2 discrete analyser
233 (SEAL analytical Inc., USA). Soil total N and C were determined by a LECO macro - CN
234 analyzer (LECO, USA).

235

236 *2.3 Soil bacterial community analyses*

237 *2.3.1 DNA extraction*

238

239 Total genomic DNA was extracted from 0.25 g of soil using the MoBio PowerSoil DNA
240 Isolation kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's
241 instruction, using a FastPrep bead beating system (Bio-101, Vista, CA, USA) at a speed of
242 5.5 m s^{-1}. The quantity and quality of extracted DNA were checked photometrically using a
243 NanoDrop® ND-2000c UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington,
244 DE, USA).

245

246 *2.3.2 Soil bacterial community abundance*

247

248 Bacterial abundance was quantified by quantitative PCR (qPCR) of the 16S rRNA gene using
249 the primers Eub338f/Eub518r (ACTCCTACGGGAGGCAGCAG/ATTACCGCGGCTGCTG
250 G) (Fierer et al., 2005). Each sample was quantified in a 10 µl reaction, including 5 µl
251 GoTaq® qPCR Master Mix (2X), 20µM each primer, 0.1 µl CXR reference dye and 10 ng of
252 template DNA. The PCR thermal cycling conditions were as follows: an initial cycle of 95^{o}C

for 15 min, 40 cycles of 95°C for 1 min, 53°C for 30 s, to 72°C for 1 min, and 1 cycle of 95°C for 15 s, 60°C for 15 s, to 95°C for 15 s (Fierer et al., 2005). Standard curves were generated using tenfold serial dilution of plasmids containing the target region of 16S rRNA genes from *Escherichia coli*. Melt-curve analyses (from 65 to 95°C) were conducted following each assay to verify the specificity of the amplification products. PCR efficiency values for the abundance of 16S rRNA were in the range of 95-101%.

2.3.3 Soil bacterial community diversity and composition

The soil bacterial diversity and community composition were examined by 16S rRNA amplicon sequencing. The V3-V4 region of the 16S rRNA gene was amplified using 341F/805R primer set (Herlemann et al., 2011) and then sequenced on the MiSeq platform (Illumina, San Diego, CA, USA) by the Next Generation Sequencing Service at Western Sydney University, NSW (Liu et al., 2017; Delgado-Baquerizo et al., 2016b). The amplification and sequencing of 16S rRNA followed the Illumina 16S Metagenomic Sequencing Library Preparation Guide (http://support.illumina.com/documents/documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf).

The sequence analysis was conducted using the Quantitative Insights into Microbial Ecology (QIIME) 1.7.0 (Caporaso et al., 2010a). The 'SeqPrep' method was used to join paired ends (https://github.com/jstjohn/SeqPrep) and regions with low quality score (Q < 20) were trimmed from the 5' end of sequences. Chimeric sequences were filtered out using USEARCH (Edgar, 2010; Edgar et al., 2011) against the Greengenes database (DeSantis et al., 2006). The average read numbers/ sample was 96901 in analysed data and the lowest

number of reads in a sample was 36359 reads. Each sample was then resampled according to the minimum sequence (36359) number for all downstream analyses. This number of reads covered most of the diversity information in our samples (S. Figure 1). Sequences were then clustered into operational taxonomic units (OTUs) at a 97% identity threshold using uclust (Edgar, 2010). Representative sequences from individual OTUs were then aligned using PyNAST (Caporaso et al., 2010b) and phylogeny was assigned using ribosomal database project (RDP) Classifier (Wang et al., 2007) based on the Greengenes database (DeSantis et al., 2006). Each sample was resampled according to the minimum sequence numbers before downstream analyses.

2.4 Soil microbial respiration

Soil respiration was examined by MicroResp^{TM} method as described by Campbell et al., (2003). Briefly, each soil sample (0.4 g) was placed into a single well of a 96-deep well micro-titre plate, and then incubated for two days at room temperature in the dark before conducting the assay. Two additional C substrates (glucose and lignin) to mimic decomposition of labile and recalcitrant C substrates were also used (1 mg ml^{-1} per well) (Colombo et al., 2016). Basal respiration was determined to examine overall rate of mineralisation which is considered a strong proxy for community functions including mineralisation (Bell et al., 2005) by using sterile deionized water. Prior to the addition of C sources into the 96-deep well plates, CO_2 detection plates were read, and then assembled with the detection plate and incubated at 25^{o}C for 6 hr. The change in optical density of the CO_2-detection plate was measured again after 6 hr of incubation. The rate of CO_2 respiration per gram of dry soil was calculated using the formula as described in MicroResp^{TM} manual (Macaulay Scientific Consulting, UK).

303 *2.5 Statistical analyses*

304 In this study, a legacy effect was determined to have occurred when historic water treatments
305 significantly affected measurable community or functional variables. One-way ANOVA was
306 used to examine the legacy effects of extreme weather events on the soil bacterial,16S rRNA
307 gene abundance, diversity, relative abundance of bacterial phyla, and microbial respiration at
308 pre-planting. Two-way ANOVA with Tukey's HSD was applied to test the effects of N-
309 addition, legacy effects of extreme weather events, and their interaction on the soil bacterial
310 16S rRNA gene abundance, diversity, relative abundance of bacterial phyla, and microbial
311 respiration at the early flowering. To determine the effects of N-addition, water treatment and
312 their interactions on 16S rRNA gene abundance over time, two-way repeated measures
313 ANOVA was carried out. 16S rRNA gene copy number was log-transformed prior to
314 statistical analysis to satisfy normality assumptions. Spearman's rank correlation analysis was
315 applied to test the relationship between soil physicochemical properties and soil microbial
316 respiration; the abundance, diversity and composition of total bacteria and microbial
317 respiration rates. Stepwise regression analysis was conducted to examine the predictors of
318 changes in the abundance, diversity and composition of total soil bacteria. Shannon index
319 was calculated to examine bacterial diversity. Principal coordinates analysis (PCOs) for Bray-
320 Curtis dissimilarity matrices was applied to visualize shifts in the microbial community
321 compositions based on the 97% OTU level across different treatments (Caporaso et al.,
322 2010a). Permutational multivariate analysis of variance (PERMANOVA) was conducted to
323 test the significance of Bray-Curtis dissimilarity. PERMANOVA was also used to examine
324 the effects of water treatment, N addition and their interaction on soil bacterial composition.
325 A value of $P < 0.05$ was considered to be statistically significant. All tests were manipulated
326 in SPSS 22 (IBM, Armonk, USA) and Primer v6 (Primer-E Ltd, Plymouth, UK).

327

3. Results

3.1 Legacy impact of extreme weather events on soil physico-chemical properties pre and post planting

Waterlogging prior to planting did not affect soil physicochemical properties (Table 1). Previous waterlogging significantly reduced soil NO_3^- content at the time of planting (-29.38%, $F_{1,30}$ = 247.218, $P < 0.001$); however, the negative effect was fully counteracted by N fertilizer addition ($P < 0.001$, Table S1). In contrast, prolonged-drought before planting established a strong legacy effect on these measurements ($P < 0.001$, Table 1). In particular, soil nutrients including inorganic N, total C and N contents were significantly lower in Post-PD soils (Table 1). Although N fertilizer supply significantly improved soil N content, prolonged-drought legacy on soil nutrients could not be counteracted completely during the growing season. Interactive effects of prolonged-drought legacy and N fertilizer addition significantly affected soil NH_4^+ and NO_3^- content ($P < 0.05$, Table S1). The significant interactive effect of Post-PD and N fertilizer on soil NO_3^- content was influenced by cotton growth stage ($P < 0.001$, Table S1).

3.2. Microbial community response to historical extreme weather events.

3.2.1 Bacterial abundance

At pre-planting, previous waterlogging events did not generate a legacy effect on soil bacterial abundance ($F_{1,30}$ = 3.423, P = 0.087), whereas bacterial abundance of Post-PD soils

was 6.7-fold lower than that of control soils ($F_{1,30}$ = 78.712, P = 0.01). After planting, bacterial abundance varied from 1.31 x 10^9 to 6.88 x 10^9 copies/g dry soils across all water and N addition treatments and was impacted by cotton growth stage (Fig. 1). Although N fertilizer addition significantly increased soil bacterial abundance ($F_{3,24}$ = 18.471, P < 0.001 for Post-PD soils and $F_{3,24}$ = 20.014, P < 0.001 for Post-WL soils), soil pre-exposed to prolonged-drought still had significantly lower bacterial abundance when compared to control soils ($F_{1,24}$ = 221.211, P < 0.001). No interactive effects between factors were observed (nitrogen x water treatment $F_{6,36}$ = 0.263, P = 0.950; time x nitrogen $F_{3,36}$ = 0.366, P = 0.778; time x water treatment $F_{2,36}$ = 0.077, P = 0.926; time x water treatment x nitrogen $F_{6,36}$ = 0.203, P = 0.974).

3.2.2. Bacterial diversity and community composition

Prior to planting, previous waterlogging did not generate a legacy effect on soil bacterial diversity (Shannon index, $F_{1,14}$ = 5.542, P = 0.068), whereas bacterial diversity of Post-PD soils was significantly lower than control soils (Shannon index, $F_{1,14}$ = 118.199, P = 0.001, Fig. 2). After planting, there were upward trends of bacterial diversity with increasing N fertilizer rates in all treatments (Table S2; Fig. 2). However, the negative effect of previous prolonged-drought event on soil bacterial diversity was persistent during the growing season (Shannon Index, $F_{1,24}$ = 31.629, P < 0.001). No interactive effects of post-PD and N fertilizer supply were observed ($F_{3,24}$ = 0.083, P = 0.943). Regarding Post-WL soils, no legacy effect was established (Shannon index, $F_{1,24}$=1.721, P=0.192). N-addition significantly affected Shannon Index of Post-WL soils ($F_{3,24}$=97.342, P=0.002). No significant interaction effects of N supply and previous waterlogging were observed ($F_{3,24}$=0.085, P=0.949). Overall, bacterial diversity was resilient to waterlogging, but not to prolonged drought.

PCO analysis of samples collected before planting explained 90.5% of variation (two axes) in bacterial community composition. Bacterial community composition was significantly different between control and Post-PD treatments (PERMANOVA, $F_{1,14}$ = 41.099, R^2 = 4.704; P = 0.001), while that of control and Post-WL treatments clustered together (PERMANOVA, $F_{1,14}$ = 9.6615, R^2 = 2.101; P = 0.002, Fig. 3a). Analysis of data collected at the early flowering stage of cotton plants by PCOs, with the two first axes explaining 81.9% of variation in bacterial community composition, indicated that an interactive effect of Post-PD and N fertilizer application on soil bacterial community composition was significant ($F_{3,24}$ = 4.776, R^2 = 1.887, P = 0.001). In addition, a strong and significant effect of Post-PD on soil bacterial community composition remained unchanged (PERMANOVA, legacy, $F_{1,24}$ = 66.299, R^2 = 3.880, P = 0.001), while bacterial communities in the Post-WL bacterial community were similar to control plots (PERMANOVA, $F_{1,24}$ = 19.181, R^2 = 2.043, P = 0.001, Fig. 3b). Nitrogen application significantly affected the bacterial community composition of Post-WL soils ($F_{3,24}$ = 4.6087, R^2 = 1.124, P = 0.001), whereas no significant effects of N addition on the bacterial composition of Post-PD soils were observed ($F_{3,24}$ = 1.4908, R^2 =0.471, P = 0.074). Overall, the bacterial community structure was resilient to waterlogging, but not to prolonged drought.

Stepwise regression analysis was applied to identify the main predictors of soil bacterial communities (Table S3). Bacterial diversity and composition were significantly related to soil NH_4^+ content and total N, respectively (R^2 = 0.485, P < 0.001 for the bacterial diversity and R^2 = 0.289, P = 0.002 for the bacterial community composition). Soil NO_3^- content was significantly related to 16S rRNA gene abundance (R^2 = 0.453, P < 0.001).

Across all soil water treatments and N addition rates, the five dominant bacterial phyla were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Plantomycetes*. At the early flowering stage, no significant differences in the relative abundance of all phyla between control and Post-WL soils, at the same rate of N supply, was observed ($P > 0.05$). Although N supply increased the relative abundance of *Proteobacteria* (+11.41%), *Planctomycetes* (+28.63%), and *Bacteroidetes* (20.63%), these phyla were significantly lower in Post-PD soils compared to control soils at all rates of N supply ($P < 0.05$). Mean decreases in relative abundance of *Proteobacteria*, *Planctomycetes* and *Bacteroidetes* due to prolonged-drought legacy were 29.1%, 50.0% and 41.1%, respectively. The relative abundance of two dominant phyla *Acidobacteria* and *Actinobacteria* decreased 17.7% and 19.2% when N fertilizer addition was increased (Fig. 4).

3.3 Impact of historical extreme weather events on microbial respiration

Before planting, basal, glucose-induced and lignin-induced respiration varied from 0.61 to 0.76, 0.79 to 0.96 and 0.87 to 1.15 μg CO_2-C/g/h, respectively, across all soil water treatments (Fig.5). Historical waterlogging did not significantly affect soil microbial respiration rates ($F_{1,30} = 1.338$, $P = 0.45$; $F_{1,30} = 2.338$, $P = 0.13$ and $F_{1,30} = 4.015$, $P = 0.098$ for basal, glucose and lignin-induced respiration, respectively), whereas previous prolonged-drought significantly reduced respiration rates (-19.73%, $F_{1,30} = 11.652$, $P = 0.005$; -17.71%, $F_{1,30} = 7.010$, $P = 0.02$ and -24.34%, $F_{1,30} = 10.476$, $P = 0.009$ for basal, glucose and lignin-induced respiration, respectively).

After planting, basal, glucose and lignin-induced respiration rates varied for control, Post-WL and Post-PD soils, respectively (Fig.5). Legacy effects caused by previous prolonged-

drought on these variables could not be removed completely ($P < 0.001$). N fertilizer addition also significantly affected microbial respiration ($P < 0.05$). There were no interactions between N supply and prolonged-drought legacy ($P > 0.05$). Spearman's rank correlation analysis indicated that microbial respiration rates were significantly negatively correlated with soil inorganic N (Table S4).

3.4. Relationship between soil bacterial community and microbial respiration

Waterlogging and prolonged-drought events prior to planting had moderate impacts on the relationships between soil bacterial community and microbial respiration. However, N fertilizer addition weakened these relationships (Table 2). At pre-planting (before N fertilization), there were statistically significant correlations between bacterial abundance and lignin-induced respiration ($r_s = 0.415$, $P = 0.012$ for control; $r_s = 0.392$, $P = 0.03$ for Post-WL; and $r_s = 0.426$, $P = 0.011$ for Post-PD); bacterial diversity and basal respiration ($r_s = 0.405$, $P = 0.01$ for control; $r_s = 0.410$, $P = 0.031$ for Post-WL; $r_s = 0.424$, $P = 0.015$ for Post-PD); bacterial diversity and glucose-induced respiration ($r_s = 0.433$, $P = 0.012$ for control; $r_s = 0.450$, $P = 0.011$ for Post-WL; $r_s = 0.462$, $P = 0.022$ for Post-PD); and bacterial composition and basal respiration ($r_s = 0.402$, $P = 0.015$ for control; $r_s = 0.412$, $P = 0.011$ for Post-WL, Table 2).

At the early flowering stage, the relationships between soil bacterial community and microbial respiration were examined for each water treatment and N fertilizer rate (Table 2). Significant positive correlations between bacterial abundance and lignin-induced respiration were observed for all soil water treatments, but only at N fertilizer of 0 kg N/ha ($r_s = 0.407$, $P = 0.014$ for control; $r_s = 0.411$, $P = 0.021$ for Post-WL; $r_s = 0.395$, $P = 0.011$ for Post-PD).

Similarly, there were significant positive correlations between bacterial diversity and basal and glucose-induced respirations for all soil water treatments without N fertilizer addition (Table 2). Bacterial diversity was also significantly correlated with glucose-induced respiration for all soil water treatments with N fertilization of 100 kg N/ha (r_s = 0.45, *P* = 0.024 for control; r_s = 0.411, *P* = 0.013 for Post-WL; r_s = 0.401, *P* = 0.017 for Post-PD). With N fertilization of 100 and 200 kg N/ha, there were significant correlations between bacterial diversity and basal respiration for control and Post-PD soils (r_s = 0.381, *P* = 0.022 and r_s = 0.397, *P* = 0.014 for control and Post-PD soils, respectively, at 100 kg N/ha; r_s = 0.385, *P* = 0.014, r_s = 0.387, *P* = 0.031 for control and Post-PD soils, respectively, at 200 kg N/ha). At fertilization of 300 kg N/ha, bacterial diversity was significantly correlated with basal respiration for all soil water treatments (r_s = 0.375, *P* = 0.025 for control; r_s = 0.371, *P* = 0.03 for Post-WL; r_s = 0.372, *P* = 0.027 for Post-PD).

Bacterial community composition was significantly correlated with basal respiration for control soils at 0 kg N/ha (r_s = 0.371, *P* = 0.021). Glucose-induced respiration was also significantly correlated with bacterial community composition for all soil water treatments without N addition (r_s = 0.471, *P* = 0.013 for control; r_s = 0.462, *P* = 0.011 for Post-WL, r_s = 0.437, *P* = 0.022 for Post-PD). Significant correlations between bacterial community composition and lignin-induced respiration for all soil water treatments were observed at 0 kg N/ha (r_s = 0.373, *P* = 0.031 for control; r_s = 0.378, *P* = 0.025 for Post-WL; r_s = 0.317, *P* = 0.027 for Post-PD).

4. Discussion

476 Our findings indicate strong legacy effects of prolonged-drought, but not waterlogging, prior
477 to planting, on the soil microbial community and microbial respiration. N fertilizer has been
478 used in fields to improve soil fertility to help counteract extreme weather events, but we
479 found that fertilizing at a rate of up to 300 kg N/ha will not fully counteract the drought
480 legacy effect on soil bacterial communities. We also found that different microbial phyla
481 respond differently to legacy effects of prolonged-drought and N fertilizer addition, and that
482 N addition inhibited soil microbial respiration rates.

483

484 Prolonged-drought- re-wetting and N fertilization in this study resulted in differential
485 microbial phyla responses. At pre-planting, the relative abundance of three dominant phyla,
486 particularly *Actinobacteria*, *Acidobacteria* and *Chloroflexi*, were resistant to prolonged-
487 drought. The phylum *Actinobacteria* is Gram-positive bacteria capable of forming spores and
488 resistant to drought conditions (Singh et al., 2007). In contrast, the relative abundance of
489 *Proteobacteria*, *Bacteroidetes* and *Planctomycetes* were negatively affected by prolonged-
490 drought treatment at either pre-sowing or early flowering stage of cotton plants. This
491 developmental stage is known as the most vulnerable period in cotton growth due to a rapid
492 increase in nutrient and water requirements (Kerby et al., 2010). *Proteobacteria and*
493 *Bacteroidetes* are Gram-negative and characterized as highly vulnerable to environmental
494 disturbances and water limitation stress (Uhlířová et al., 2005; Schimel et al., 2007). The
495 phylum *Planctomycetes* is Gram-negative and slow-growing, and hence may take a long time
496 to recover after environmental stresses (Buckley et al., 2006). This may explain why the
497 microbial community was less resilient to a historical exposure of prolonged drought. Our
498 results showed that N fertilizer supply gradually increased the relative abundance of these
499 phyla across all soil water treatments, while that of *Acidobacteria* and *Actinobacteria* phyla
500 decreased. Increases in some bacterial phyla in our study are supported by previous

observations, where the resilience of microbial communities to an environmental disturbance was observed when environmental conditions were improved (Lu et al., 2006; Singh et al., 2007). Stepwise regression analysis indicated soil NH_4^+, NO_3^- and total N were linked to changes in total bacterial diversity, abundance, and composition, respectively. Thus, this supports our hypothesis that changes in soil physicochemical properties due to legacy effects of extreme weather events and N addition could explain shifts in soil bacterial communities.

Prolonged-drought prior to planting established a legacy effect on soil microbial respiration rates in our study. In addition, soil microbial respiration was observed to decrease with increased N fertilizer doses across all water treatments, in agreement with previous studies showing the inhibition of soil microbial respiration to N supply (Thirukkumaran and Parkinson, 2000; Bowden et al., 2004; Craine et al., 2007; Ramirez et al., 2010). Our findings show significant negative correlations between soil NH_4^+, NO_3^- and microbial respiration, which supports our hypothesis that soil microbial respiration will respond to legacy effects, but N addition may partially modulate those responses. This finding agrees with Ramirez *et al*., (2010) who observed negative direct effects of increased N availability, due to N fertilizer supply, on soil microbial respiration rates.

Interestingly, N fertilizer supply increased the abundance and diversity of total soil bacteria, but decreased microbial respiration rates. The decreased microbial respiration rates due to N fertilizer addition could be attributed to (i) inhibited C-degrading enzyme activities (Berg and Matzner, 1997; Gallo et al., 2004; Sinsabaugh et al., 2005; Waldrop and Zak, 2006) or (ii) shifts in microbial community composition (Fontaine et al., 2003; Fierer et al., 2007; Fierer et al., 2012). In this study, no significant correlations between soil bacterial community

composition and microbial respiration were observed for control, Post-WL and Post-PD soils with N fertilizer application, although there were changes in bacterial community composition. Thus, our findings support the premise that N supply inhibits enzymes involved in decomposing recalcitrant C, thereby reducing microbial respiration rates (Gallo et al., 2004). Waterlogging events prior to planting did not generate legacy effects on soil bacterial community and respiration. It could be that bacterial communities in irrigated cotton soils were tolerant to waterlogging stress or capable of recovering completely. Overall, our data suggest that microbial communities and functions were more resilient to the legacy impact of waterlogging than to prolonged drought. Unlike Post-WL samples, alpha diversity and community composition did not recover in Post-PD samples during the experimental period, which was reflected in the rate of respiration. However, the Post-PD and Post-WL treatments were applied at different times and handled differently (field vs laboratory); subsequently, the comparisons between Post-PD and post-WL samples should be interpreted with caution. Note that the primary focus of this study was to identify potential legacy effects of prolonged drought and waterlogging treatments relative to controls.

Several statistically significant correlations between soil bacterial communities and respiration rates were observed in our study. Overall, the relationships between soil bacterial community and respiration were not much different among water treatments before planting and at the early flowering stage at each N fertilizer rate. However, increases in N fertilizer addition doses weakened these relationships suggesting that extreme weather event legacy did not involve controlling these relationships in irrigated cotton soils. Our results are consistent with the finding in a study by Liu et al., (2017) showing N input influenced the relationship between microbial community structure and function. Therefore, our study provides evidence to support the role of N fertilization in modulating microbial structure-

function relationship in agroecosystems. Our study also suggests that increased N fertilizer supply is an appropriate management practice to improve soil function, thereby potentially enhancing agricultural productivity within the context of extreme weather event legacies.

5. Conclusions

The prolonged-drought period established legacy effects on soil bacterial communities and microbial respiration, whereas only marginal or no legacy effects of waterlogging events were observed in these variables. This suggests that the microbial community structure and function are less resilient to historical exposure to prolonged-drought than to waterlogging. Different groups of bacteria responded differently to these legacy effects; three phyla (*Proteobacteria*, *Bacteroidetes* and *Planctomycetes*) were significantly decreased after Post-PD treatment. N fertilizer supply could not completely diminish these negative legacy effects on soil bacterial abundance and diversity, suggesting that they will take a long time to recover from prolonged drought, with potential consequences for agriculture productivity. N supply increased microbial abundance but decreased soil respiration rates, thereby limiting C loss from soils. The dominant phyla *Acidobacteria* and *Actinobacteria* decreased with increased N fertilizer addition rates, which coincided with low microbial respiration rates, suggesting that the availability of N can modulate the microbial structure-function relationship. A greater understanding of soil bacterial communities and respiration, in response to legacy effects due to extreme weather events, will help to develop adaptation and management strategies to sustain soil function and fertility, and thus help maintain crop yields.

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Figure Legends

Fig. 1 The abundance of total bacteria across all soil treatments and N fertilizer rates at (**A**) the early squaring, (**B**) the early flowering, and (**C**) harvest. Values represent mean ± SE (n=4) of each soil water treatment at each N level. Different letters indicate significant differences between control and Post-WL soils, and control and Post-PD soils at each N fertilizer level and among N fertilizer levels. Post-WL = Post-waterlogging, Post-PD = Post-prolonged drought.

Fig. 2 Shannon index of soil total bacteria community at pre-planting and at the early flowering stage across all soil water treatments and N-addition rates. Values represent mean ± SE (n=4) of each soil water treatment at each N level. Different letters indicate significant differences between control and Post-WL soils, and control and Post-PD soils at pre-planting and at the early flowering stage. Post-WL=Post-waterlogging, Post-PD= Post-prolonged drought.

Fig. 3 Principal coordinates analysis (PCO) were derived from the Bray-Curtis dissimilarity matrices, based on the 97% OTU level of bacterial community compositions across all soil water treatments and N-addition at (**A**) pre-planting and (**B**) the early flowering stage. Post-WL = Post-waterlogging, Post-PD = Post-prolonged drought. OTU = Operational Taxonomic Unit.

Fig. 4 Changes in the bacterial community compositions at the phylum level across all soil water treatments and N-addition at pre-planting and the early flowering stage. Post-WL=Post-waterlogging, Post-PD=Post-prolonged drought.

Fig. 5 Microbial respiration at (**A**) pre-planting and the early flowering stage across all soil treatments and different nitrogen fertilizer rates: (**B**) Basal respiration, (**C**) Glucose-induced respiration, and (**D**) Lignin-induced respiration. Values represent mean ± SE (n=4) of each soil water treatment at each N level. Different letters indicate significant differences between treatments at pre-planting and the early flowering stage. Post-WL = Post-waterlogging, Post-PD = Post-prolonged drought.

Table 1 Soil physicochemical properties before planting. Data are presented as means ± SE (N=4) of each treatment. Different letters within the same column indicate significant differences between treatments after multiple comparisons (Tukey HSD). Post-WL = Post-waterlogging, Post-PD = Post-prolonged drought, NH_4^+ = Ammonium, NO_3^- = Nitrate, Total N = Total nitrogen, Total C = Total carbon, SE = Standard error.

Treatment	Soil moisture (%)	pH	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	Total N (g kg^{-1})	Total C (g kg^{-1})
Control	24.23 ± 0.71a	8.00 ± 0.09a	8.47 ± 0.27a	24.84 ± 0.69a	0.52 ± 0.01a	8.18 ± 0.12a
Post-WL	24.86 ± 0.49a	7.92 ± 0.08a	8.41 ± 0.12a	17. 54 ± 0.71b	0.50 ± 0.01a	8.02 ± 0.11a
Post-PD	24.35 ± 0.26a	8.02 ± 0.08a	5.69 ± 0.11b	17.13 ± 0.55b	0.41 ± 0.008b	7.06 ± 0.08b

Table 2 Correlation coefficients between soil bacterial abundance and microbial respiration (basal, glucose and lignin-induced respiration) at each N fertilizer rate during growing season; and soil bacterial diversity, composition, and microbial respiration (basal, glucose and lignin-induced respiration) at each N fertilizer rate at the early flowering stage. Significant differences at $P<0.01$(**) and $P<0.05$ (*) are in bold. Post-WL = Post-waterlogging, Post-PD = Post-prolonged drought.

	0 kg N/ha			**100 kg N/ha**			**200 kg N/ha**			**300 kg N/ha**		
	Control	**Post-WL**	**Post-PD**	**Control**	**Post-WL**	**Post-PD**	**Control**	**Post-WL**	**Post-PD**	**Control**	**Post-WL**	**Post-PD**
Variables	**Basal respiration**											
Abundance	0.315	0.320	0.295	0.311	0.306	0.301	0.318	0.307	0.312	0.264	0.262	0.258
Diversity	**0.461***	**0.442***	**0.415***	**0.381***	**0.397***	0.332	**0.385***	**0.387***	0.317	**0.375***	**0.371***	**0.372***
Composition	**0.371***	0.333	0.327	0.312	0.311	0.317	0.301	0.296	0.292	0.284	0.303	0.266
	Glucose-induced respiration											
Abundance	0.328	0.335	0.275	0.309	0.311	0.284	0.267	0.266	0.247	0.271	0.264	0.187
Diversity	**0.421***	**0.418***	**0.423***	**0.405***	**0.411***	**0.401***	0.386	0.397	**0.403***	0.275	0.302	0.294
Composition	**0.471****	**0.462***	**0.437***	0.365	0.342	0.348	0.303	0.311	0.306	0.276	0.285	0.274
	Lignin-induced respiration											
Abundance	**0.407***	**0.411***	**0.395***	0.354	0.342	0.329	0.333	0.301	0.312	0.301	0.297	0.315
Diversity	0.311	0.309	0.307	0.275	0.301	0.254	0.295	0.284	0.291	0.253	0.248	0.254
Composition	**0.373***	**0.378***	**0.371***	0.284	0.282	0.262	0.302	0.267	0.244	0.238	0.221	0.224

Figure

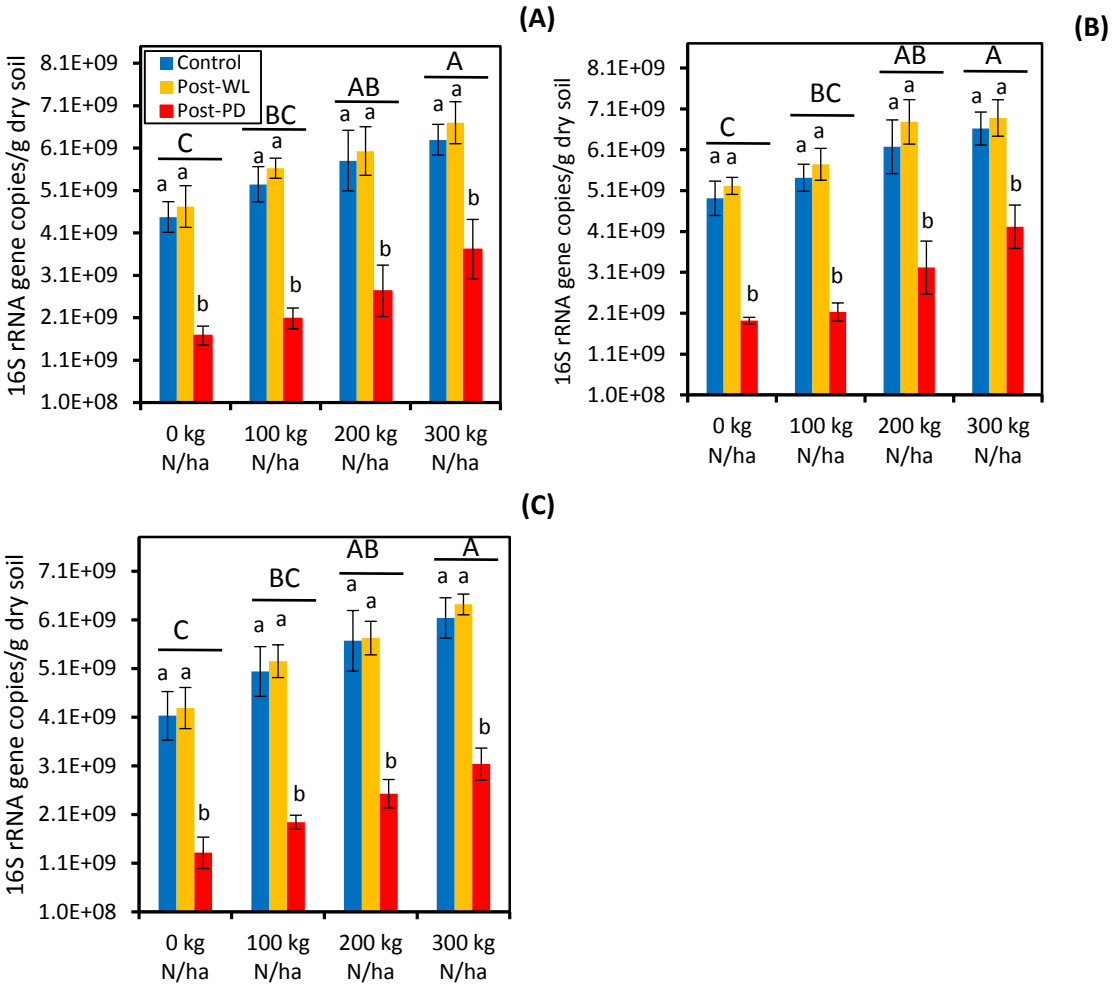
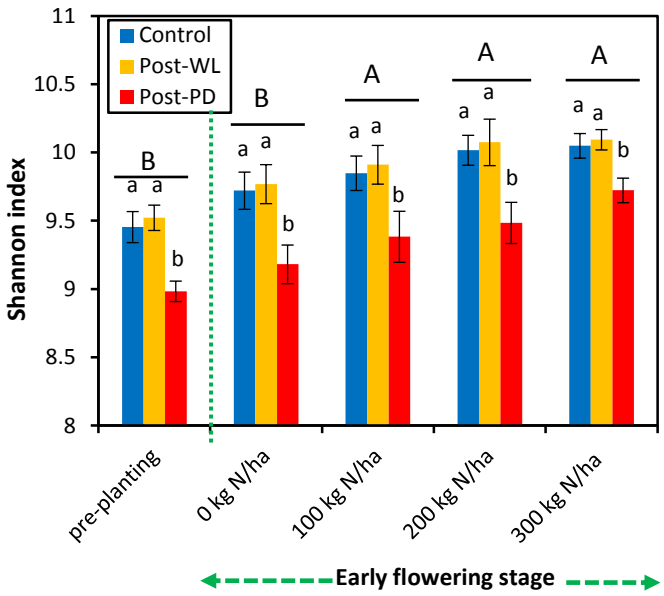


Fig. 1 The abundance of total bacteria across all soil water treatments and N fertilizer rates at (**A**) early squaring; (**B**) early flowering; and (**C**) harvest. Values represent mean ± SE (n=4) of each soil water treatment at each N level. Different small and capital letters indicate significant differences between soil water treatments at each N fertilizer level and across N fertilizer levels, respectively. Post-WL = Post-waterlogging, Post-PD = Post-prolonged drought, SE = Standard error, N = Nitrogen.



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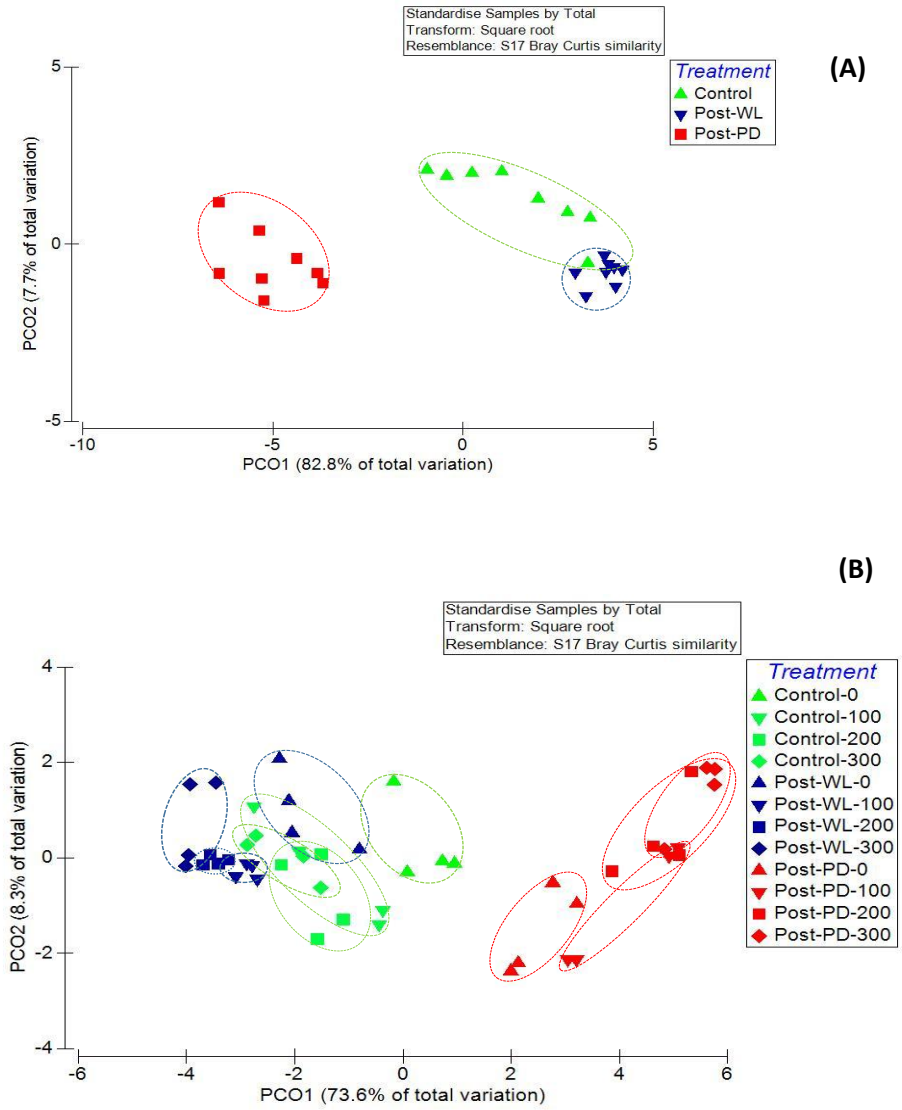

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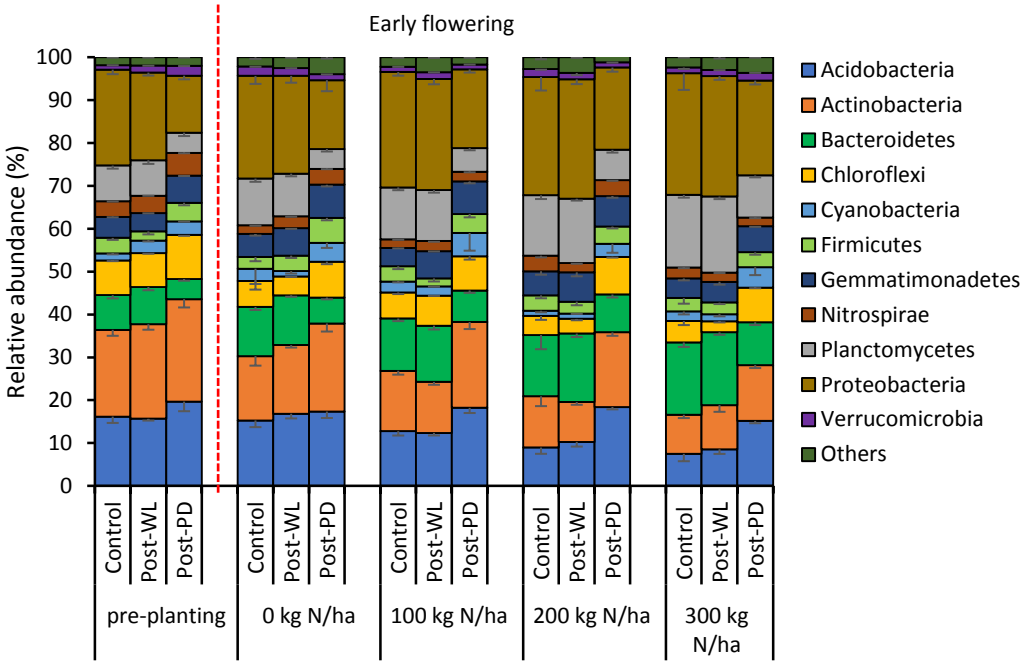
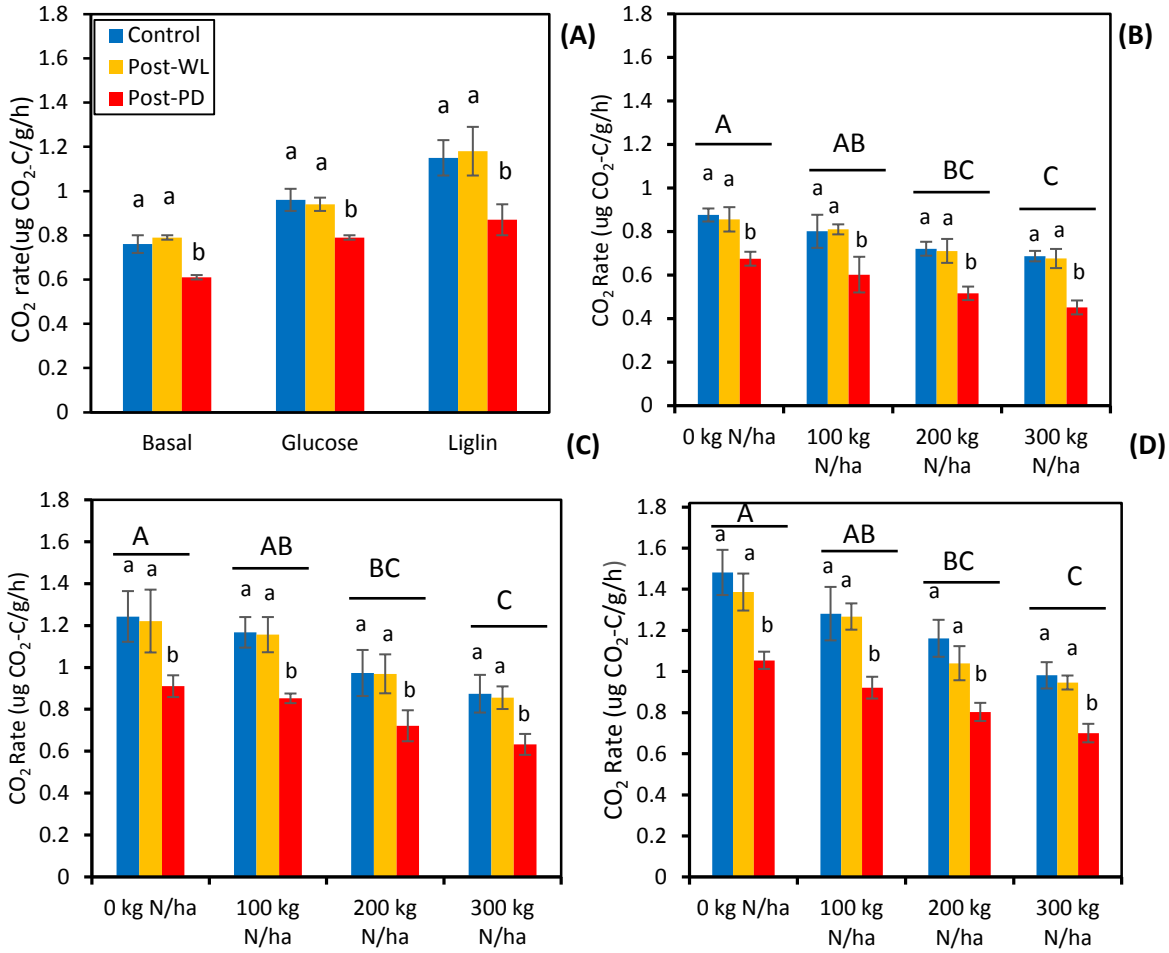


Fig. 4 Changes in the bacterial community composition at the phylum level across all soil water treatments and N-additions at pre-planting and the early flowering stage. Post-WL=Post-waterlogging, Post-PD=Post-prolonged drought.



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