Australian Government
Cotton Research and Development Corporation

FINAL REPORT 2016

Choose an item.

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: **UWS1301**

Project Title: Cotton Industry adaptation to extreme weather and climate change

Project Commencement Date: 07/2012 **Project Completion Date:** 12/2015

CRDC Program: Farming Systems Choose an item.

Part 2 – Contact Details

Administrator: Teresa Kresevic, Research, Engagement, Development and Innovation (REDI)
Organisation: Western Sydney University
Postal Address: Western Sydney University, Locked Bag 1797, Penrith, NSW 2751
Ph: 02 9678 7256 **Fax: N/A** **E-mail:** t.kresevic@westernsydney.edu.au

Principal Researcher: Prof Brajesh Singh
Organisation: Western Sydney University
Postal Address: Western Sydney University, Locked Bag 1797, Penrith, NSW 2751
Ph: 02 4570 1329 **Fax:** 02 4570 1103 **E-mail:** b.singh@westernsydney.edu.au

Supervisor: Prof Brajesh Singh
Organisation: Western Sydney University
Postal Address: Western Sydney University, Locked Bag 1797, Penrith, NSW 2751
Ph: 02 4570 1329 **Fax:** 02 4570 1103 **E-mail:** b.singh@westernsydney.edu.au

Signature of Research Provider Representative: _____

Date Submitted: _____

Part 3 – Final Report

Table of contents (of Part 3)

Project background
Project objectives
Methodology/Experimental approaches
Outcomes (against milestones)
Results

1. Glasshouse experiment (season 1)
    - Background/aims
    - Methods
    - Results
    - Discussion/Conclusion
2. Glasshouse experiment (season 2)
    - Background/aims
    - Methods
    - Results
    - Discussion/Conclusion
3. Glasshouse experiment (season 1&2) – soil microbial response
    - Background/aims
    - Methods
    - Results
    - Discussion/Conclusion
4. Field waterlogging experiments (PhD thesis)
    - Background/aims
    - Methods
    - Results
    - Discussion/Conclusion
5. Field chamber experiment (PhD thesis)
    - Background/aims
    - Methods
    - Results
    - Discussion/Conclusion

Extension opportunities

Project background

1. Outline the background to the project.

Adapting to extreme weather events under current and future climate conditions will be necessary to maintain industry profitability, sustainability, and reduce greenhouse gas (GHG) emissions under more stringent carbon-accounting regulations. The intensity and frequency of extreme weather events are predicted to increase under future climate scenarios, potentially reducing cotton productivity due to loss of soil fertility and function. Recent droughts and floods have highlighted these challenges (e.g. loss of soil fertility and structure after flooding). To develop strategies to assist recovery from these extreme climate events, a robust adaptation knowledge framework must be developed within the context of climate change scenarios, including the independent and interactive effects of alterations in CO_2 and temperature.

Therefore, we examined i) the impact of extreme events under current and future climate (elevated CO_2 and temperature) on soil fertility and function; and ii) how these changes in soil processes affect cotton productivity through better understanding of soil-plant interaction and environmental sustainability through differences in GHG emissions.

Project objectives

2. List the project objectives and the extent to which these have been achieved.

The overarching aim of the project was to create knowledge for the development of a robust adaptation framework suitable for informing technological, societal and political action to address increasing frequency of extreme weather events associated with climate change and subsequent impacts on:

1. Reductions in soil fertility and function
2. Subsequent consequences for crop productivity (including WUE and NUE) and environmental footprints in terms of greenhouse gas flux (GHG) and soil carbon (C).

This project investigated the impacts of extreme events (prolonged drought and flooding) on soil fertility (plant available nutrients and water), function (structure, carbon sequestration, habitat for soil biota) and plant growth and investigated, for the first time, how these impacts differed under future climate scenarios. These data are available to be utilised within the OZCOT and APSIM models to quantify agronomic management requirements for recovery of soil fertility and to inform adaptation strategies for the cotton industry in response to predicted increased frequency of extreme climate events.

Methodology/Experimental approaches

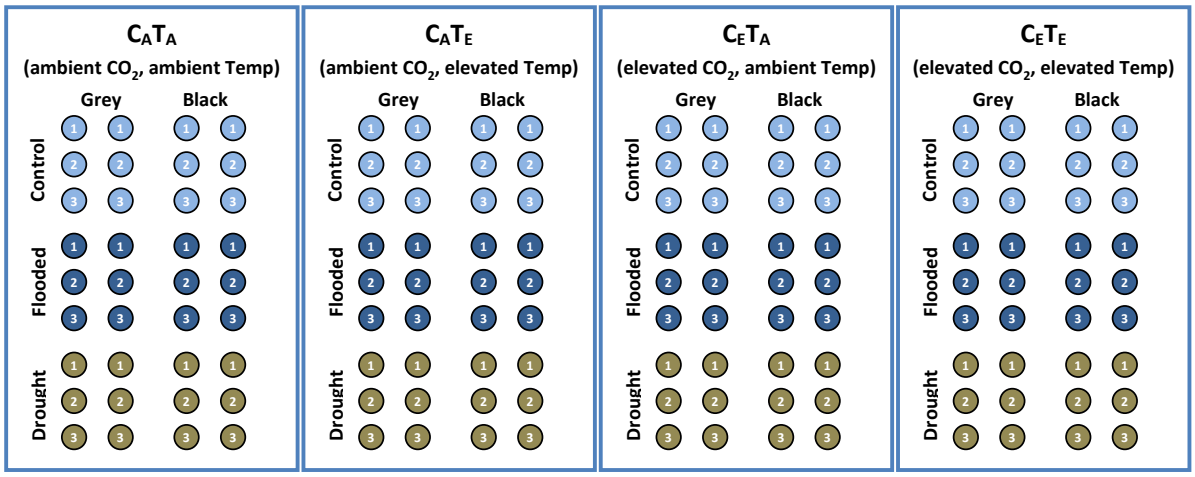
3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

Our methodology included the following:

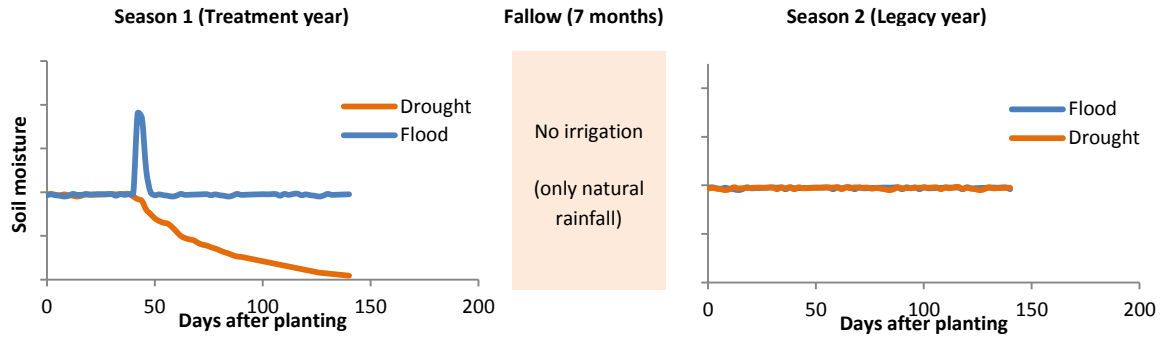
(1) **Defining extreme climate events of relevance to cotton farming** based upon existing and projected future climate scenarios, which we implemented in our experimental design (see materials and methods in the result section).

(2) Establishing manipulative experiments in state-of-the-art climate change research facilities at WSU. Our experimental design assessed the impact of different soil types (grey and black vertosols), soil moisture conditions (flooding *vs* drought), and climate treatments (elevated CO_2 and temperature) on soil fertility and function, and subsequent effects on cotton productivity. We imposed flooding and drought treatments at the early flowering stage of cotton growth in the first season (2013). After the harvest, the remaining aboveground biomass was turned into fresh mulch and incorporated back into each pot. Using these "conditioned" soils, we repeated the experiment in the following season under the same climatic conditions as the previous year's to examine the legacy of climate change as well as that of extreme weather events on cotton productivity under well-watered conditions.

Experimental set up in the glasshouse



Timeframe



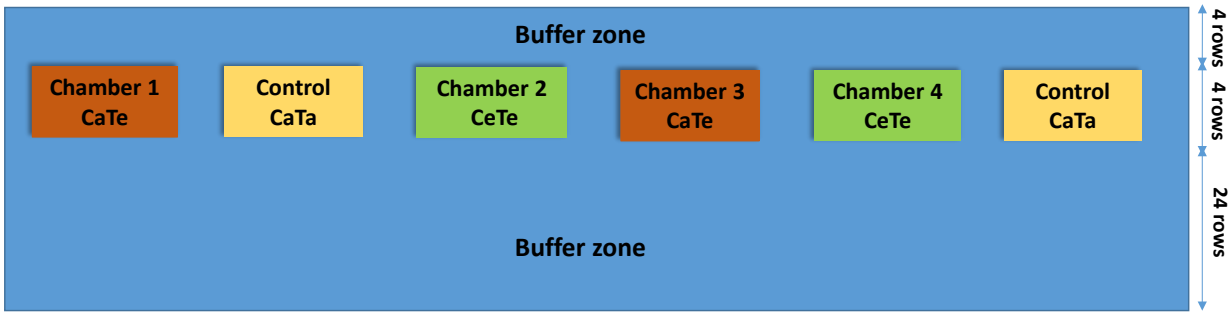
(3) Generating flooding events in cotton fields. We used recent floods (2010/11) as an initial benchmark for treatment design.

Experimental set up for field waterlogging trial 2014 (ACRI)

Block 1			Block 2			Block 3			Block 4		
Plot 1 +WL2	Plot 2 +WL1	Plot 3 Control	Plot 4 Control	Plot 5 +WL2	Plot 6 +WL1	Plot 7 +WL1	Plot 8 +WL2	Plot 9 Control	Plot 10 Control	Plot 11 +WL1	Plot 12 +WL2
8 rows	8 rows	8 rows	8 rows	8 rows	4 rows	4 rows	8 rows	8 rows	8 rows	8 rows	8 rows
D	D	D	C	C			B	B	A		A
C	C	C	D	D			A	A	B		B
B	B	B	A	A		A	D	D	C		C
A	A	A	B	B	B		C	C	D		D
		D	C		C	B		B	A	A	
		C	D		D			A	B	B	
		B	A		A	D		D	C	C	
		A	B			C		C	D	D	

78 m

(4) **Establishing manipulative experiments in cotton fields using field chambers.** We monitored soil fertility and function in climate change studies located in the field at Narrabri.



(5) **Data are available to be used to calibrate the OZCOT (for crop productivity) and APSIM (for nutrient availability and GHG emission) models** to improve prediction and provision for potential management solutions following extreme flooding and drought events.

Throughout the course of this project, we measured a range of variables to identify the impact of our treatments on soil- plant interactions, plant productivity, and GHG emission. This included the impact on important soils processes including nutrient cycling (C, N and P), soil fertility (plant available N and P, soil water retention, organic C, dissolved organic carbon and dissolved organic nitrogen), soil structure, total GHG emission (carbon dioxide, methane and nitrous oxide), rate of nitrification, denitrification and mineralisation. To investigate soil function and microbial community, we have originally proposed to use a novel patented molecular approach (USA patent 11/640066) to simultaneously characterise functional biota engaged in specific processes (e.g. nitrification, denitrification, methane emission, mineralisation, etc.). Since the proposal was made, a newer and better molecular technique became readily available, therefore we have upgraded our molecular approach to characterise soil microbial community (cost associated with this approach [~ $110, 000 staff cost plus consumables] was funded by Global Centre for Land-Based Innovation, at Western Sydney University). Plant productivity and recovery after treatments were analysed by monitoring plant establishment, growth rates (above- and below-ground biomass), plant physiology (e.g. leaf photosynthesis), nutrient uptake (leaf nutrient content and NUE) and lint yield.

Outcomes

4. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

Obj No.	Objective	No.	Milestone	Performance Indicator	Achieved?
1	Recruit new personnel for the project	1.1	Recruitment of a Postdoctoral scientist and a PhD student	Recruitment successfully completed	Yes
2.	Define extreme	2.1	Desktop activity and	Future climate and	Yes

	weather events which will have direct impacts on cotton farming		discussion with industry to define the frequency and length of the extreme weather events.	frequency and length of extreme weather identified and experimental design formulated.	
3	Measure effects of extreme weather events (flooding, prolonged drought) with and without future climate scenarios on soil fertility, nutrient cycling and crop capacity to recover.	3.1	Set up experimental conditions mimicking predicted extreme weather events under controlled environments (drought within season; flooding within and between the seasons)	Three separate experiments established under controlled environmental conditions at UWS facilities.	Yes (results are presented below)
		3.2	Quantify effect on soil fertility by measuring soil carbon (for water retention), water availability, plant available N and P	All characteristics responsible for soil fertility measured, data analysed and documented	Yes (results are presented below)
		3.3	Measure N and P cycling and its consequences for plant productivity in terms of growth and yields. Response in terms of water and nitrogen use efficiency	All characteristics responsible for nutrient cycling and plant productivity measured, data analysed and documented. Guideline published as an extension article.	Yes (results are presented below)
		3.4	Examine response of soil biota responsible for N and P cycling and consequences for plant available N and P.	Response of functional biota measured and linked to plant productivity, data analysed and documented. Complete controlled environmental experiment at UWS.	Yes (results are presented below)
4	Measure effects of extreme weather and climate change in field experiments.	4.1	Set up of extreme events/flooding field experiments at selected cotton sites throughout the industry.	Experiments established in Narrabri to validate controlled environment experiment. Soil fertility, nutrient cycling and plant productivity variables (as listed above) quantified, data analysed and documented. Guideline published as an extension article.	Yes (results are presented below). Guideline provided as a part of extension activities (masterclasses, workshops, book and industry magazine articles).
		4.2	Poly-tunnel experiment in	Soil fertility, nutrient cycling and plant	Yes (results are presented

			Narrabri. Measure all processes and biota listed above.	productivity variables (as listed above) quantified, data analysed and documented. Guideline published as an extension article.	below). Guideline provided as a part of extension activities (masterclasses, workshops, book and industry magazine articles).
5	OZCOT and APSIM model validation and improvement based on data obtained from above	5.1	Plant growth, physiology and yield data incorporation in OZCOT model for improved prediction and basis for DSS.	Data obtained from control environment and field experiment for plant productivity and yield incorporated on OZCOT simulation.	Data are available to be incorporated into OZCOT simulation.
		5.2	Nutrient availability, form and rate of processes incorporated into APSIM model to assess future water and N use, and GHG emission.	Data obtained from control environment and field experiment for nutrient cycling and GHG emission incorporated in APSIM simulation.	Data are available to be incorporated into APSIM simulation.
6	Communicate results of studies to scientific community and industry	6.1	Publish articles and participate in conference and/or industry presentations	2 Peer-reviewed journal articles, two scientific conference presentations, 2 cotton grower articles, at least one major industry presentation per year.	Yes (see below for detail)
		6.2	Updated OZCOT/ APSIM simulation models and provide knowledge for adaptation framework with knowledge developed in this project, in order to help industry planning and action for adaptation to climate change. Final report submission to CDRC	Improved OZCOT and APSIM models validated and knowledge for adaptation framework provided for future action by farming community. Guideline published as an extension article. Final report submitted to CRDC.	Data are available to be incorporated into OZCOT and APSIM simulations.

Results

5. Detail and discuss the results for each objective including the statistical analysis of results.

Expt. 1 – Glasshouse experiment (season 1)

The impact of climate change and extreme weather events on cotton productivity and soil nutrients.

Background and aims

Projected increases in global atmospheric concentration of CO_2, temperature and extreme weather events are expected to affect agricultural production. However, our knowledge on the interactions between extreme weather events, elevated CO_2 (C_E) and temperature (T_E) on agricultural system is limited, thus potentially underestimating the impact of future climates on agriculture. Using a large glasshouse experiment, we examined 1) the main and interactive effect of elevated CO_2 and temperature on cotton productivity and soil nutrients, and 2) whether flooding/drought impacts on cotton productivity and soil fertility are exacerbated or ameliorated in the future CO_2 and temperature regimes, and whether the responses differ between the two vertosols.

Materials and methods

Soil and plant materials and climate conditions in the glasshouse

A large glasshouse experiment was set up in 2013 using two soils (grey and black vertosols) collected from two adjacent cotton growing regions in New South Wales, Australia. The majority of irrigated cotton production in Australia occurs on heavy clay soils (Cattle and Field 2013). The grey vertosol (USDA Soil Taxonomy: Typic Haplustert) was collected at the Australia Cotton Research Institute in Narrabri (30°10'S, 149°40'E) and black vertosol

(Ustic Pellustert) was collected from a farm in Spring Ridge (31°21'S, 150°12'E). Top-soil (0 – 20 cm) and sub-soil (20 – 40 cm) were collected separately at each of the two field sites, and were re-assembled to re-construct the soil profile and placed into large pots (26 x 26 x 40 cm deep). These soils differed in physical and chemical properties (Table 1). The pots were watered to field-capacity and allowed to drain for two weeks prior to planting cotton seeds.

Cotton seeds (*Gossypium hirsutum* L. Cv, 71BRF [Bollgard II® Roundup Ready Flex®], CSIRO Australia, Stiller 2008) were sown into pots filled with grey or black vertosol, and maintained under [CO_2] and temperature treatments for *ca.* six months. Pots were fertilised with Multigro® fertiliser (8 g, 10.1% N, 3.5% P, 5.5% K, 16.3% S, 7.8% Ca, Incitec Pivot Ltd, Melbourne) and 500 mL of Aquasol® (1.6 g/L, 23.0% N, 40% P, 18.0% K, 0.05% Zn, 0.06% Cu, 0.0013% Mo, 0.15% Mn, 0.06% Fe, 0.011% B, Hortico, Vic) to achieve a N fertiliser rate of 190 kg N ha^{-1}, which is commonly applied to irrigated cotton in the field (Braunack 2013) in addition to the pre-existing soil inorganic N. The pre-existing inorganic N for grey and black vertosols were 74 kg N ha^{-1} and 100 kg N ha^{-1} respectively. Fertiliser was applied once before cotton seeds were sown.

Table 1 Physical and chemical characteristics of top-soil (0-20cm) and sub-soil (20-40cm) of grey and black vertosol before planting (before fertiliser application). Values are means with standard errors in parenthesis. The results of analysis of variance is also presented showing probability values with significant values (P<0.05) in bold, marginally significant values (P<0.1) in italics and non-significant results (P>0.1) as n.s.

	Grey vertosol		Black vertosol		*P* values		
	Top-soil	Sub-soil	Top-soil	Sub-soil	Soil	Depth	S x D
Particle size							
% Clay	44.0 (2.0)	48.7 (0.7)	47.0 (4.4)	54.3 (0.9)	n.s.	**0.04**	n.s.
% Silt	22.7 (1.3)	20.3 (0.3)	7.7 (2.7)	9.7 (0.3)	**<0.0001**	n.s.	n.s.
% Sand (Fine)	26.0 (2.1)	26.7 (1.3)	30.7 (0.3)	23.7 (0.3)	n.s.	**0.04**	**0.02**
% Sand (Coarse)	7.3 (1.5)	4.7 (0.3)	15.3 (2.2)	12.7 (1.3)	**0.001**	n.s.	n.s.
Chemical properties							
pH (Water)	7.9 (0.07)	8.3 (0.06)	8.5 (0.10)	8.8 (0.03)	**<0.0001**	**0.001**	n.s.
EC (dS/m)	0.1 (0.01)	0.1 (0.00)	0.2 (0.00)	0.2 (0.01)	**<0.0001**	**0.001**	**0.002**
ECSE (dS/m)	0.6 (0.03)	0.9 (0.03)	1.2 (0.00)	1.2 (0.03)	**<0.0001**	**0.0004**	**0.002**
PBI	88.0 (3.61)	96.3 (3.67)	160.0 (10)	173.3 (6.67)	**<0.0001**	n.s.	n.s.
CEC	29.2 (1.08)	34.4 (0.76)	53.7 (2.55)	56.5 (1.07)	**<0.0001**	**0.03**	n.s.
Macronutrients							
Nitrate (mg/kg)	16.3 (3.4)	10.4 (1.7)	23.3 (2.9)	15.3 (0.9)	**0.04**	**0.02**	n.s.
Ammonium (mg/kg)	4.2 (2.4)	1.4 (0.7)	3.5 (1.2)	2.2 (0.8)	n.s.	n.s.	n.s.
Potassium (%)	4.3 (0.24)	2.5 (0.25)	1.2 (0.35)	0.6 (0.04)	**<0.0001**	**0.001**	**0.04**
Potassium (cmol(+)/kg)	1.3 (0.03)	0.9 (0.06)	0.6 (0.17)	0.4 (0.03)	**0.0003**	**0.01**	n.s.
Available potassium (mg/kg)	490 (5.8)	333 (23.3)	250 (69.3)	140 (11.6)	**0.0004**	**0.01**	n.s.
Calcium (%)	70.7 (0.67)	74.7 (0.88)	70.3 (2.33)	63.3 (2.40)	**0.01**	n.s.	**0.01**
Calcium (cmol(+)/kg)	20.7 (0.88)	25.7 (0.88)	37.7 (0.88)	35.7 (0.88)	**<0.0001**	n.s.	**0.004**
Magnesium (%)	24.7 (0.33)	22.3 (0.88)	27.3 (2.60)	34.7 (1.86)	**0.002**	n.s.	**0.02**
Magnesium (cmol(+)/kg)	7.2 (0.24)	7.7 (0.07)	15.0 (2.08)	19.7 (1.33)	**<0.0001**	*0.07*	n.s.
Phosphate (mg/kg)	65.7 (1.2)	40.3 (3.0)	19.3 (5.8)	7.7 (0.3)	**<0.0001**	**0.001**	*0.07*
Sulfate (mg/kg)	3.4 (0.4)	2.9 (0.1)	12.0 (1.0)	10.7 (1.4)	**<0.0001**	n.s.	n.s.
Micronutrients							
Iron (mg/kg)	11.7 (0.4)	9.3 (0.9)	9.3 (0.1)	8.6 (0.2)	**0.01**	**0.01**	n.s.
Manganese (mg/kg)	6.8 (0.4)	5.3 (0.7)	4.4 (1.0)	2.9 (0.2)	**0.01**	**0.05**	n.s.
Zinc (mg/kg)	0.4 (0.06)	0.2 (0.05)	0.5 (0.20)	0.1 (0.00)	n.s.	**0.02**	n.s.
Copper (mg/kg)	1.3 (0.1)	1.3 (0.1)	1.4 (0.0)	1.5 (0.0)	**0.02**	n.s.	n.s.
Boron (mg/kg)	1.1 (0.0)	1.1 (0.0)	2.2 (0.6)	3.3 (0.6)	**0.003**	n.s.	n.s.
Chloride (mg/kg)	<10 (0.0)	<10 (0.0)	13.3 (1.2)	15.3 (1.9)	**0.004**	n.s.	n.s.
Others							
Organic carbon (%)	1.1 (0.0)	0.9 (0.02)	1.1 (0.16)	0.8 (0.05)	n.s.	**0.02**	n.s.
Sodium (%)	0.3 (0.02)	0.7 (0.06)	0.8 (0.06)	1.5 (0.34)	**0.01**	**0.02**	n.s.
Sodium (cmol(+)/kg)	0.1 (0.03)	0.2 (0.02)	0.4 (0.04)	0.8 (0.21)	**0.002**	**0.02**	n.s.
Water holding capacity (%)	55.2 (2.9)	33.1 (1.8)	71.3 (1.6)	42.8 (1.0)	**0.001**	**0.002**	n.s.

EC=electrical conductivity, EC_{SE}=electrical conductivity of saturated soil extract, PBI=phosphorus buffer index, CEC=cation exchange capacity.

Four naturally sun-lit glasshouse compartments were used to simulate four climate change treatments: (ambient [CO_2], ambient temperature; C_AT_A), (ambient [CO_2], elevated temperature; C_AT_E), (elevated [CO_2], ambient temperature; C_ET_A), and (elevated [CO_2], elevated temperature; C_ET_E). C_A was targeted at 400 ppm (averaged 421±1.3 ppm) and C_E was targeted at 640 ppm (646±7.3 ppm) [CO_2]. The target temperatures (day/night) for T_A were 28/16 °C (averaged 28.8±0.06/16.8±0.06 °C) and for T_E were 32/20 °C (averaged

33.2±0.06/20.0±0.01 °C) throughout the experiment. We simulated diurnal changes in temperature within each compartment by ramping up temperatures during the day and ramping temperatures down in the night; this occurred five times over each 24h period. Humidity was not controlled, and allowed to vary in each glasshouse compartment, as expected in the field. Subsequently, vapour pressure deficit (VPD) differed between the temperature treatments, with the mean of 1.06±0.003 kPa (ranged 0.61 – 2.72 kPa) for T_A and 1.25±0.003 kPa (ranged 0.61 – 3.15 kPa) for T_E. VPD did not vary between CO_2 treatments. Pots were rotated within and between glasshouse compartments on a monthly basis to avoid pseudoreplication and minimise potential effects on plant performance associated with environmental conditions in each glasshouse compartment (see Ghannoum *et al.* (2010) for details on glasshouse environmental control).

Four cotton seeds (*Gossypium hirsutum* L. Cv, 71BRF [Bollgard II® Roundup Ready Flex®], CSIRO Australia; Stiller, 2008) were sown into each pot filled with grey or black vertosol and were thinned to one plant per pot at the 1 to 2 leaf stage. Pots were maintained under the [CO_2] and temperature treatments until plants were harvested (at 142 days after planting for T_E and 196 days after planting for T_A).

Extreme weather events

Extreme weather events were imposed at the early flowering stage when environmental stresses are most likely to affect the reproductive growth of cotton (Bange et al. 2004; Snowden et al. 2014). The timing of extreme weather events was based on the developmental stage rather than on a particular point in time after sowing. This was done to minimise the confounding effect of differences in plant size and developmental stage, which were generated by the different CO_2 and temperature treatments, at any given time period. Prior to the early flowering stage, when 90% of plants in each climate condition produced the first flower, all plants were well-watered to maintain optimal volumetric soil water content (40 – 60%). Plants in each climate condition were then divided into two extreme weather treatments (flooding, drought) and a well-watered control for each soil, with six replicates for each treatment combination (144 plants in total).

Flooding treatment

Flooding conditions were created by maintaining 5-10 cm of standing water above the soil surface for six days to ensure a significant flooding event. Soils can remain saturated for this period of time where a significant rain event follows furrow irrigation. At the end of the flooding period, all pots were left to drain naturally and watered regularly to maintain optimal volumetric water content (40 – 60%) until the end of the experiment. Plant height was measured before, during and after the flooding event to examine the treatment effect on plant growth. Vegetative growth was calculated for both the pre-flooding and post-flooding periods. Pre-flooding vegetative growth rate was calculated by dividing the plant height at the early flowering stage (pre-flooding) by the number of days to achieve it. Post-flooding vegetative growth rate was calculated by subtracting pre-flooding plant height from the final plant height, and then dividing it by the number of days between the two measurement points.

Plants were harvested when 90% of well-watered control plants in each climate condition (i.e. C_AT_A, C_AT_E, C_ET_A and C_ET_E) produced the first open boll. Seed cotton was harvested from both open and closed bolls and weighed, after being oven-dried at 70 °C for a week.

Net photosynthesis and stomatal conductance were measured at the early flowering stage before the flooding event on recently fully expanded leaves using a portable open gas exchange system (LI-6400XT, LI-COR, Lincoln, NE, USA). The measurements were taken around midday (between 1100 h and 1400 h) at saturating light (photosynthetic photon flux density of 1800 μmol m^{-2} s^{-1}), using mid-day growth temperature (28 °C or 32 °C) as the air temperature in the cuvette, and CO_2 concentration (400 ppm or 640 ppm) of each climate condition. The impacts of flooding on net photosynthesis and stomatal conductance were measured at the end of the six-day flooding period.

A leaf sample was taken from each plant at seven days after the end of the flooding period (from recently fully expanded leaves) for the determination of total C and N concentrations. Leaf samples were oven-dried at 70 °C and ground to powder before being analysed using an elemental analyser (CE1110 CHN-S, Carlo Erba, Milan, Italy).

The impact of flooding on soil N availability was examined by collecting soil samples before the flooding event (at early flowering) and 7 days after the end of flooding from both well-watered control and flooded soils. Plant available N was assessed by measuring ammonium (NH_4^+) and nitrate (NO_3^-) concentrations using the 2M KCl extraction method (Rayment and Lyons 2010). The extracts were analysed for NH_4^+ and NO_3^- concentrations using an AQ2

discrete analyser (SEAL Analytical, Wisconsin, USA). Plant available N predominantly existed as NO_3^- as the concentration of NH_4^+ remained low (less than 5 mg kg $soil^{-1}$) throughout the experiment. Thus, only the response of NO_3^- is presented.

Drought treatment

We simulated an extreme drought event by discontinuing water supply to the plants until the harvest. The treatment simulated an extreme weather event in which a severe water shortage limited the use of irrigation. This was different from episodic water stress that crops may experience during the season. Similar to the flooded plants, the timing of the harvest was determined when 90% of well-watered control plants in each climate change treatment produced the first open boll. Leaf gas exchange was measured before the drought event and six days into the drought treatment, as described above. Plant growth measurements and leaf nutrient analyses were also performed, as described above. The impact of drought on soil N availability was examined by collecting soil samples before the drought treatment (at early flowering) and at harvest from both well-watered controls and drought soil. Plant available N was assessed as described above.

Well-watered control

Plants under well-watered conditions were watered regularly to maintain optimal volumetric soil water content (40 – 60 %) throughout the experiment. Plant growth measurements and leaf nutrient analyses were performed, as described above. Leaf gas exchange and soil N availability were measured concomitantly with the measurements taken for flooding and drought treatments.

Statistical analyses

Climate change impacts under well-watered conditions

Data from well-watered control treatments were analysed by three-way analysis of variance (ANOVA) in R statistical software (ver. 3.2.2, R Core Team 2014) to test the main and interactive effects of soil, C_E and T_E. All data were further analysed by two-way ANOVA for each soil to compare means using the Tukey's *post hoc* comparison. Plant height and node

data were analysed by repeated measures ANOVA to test the temporal effect of the main and interactive effects of soil, C_E and T_E. All data were checked for normality and heteroscedasticity, and log-transformed where necessary. Relationships between the seed cotton yield and measured variables were examined by Spearman's correlation (*r*) and further by partial correlation analyses while controlling for the effect of C_E and T_E using 'ggm' package in R 3.2.2 (Marchetti et al. 2015).

Extreme weather impacts under climate change treatments

To compare the magnitude of extreme weather events on plant and soil responses between the four climate change treatments, we calculated the response ratio between the well-watered control and flooded/drought plants, under each climate change treatment in each soil, to be used as an effect-size metric (Hedges et al. 1999; Borenstein et al. 2009). We calculated the natural logarithm of the response ratio (InR) as,

$$InR = ln(\bar{X}_t/\bar{X}_c) \quad (1)$$

where $\bar{X}_c$ is the mean of well-watered control and $\bar{X}_t$ is the mean of extreme weather treatment. The variance of the log response ratio ($VlnR$) is computed as,

$$VlnR = S^2_{Pooled}\left(\frac{1}{n_c(\bar{X}c)^2} + \frac{1}{n_t(\bar{X}_t)^2}\right), \quad (2)$$

$$\text{and } S^2_{Pooled} = \sqrt{\left(\frac{(n_c-1)S_C^2 + (n_t-1)S_t^2}{n_c + n_t - 2}\right)} \quad (3)$$

where S^2_{Pooled} is pooled standard deviation, n_c and S_c are the sample size and standard deviation of the well-watered control and n_t and S_t are the sample size and standard deviation of the extreme weather treatments. The 95% confidence interval is calculated from *t*-distribution. An effect size is considered significant if the confidence interval does not overlap with 1 in the back-transformed response ratio. Values >1 and <1 indicate positive and negative responses, respectively.

We also tested the statistical significance of the extreme weather treatments and their interactions with CO_2, temperature and soil by using analysis of variance (ANOVA) in R statistical software package (ver. 3.2.2, R Core Team 2014). Firstly, we performed two-way ANOVA to test the effects of flooding/drought and soil on crop and soil responses in the

current CO_2 and temperature regime (i.e. C_AT_A). We then used four-way ANOVA to test whether the impacts of flooding/drought were changed in the future CO_2 and temperature regimes by examining the significance of interactions between flooding/drought, CO_2, temperature and soil. The effects of extreme weather treatment on soil nitrate (for flooding) and residual soil nitrate (for drought) were further assessed by comparison between the pre-flood/drought treatment and post-flood/drought treatment data using four-way ANOVA with sampling (pre and post), soil, CO_2 and temperature as factors for each extreme weather treatment. *P* values were obtained for minimal adequate models by deleting non-significant ($P>0.1$) factors from full models (Crawley 2013). All data were checked for normality and heteroscedasticity and log-transformed where necessary.

To examine the relationship between the immediate crop physiological and soil N responses to extreme weather treatments and vegetative growth and yield responses, Pearson's correlation analyses were performed on the response ratios calculated as above.

Results

Climate change impacts on cotton development and vegetative growth

Plant development and vegetative growth

Plant development (number of days to attain phenological stages) and vegetative growth (plant height and number of nodes) were significantly influenced by C_E and T_E (Table 2, 3), with a strong interaction between C_E and T_E on plant development and plant height in both soils (CO_2 x temperature interaction, $P<0.0001$ and $P=0.0002$, respectively). For plants grown at C_AT_E, the rate of early plant development was increased by more than 50% relative to C_AT_A, with plants reaching first square on average 51 and 61 days earlier in grey vertosol and black vertosol, respectively; this difference was maintained until harvest (Table 2, Fig. 1). Plants grown in C_ET_A also had increased rates of early development compared to C_AT_A; 24% and 28% faster in grey vertosol and black vertosol, respectively. Plants grown in C_ET_E showed a similar response to that of C_AT_E, thus the effect of C_E was only evident at T_A and not at T_E in both soils. Plant development and vegetative growth (plant height and number of nodes) differed significantly between the two soils, with faster growth observed for plants

grown on grey vertosol than black vertosol (Table 2, 3). Soil interacted with T_E to the first phenological event (i.e. the development of first square), with a greater difference between the two soils at T_A (temperature x soil interaction, *P*=0.03).

Table 2 Climate change impact on the rate of crop development (the number of days) to each developmental stage with standard errors in parenthesis (n=6) and the effect size relative to the ambient CO_2 and ambient temperature treatment (C_AT_A) given as a percentage change. Different letters within columns indicate significant differences between the treatments within each soil (*P*<0.05).

Soil	Treatment	Number of days			Percentage change		
		1st square	1st flower	1st open boll	1st square	1st flower	1st open boll
Grey	C_AT_A	97.2 (3.3)^a	131.2 (2.3)^a	186.5 (2.6)^a	-	-	-
	C_AT_E	45.8 (1.0)^c	76.0 (1.4)^c	136.5 (1.4)^b	-52.9	-42.1	-26.8
	C_ET_A	73.5 (2.0)^b	109.2 (2.6)^b	191.3 (1.9)^a	-24.4	-16.8	2.6
	C_ET_E	44.8 (0.8)^c	72.5 (1.4)^c	129.3 (1.6)^b	-53.9	-44.7	-30.7
Black	C_AT_A	109.8 (3.6)^a	139.3 (1.3)^a	193.7 (0.4)^a	-	-	-
	C_AT_E	48.7 (0.8)^c	77.3 (1.0)^c	138.5 (2.0)^b	-55.6	-44.5	-28.5
	C_ET_A	79.2 (1.7)^b	115.3 (1.3)^b	195.7 (0.4)^a	-27.9	-17.2	1.0
	C_ET_E	47.0 (0.9)^c	77.0 (1.0)^c	134.5 (2.8)^b	-57.2	-44.7	-30.6

The effect of C_E and T_E on the rate of vegetative growth measured as change in plant height reflected the differences in the rate of phenological development; however, the strength of their effects changed with time (repeated ANOVA, time x CO_2 x temperature interaction, *P*<0.0001), with the greatest differences between the treatments observed around 100 days after planting (DAP) in both soils (Fig. 1a, b). T_E accelerated the rate of vegetative growth at C_A and C_E, with plants reaching their maximum height at 109 DAP in both soils (Fig. 1a, b). Plants grown in C_ET_A reached their maximum height at 142 DAP compared to 161 DAP for plants grown in C_AT_A. C_E and T_E, either singly or in combination, increased the final height of the plants by 22% on average for both soils (CO_2 x temperature interaction, *P*=0.0002, Table 2). The rate of vegetative growth also reflected the difference in the rate of development between the two soils and was faster in the grey than black vertosol soils throughout the experiment (repeated ANOVA, *P*=0.01).

The effect of C_E and T_E on node development interacted with time in both soils (repeated ANOVA, time x CO_2 x temperature interaction, *P*<0.0001), with the greatest differences between the treatments being observed around 100 DAP (Fig. 1c, d). T_E increased the rate of

node development at C_A and C_E, in both soils and C_E also increased the rate of node development, but only at T_A. Although C_E and T_E increased the rate of node development, both reduced the total number of nodes produced per plant (P=0.001, P=0.0002 respectively, Table 1). The final node number was higher in black vertosol compared to grey vertosol (P=0.001); however, the effect of C_E and T_E on final node number did not differ between the two soils. Plants grown in the grey and black vertosol also differed in the rate of node development, although the nature of this difference changed with time (repeated ANOVA, time x soil interaction, P=0.0004). Node development was faster for plants grown on grey vertosol compared to those in black vertosol at early stages of growth; however, this difference disappeared by the flowering stage and eventually reversed by harvest (Fig. 1c, d).

Table 3 Results of analysis of variance (ANOVA) showing the effect of soil, CO_2, temperature and their interactions on phenology, vegetative growth, yield components, physiology, leaf nutrients and soil nutrients. Values are probability with significant results (P<0.05) shown in bold and marginally significant results (P<0.1) in italics.

			CO_2	Temp	Soil	CO_2 x Temp	CO_2 x Soil	Temp x Soil	CO_2 x Temp x Soil
Phenology	1st square		**<0.0001**	**<0.0001**	**0.0002**	**<0.0001**	0.19	**0.03**	0.28
	1st flower		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.80	*0.07*	0.27
	1st open boll		0.41	**<0.0001**	**0.001**	**0.001**	0.95	0.41	0.26
Vegetative growth	Final height		0.32	**<0.0001**	0.35	**0.0002**	0.84	0.37	0.66
	Final node number		**0.001**	**0.0002**	**0.001**	0.36	0.82	0.50	0.12
Yield components	Seed cotton		**0.001**	**<0.0001**	*0.07*	0.88	0.41	0.13	0.88
	Boll size		*0.08*	**<0.0001**	**0.0002**	*0.06*	**0.05**	**0.01**	0.99
	Boll number		**0.0003**	**0.001**	0.12	**<0.0001**	**0.001**	0.81	**0.03**
Physiology	Photosynthetic rate		**<0.0001**	0.001	0.33	**<0.0001**	0.90	0.98	0.24
	Stomatal conductance		**0.03**	**<0.0001**	**0.05**	**<0.0001**	0.68	0.54	0.29
	iWUE		**<0.0001**	**<0.0001**	0.18	**<0.0001**	0.33	*0.08*	*0.09*
Leaf nutrients	C	Flowering	**<0.0001**	**<0.0001**	0.30	**0.001**	0.20	**0.05**	*0.06*
		Harvest	**<0.0001**	**<0.0001**	0.22	**<0.0001**	0.57	**0.01**	**0.02**
	N	Flowering	**<0.0001**	**0.001**	*0.06*	**0.004**	0.34	0.49	0.53
		Harvest	**<0.0001**	**<0.0001**	**0.001**	**<0.0001**	0.96	**0.02**	0.58
	C:N	Flowering	**<0.0001**	**<0.0001**	0.10	**0.02**	0.63	0.22	0.96
		Harvest	**<0.0001**	**<0.0001**	**0.003**	**<0.0001**	0.47	0.22	0.60
Soil nutrients	NO_3^-	Early flowering	0.85	**0.001**	**0.001**	*0.08*	0.57	0.41	0.55
		Late flowering	0.65	**<0.0001**	**0.03**	0.98	0.10	0.50	0.90
		Harvest	**0.01**	**0.01**	0.19	**<0.0001**	0.63	0.74	0.11
	PO_4^-	Early flowering	0.78	**0.004**	**<0.0001**	0.10	0.63	0.49	0.42
		Late flowering	**0.003**	0.12	**<0.0001**	0.30	0.73	0.77	0.29
		Harvest	0.38	0.41	**<0.0001**	**<0.0001**	0.81	**0.003**	0.47

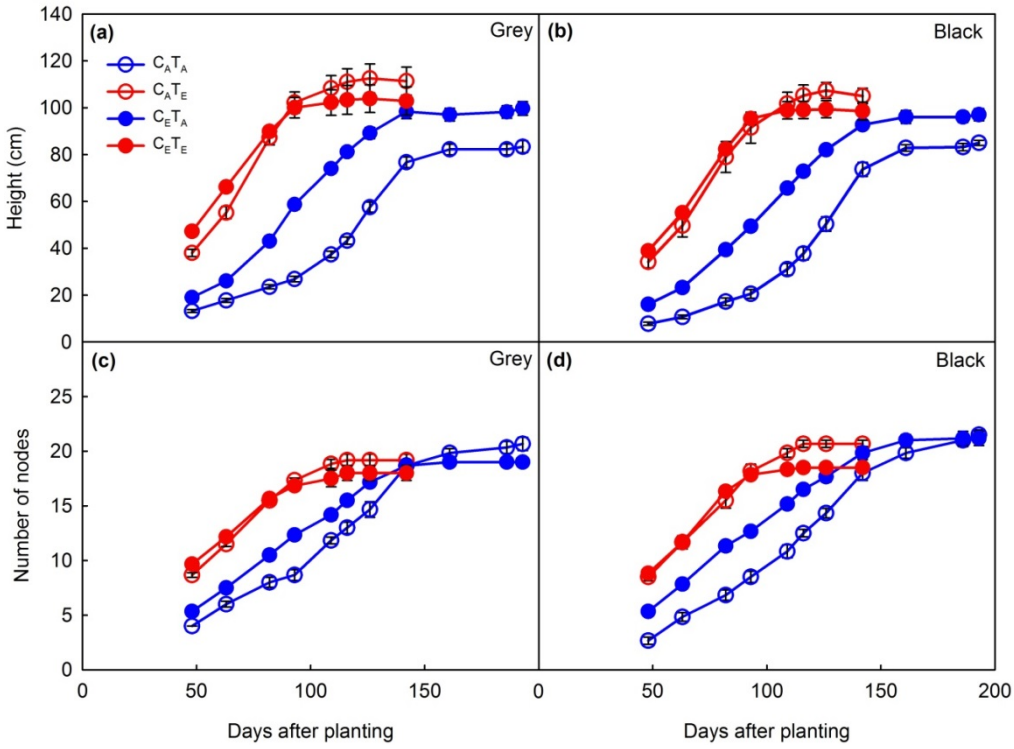


Fig. 1 Climate change impact on plant height (a, b) and number of nodes (c, d) of cotton plants grown on grey vertosol and black vertosol under the four climate change treatments (n=6). Plants were harvested at 142 and 196 days after planting (DAP) for T_E and T_A respectively.

Leaf physiology

There was a strong positive effect of C_E on net photosynthetic rate in both soils (P<0.0001, Table 3, Fig. 2a); however, the magnitude of this effect depended on the temperature treatment (CO_2 x temperature interaction, P<0.0001), as the positive effect of C_E was significantly greater at T_A compared to T_E, in both soils. There were no significant differences in net photosynthetic rate between plants grown on grey and black vertosol (Table 3). Stomatal conductance was significantly increased by T_E (P<0.0001, Fig. 2b), particularly at C_A (CO_2 x temperature interaction, P<0.0001), which more than doubled stomatal conductance in plants grown at C_AT_E when compared to plants in C_AT_A in both soils. There was also a significant difference in stomatal conductance between the two soils (Table 3), where plants grown on grey vertosol had a higher stomatal conductance than plants grown on black vertosol. The instantaneous water use efficiency (iWUE) calculated from the net photosynthetic rate over transpiration rate was strongly increased by C_E in both soils (P<0.0001) particularly at T_A (CO_2 x temperature interaction, P<0.0001), with the effect of C_E and T_E being antagonistic (Fig. 2c). C_ET_A increased the iWUE by 90% and 79% in the

grey and black vertosol, respectively, compared to C_AT_A, while C_ET_E increased iWUE by 33% and 24 % in the grey and black vertosol, respectively, compared to C_AT_A. There were no differences in iWUE between the two soils (Table 2).

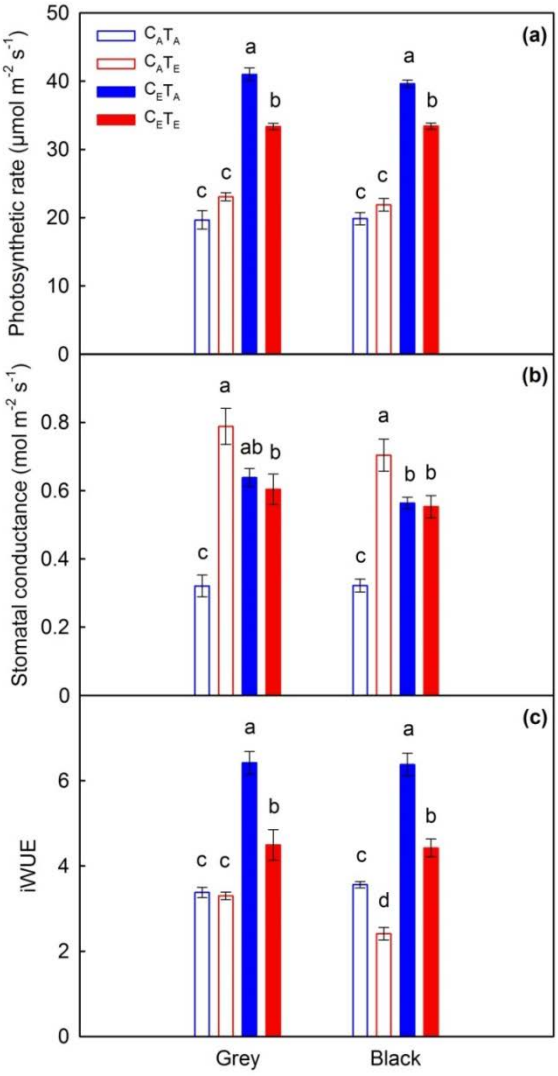


Fig. 2 Climate change impact on leaf photosynthetic rate (a), stomatal conductance (b) and instantaneous water use efficiency (c) of cotton plants grown on grey vertosol and black vertosol under the four climate change treatments (n=6). Measurements were taken over 3 days at early flowering stage of cotton growth. Different letters indicate significant differences (P<0.05) between the treatment within each soil.

Cotton yield and yield components

There were significant positive effects of T_E and C_E on seed cotton yield (P<0.0001, P=0.001 respectively, Table 3, Fig. 3a), which did not interact with each other nor with soils. The highest yield was observed at C_ET_E with more than a 2-fold increase compared to C_AT_A, followed by C_AT_E and C_ET_A (Fig. 3a). There was only a marginal difference in seed cotton

yield between the two soils (P=0.07) where plants grown on grey vertosol produced more seed cotton yield than that of black vertosol. The total number of bolls produced per plant was significantly influenced by the interaction between CO_2, temperature and soil (CO_2 x temperature x soil interaction, P=0.03), giving no consistent pattern in the effect of climate change treatments (Fig. 3b). In both soils, however, plants grown at C_AT_A produced the least number of bolls. The average size of each boll differed between CO_2 and temperature treatments; however, each treatment also interacted with soil (CO_2 x soil interaction, P<0.05, temperature x soil interaction, P=0.01), largely driven by the lack of C_E effect and greater effect of T_A on boll size in black compared to grey vertosol (Fig. 3c).

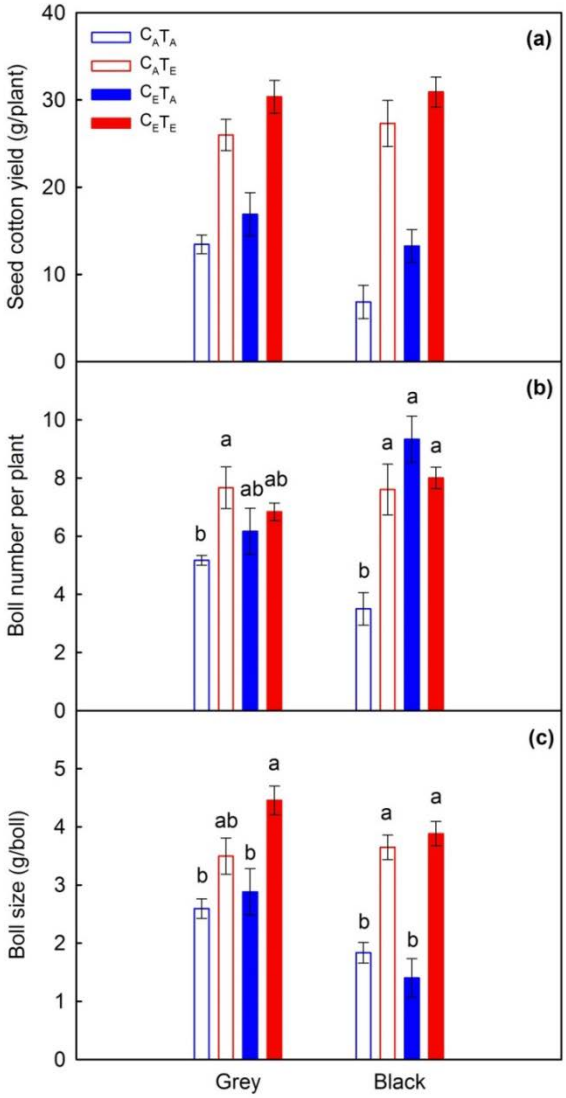


Fig. 3 Climate change impact on cotton yields (a), the number of bolls per plant (b) and boll size (c) of cotton plants grown on grey vertosol and black vertosol under the four climate change treatments (n=6). Plants were harvested at 142 and 192 days after planting for T_E and T_A respectively. Different letters indicate significant differences (P<0.05) between the treatment within each soil (shown only when CO_2 x temperature interactions were significant).

Leaf nutrients

Leaf total C and N concentrations and C:N ratios differed substantially between developmental stages (Table 3, 4). At flowering, C_E significantly increased C concentrations, but only at T_A (CO_2 x temperature interaction, P=0.001) in both soils. C_E and T_E alone or in combination increased leaf N concentration in both soils (CO_2 x temperature interaction, P=0.004) by 16% compared with C_AT_A. This increase was largely reflected in leaf C:N ratios with plants grown at C_AT_E, C_ET_A and C_ET_E having lower C:N ratios than plants grown at C_AT_A (CO_2 x temperature interaction, P=0.02). There were no significant differences in leaf C and N concentrations and C:N ratios between the plants grown on the two soils, although T_E decreased leaf C concentrations only in black vertosol (temperature x soil interaction, P<0.05). At harvest, leaf C contents were higher than during flowering but generally showed a similar pattern to that of flowering, although there was a significant CO_2 x temperature x soil interaction (P<0.0001). Leaf N concentration on the other hand showed a substantial decrease at harvest (by 43% on average). C_E and T_E alone or in combination reduced leaf N concentration at harvest in both soils (CO_2 x temperature interaction, P<0.0001). Leaf C:N ratios reflected this change in the effect of C_E and T_E, with plants grown at C_AT_E, C_ET_A and C_ET_E having higher C:N ratios that that of C_AT_A (CO_2 x temperature interaction, P<0.0001). There were significant differences in leaf N concentrations and C:N ratios between the two soils (P=0.001 and P=0.003, respectively).

Table 4 Climate change impact on leaf C, N and C:N ratios of cotton plants grown on grey vertosol and black vertosol under the four climate change treatments. Leaf samples were collected at flowering and at harvest. Values are means with standard errors in parenthesis. Different letters within columns indicate significant differences (P<0.05) between the treatment within each soil (shown only when CO_2 x temperature interactions were significant).

		C (%)		N (%)		C:N	
Soil	Treatment	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest
Grey	C_AT_A	39.92 (0.28)^b	41.75 (0.32)^b	3.96 (0.08)^b	3.41 (0.08)^a	10.09 (0.16)^a	12.26 (0.28)^b
	C_AT_E	40.35 (0.19)^b	42.30 (0.26)^b	4.37 (0.09)^a	2.48 (0.10)^b	9.25 (0.22)^b	17.19 (0.72)^a
	C_ET_A	41.39 (0.13)^a	44.16 (0.21)^a	4.46 (0.11)^a	2.43 (0.07)^b	9.31 (0.22)^b	18.23 (0.59)^a
	C_ET_E	39.94 (0.18)^b	41.69 (0.21)^b	4.41 (0.06)^a	2.43 (0.12)^b	9.07 (0.16)^b	17.42 (1.12)^a
Black	C_AT_A	40.54 (0.06)^b	42.43 (0.40)^b	4.02 (0.03)^b	4.07 (0.20)^a	10.09 (0.08)^a	10.55 (0.47)^b
	C_AT_E	39.65 (0.25)^b	40.91 (0.27)^c	4.44 (0.10)^a	2.54 (0.12)^b	8.95 (0.17)^b	16.24 (0.68)^a
	C_ET_A	41.81 (0.43)^a	44.08 (0.18)^a	4.56 (0.12)^a	2.97 (0.19)^b	9.20 (0.21)^b	15.16 (1.04)^a
	C_ET_E	40.31 (0.17)^b	41.51 (0.29)^{bc}	4.67 (0.06)^a	2.59 (0.15)^b	8.64 (0.15)^b	16.25 (0.82)^a

Soil nutrients

Soil nitrate concentrations declined gradually as plants matured (Table 5). The rate of soil nitrate depletion was greater at T_E in both soils (P=0.0001). Soil nitrate concentrations were 47% and 128% higher under T_E than T_A for grey and black vertosol at early and late flowering, respectively. By harvest, little nitrate remained in the soil, and nitrate concentrations were particularly low under C_ET_A for both soils (CO_2 x temperature interaction, P<0.0001). Soil ammonium concentrations were less than 5 mg N kg soil^{-1} for both soils and did not change throughout the experiment (data not shown). Soil phosphate concentrations differed significantly between the soils (P<0.0001) and fluctuated throughout the experiment, with no consistent effect from C_E or T_E. Soil inorganic N and P concentrations differed significantly between the two soils; however, responses to climate change treatments were generally similar between soils.

Table 5 Climate change impact on soil nitrate and phosphate concentrations at early flowering, late flowering and harvest. Values are means and standard errors in parenthesis. Different letters within columns indicate significant differences (P<0.05) between the treatment within each soil (shown only when CO_2 x temperature interactions were significant).

		Soil nitrate (mg N/kg soil dwt)			Soil phosphate (mg P/kg soil dwt)		
Soil	Treatment	Early flower	Late flower	Harvest	Early flower	Late flower	Harvest
Grey	C_AT_A	49.4 (9.7)	25.4 (8.6)	4.1 (0.6)^{a}	139.5 (13.8)	130.9 (15.7)	133.3 (10.5)^{ab}
	C_AT_E	81.7 (10.6)	64.4 (11.0)	3.3 (0.4)^{a}	137.9 (9.9)	97.7 (9.3)	145.1 (8.8)^{a}
	C_ET_A	52.9 (11.9)	33.3 (8.8)	1.1 (0.1)^{b}	121.8 (2.9)	89.9 (14.7)	151.9 (6.8)^{a}
	C_ET_E	65.9 (4.7)	74.5 (5.0)	4.1 (0.3)^{a}	171.8 (21.1)	91.9 (3.8)	113.8 (3.3)^{b}
Black	C_AT_A	62.3 (11.8)	48.7 (4.8)	5.0 (0.9)^{ab}	56.5 (9.1)	56.6 (16.0)	50.2 (6.7)^{b}
	C_AT_E	117.7 (17.5)	99.7 (16.3)	2.9 (0.3)^{bc}	86.2 (19.8)	46.0 (13.1)	105.8 (12.4)^{a}
	C_ET_A	84.6 (10.0)	33.7 (7.1)	1.3 (0.2)^{c}	45.3 (10.4)	27.5 (8.6)	79.6 (5.8)^{ab}
	C_ET_E	101.4 (10.9)	83.1 (16.2)	5.2 (0.6)^{a}	93.1 (20.0)	16.5 (4.1)	69.0 (5.4)^{b}

Relationships between yields, vegetative growth, plant physiology and leaf and soil nutrient status

Correlation analyses were performed to examine the relationship between the seed cotton yield and yield components, vegetative growth, leaf physiology and leaf and soil nutrient status (Table S1). Seed cotton yield was correlated with boll size (r=0.84, P<0.0001, Fig. 4a) and also correlated with vegetative growth rate (calculated as the height at first flowering divided by the number of days to reach the stage of first flowering), with T_E driving this

relationship (r=0.86, P<0.0001, Fig. 4b). There were also significant relationships between seed cotton yield and soil nitrate concentrations at late flowering (r=0.51, P=0.0002, Fig. 4c) and leaf N concentrations at harvest, (r=–0.69, P<0.0001, Fig. 4d), with T_E also driving these relationships. We further examined these relationships using partial correlation analysis while controlling for the effect of climate change treatment and found that significant relationships were maintained for boll size and leaf N concentrations at harvest (Table S1). However, this was not the case for vegetative growth rate and soil nitrate concentrations at late flowering, suggesting that climate change treatments were the underlying drivers of these relationships (Table S1).

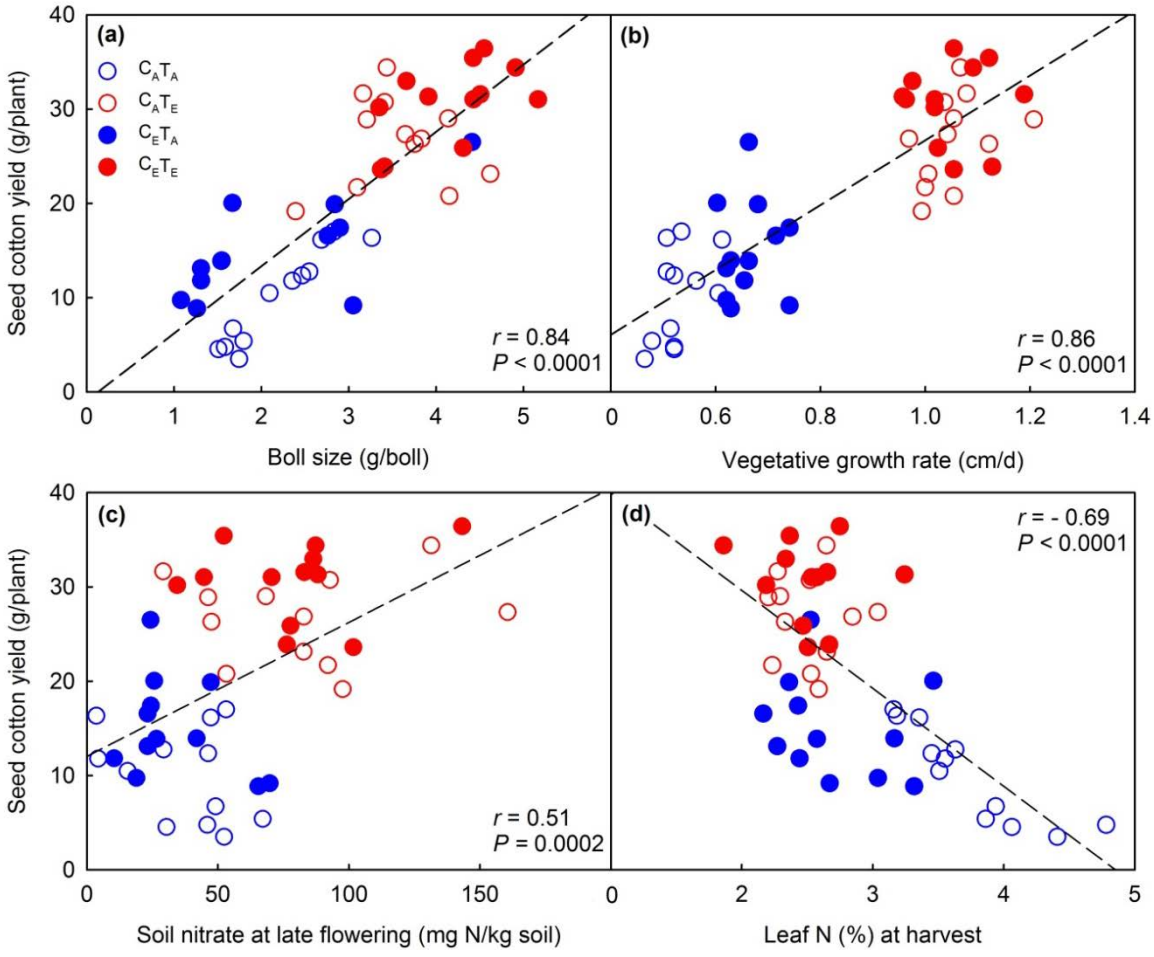


Fig. 4 Relationship between seed cotton yield and boll size (a), vegetative growth rate (b), soil nitrate concentrations at late flowering (c) and leaf N concentrations at harvest (d) of cotton plants grown on grey vertosol and black vertosol under the four climate change treatments.

Extreme weather impacts under current and future CO_2 and temperature regimes

The impact of flooding at early flowering on leaf physiology in the current and future CO_2 and temperature regimes

In the current CO_2 and temperature regime (C_AT_A), flooding reduced photosynthetic rate in both soils (*P*=0.03, Table 6), with 12% and 13% reduction in grey and black vertosol, respectively (Fig. 5). Flooding marginally interacted with soil (*P*=0.08) and reduced stomatal conductance by 21% in grey vertosol, while no difference was observed in black vertosol.

Table 6 Results of analysis of variance (ANOVA) showing the immediate impact of flooding and drought on photosynthetic rate (*A*), stomatal conductance (*gs*), leaf nitrogen and soil nitrate (flooding only) and their consequences on the post-flowering growth rate, seed cotton yield and yield components of cotton plants and residual soil nitrate (drought only) between the two soils under the current CO_2 and temperature regime (C_AT_A). Values are probability with significant results (*P*<0.05) shown in bold, marginally significant results (*P*<0.1) in italic and non-significant results (*P*>0.1) as n.s.

	Immediate impact				Consequences				
	A	*gs*	Leaf N	Soil nitrate	Post-flower growth rate	Seed cotton yield	Boll number	Boll size	Residual soil nitrate
Flooding treatment									
Flood	**0.03**	n.s.	**0.01**	**<0.0001**	**0.003**	n.s.	**0.01**	n.s.	-
Soil	n.s.	n.s.	n.s.	**0.03**	n.s.	**0.0001**	n.s.	**<0.0001**	-
Flood x Soil	n.s.	*0.08*	n.s.	**0.03**	n.s.	n.s.	**0.04**	*0.08*	-
Drought treatment									
Drought	**<0.0001**	**<0.0001**	**0.0002**	-	**0.0001**	**0.0003**	**0.01**	**0.002**	**<0.0001**
Soil	n.s.	n.s.	n.s.	-	n.s.	**0.001**	**0.04**	**0.0005**	n.s.
Drought x Soil	n.s.	n.s.	n.s.	-	*0.08*	n.s.	n.s.	n.s.	n.s.

These physiological responses to flooding were altered in future CO_2 and temperature regimes, particularly at C_E (Fig. 5, Table 7). The effect of flooding on photosynthetic rate interacted with CO_2 in both soils (flood x CO_2 interaction, *P*=0.01) in that the relative reductions in photosynthetic rate between well-watered and flooded plants were greater at C_E (10% and 15% in grey and black vertosol, respectively) compared to C_A (8% and 10% respectively). The impact of flooding on photosynthetic rate was marginally reduced at T_E (flood x temperature interaction, *P*=0.08), as the relative reductions in photosynthetic rate between well-watered and flooded plants were lower at T_E (5% and 10% in grey and black vertosol, respectively) compared to T_A (13% and 16%, respectively).

The impact of flooding on stomatal conductance interacted with CO_2 (flood x CO_2 interaction, *P*=0.02). The relative reductions in stomatal conductance between well-watered and flooded plants were greater at C_E (39% and 37% in grey and black vertosol, respectively) compared to C_A (20% and 7%, respectively). Flooding impact was also marginally influenced by temperature (flood x temperature interaction, *P*=0.07); the relative reduction in stomatal conductance by flooding was greater at T_E (35% and 31% in grey and black vertosol respectively) than at T_A (24% and 14%, respectively).

CO_2 and temperature exhibited significant interactive effects on photosynthetic rate (CO_2 x temperature interaction, *P*<0.0001, Table 7, Fig. S2) and stomatal conductance (CO_2 x temperature interaction, *P*<0.0001), and also between soils (soil x CO_2 x temperature interaction, *P*=0.04); however, CO_2 and temperature did not interact with flooding.

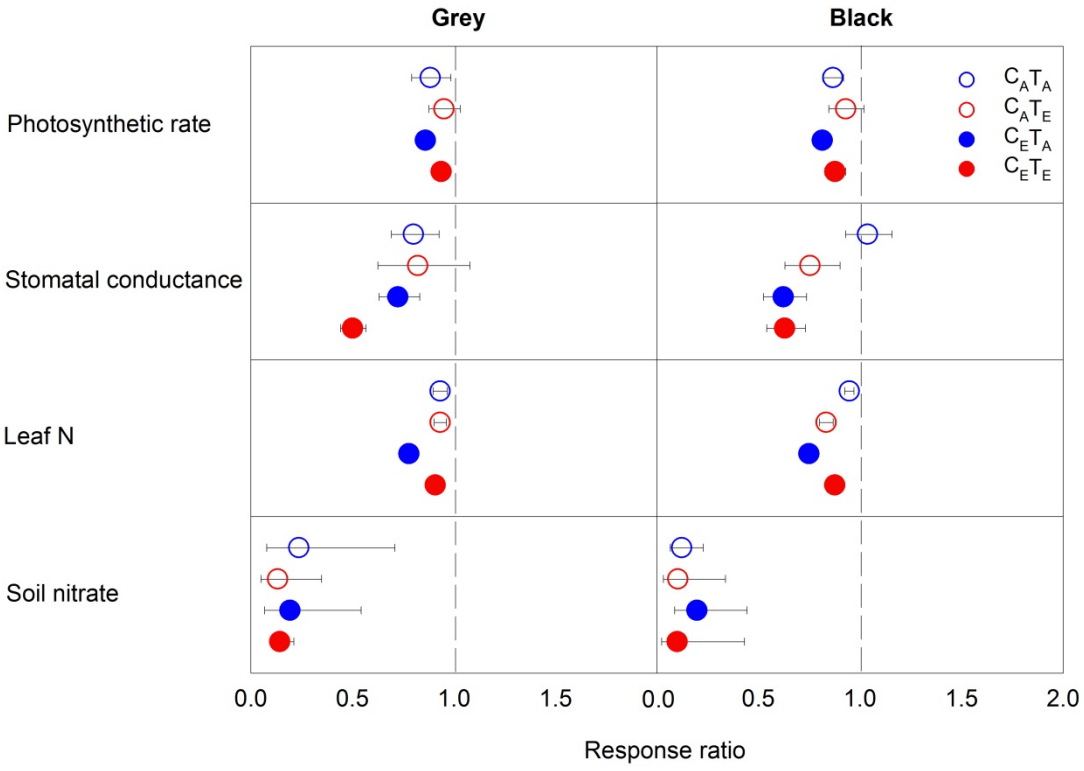


Fig. 5 The effect of flooding on leaf physiology at the end of flooding treatment and leaf and soil N status at 7 days after the end of flooding period from grey vertosol and black vertosol planted with cotton under the four climate treatments. The effect is expressed in response ratios calculated from comparisons between well-watered plants and flooded plants. Values are mean response ratios with ± 95% confidence intervals (CI). Values >1 and <1 indicate positive and negative responses, respectively. Effects are considered significant when CI does not overlap 1.

Table 7 Results of analysis of variance (ANOVA) showing the immediate impact of flooding and its interactions with CO_2, temperature and soil on leaf physiology, leaf nitrogen and soil nitrate and their consequences on the post-flooding growth rate, seed cotton yield and yield components of cotton plants. Values are probability with significant results ($P<0.05$) shown in bold, marginally significant results ($P<0.1$) in italics and non-significant results ($P\geq0.1$) as n.s.

	Immediate impact				Consequences			
Factors	Photosynthetic rate	Stomatal conductance	Leaf N	Soil nitrate	Post-flood growth rate	Seed cotton yield	Boll number	Boll size
Flooding effects								
Flood	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.01**	**0.0002**	n.s.
Flood x Soil	n.s.	n.s.	*0.07*	**0.03**	n.s.	n.s.	n.s.	n.s.
Flood x CO_2	**0.01**	**0.02**	**<0.0001**	n.s.	**0.01**	*0.07*	*0.09*	n.s.
Flood x Temp	*0.08*	*0.07*	n.s.	**0.003**	**0.0005**	**0.01**	**<0.0001**	n.s.
Flood x Soil x CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	**0.03**	**0.01**	n.s.
Flood x Soil x Temp	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*0.10*	n.s.
Flood x CO_2 x Temp	n.s.	n.s.	**<0.0001**	n.s.	**0.01**	n.s.	**0.02**	*0.07*
Flood x Soil x CO_2 x Temp	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Non-flooding effects								
Soil	**0.002**	**0.02**	n.s.	*0.05*	n.s.	**<0.0001**	**0.04**	**<0.0001**
CO_2	**<0.0001**	n.s.	**0.01**	n.s.	n.s.	**0.01**	**0.0002**	n.s.
Temp	**0.01**	**<0.0001**	**<0.0001**	**<0.0001**	n.s.	**<0.0001**	n.s.	**<0.0001**
Soil x CO_2	n.s.	n.s.	n.s.	**0.02**	n.s.	n.s.	**0.01**	**0.04**
Soil x Temp	n.s.	n.s.	n.s.	n.s.	n.s.	**<0.0001**	**0.05**	**<0.0001**
CO_2 x Temp	**<0.0001**	**<0.0001**	n.s.	n.s.	**<0.0001**	n.s.	**0.0001**	n.s.
Soil x CO_2 x Temp	n.s.	**0.04**	**0.04**	n.s.	n.s.	n.s.	**0.004**	n.s.

The impact of flooding at early flowering stage on leaf and soil nutrients in current and future CO_2 and temperature regimes

In the current CO_2 and temperature regime (C_AT_A), flooding significantly decreased leaf N concentrations of cotton plants in both soils (P=0.01, Fig. 5, Table 6), with 7% and 5% reduction in leaf N in grey and black vertosol, respectively, when compared with well-watered plants. The strongest negative impact of flooding was observed in its effect on soil nitrate concentrations (P<0.0001), which differed between the two soils (flood x soil interaction, P=0.03, Fig. 5, Table 6). While soil nitrate concentrations showed a small reduction in well-watered plants due to plant uptake, the reduction was far greater for soils from flooded plants (Fig. S3, Table S2). The reduction in soil nitrate concentrations by flooding was greater in black vertosol (88%) than grey vertosol (76%) at C_AT_A.

In the future CO_2 and temperature regimes, flooding impact on leaf N strongly interacted with CO_2 (flood x CO_2 interaction, P<0.0001, Table 7, Fig. 5); however, this also interacted with temperature (flood x CO_2 x temperature interaction, P<0.0001) in both soils. This was largely driven by the stronger reduction in leaf N by flooding at C_ET_A (22% and 25% reduction in grey and black vertosol, respectively) compared to C_ET_E (9% and 13% respectively). Flooding impact on soil nitrate concentration was significant in the future CO_2 and temperature regimes (P<0.0001, Fig. 5, Fig. S3, Table 7). Flooding impact on soil nitrate concentration was not altered by CO_2; however, it interacted with temperature (flood x temperature interaction, P=0.003, Table 7, Table S2) in both soils. The reduction in soil nitrate concentration by flooding was greater at T_E (86% and 90% in grey and black vertosol, respectively) compared to T_A (79% and 84%, respectively). Leaf N concentrations were also influenced by soil x CO_2 x temperature interaction (P=0.04, Table 7, Fig. S2).

The consequences of flooding on cotton yield and yield components in the current and future CO_2 and temperature regimes

In the current CO_2 and temperature regime (C_AT_A), the rate of vegetative growth following the 6-day flooding event was significantly reduced in both soils (P=0.003, Fig. 6, Table 6), with 48% and 77% reduction in post-flooding vegetative growth rate in grey and black vertosol, respectively. However, flooding had no effect on seed cotton yield in either soil (Fig. 6, Table 6). Flooding impact was evident in boll number and boll size, but only in black vertosol (flood x soil interaction, P=0.04, P=0.08 respectively); flooding increased the

number of bolls by 81%, but reduced boll size by 24% in black vertosol. Flooding had no impact on the number and size of bolls in grey vertosol.

Flooding impact on vegetative growth, seed cotton yield and yield components were significantly altered by future CO_2 and temperature regimes (Fig. 6, Table 7). Flooding impact on vegetative growth rate was altered by CO_2 (flood x CO_2 interaction, P=0.01), temperature (flood x temperature interaction, P=0.0005) and their interaction (flood x CO_2 x temperature interaction, P=0.01) in both soils. The impact of flooding on vegetative growth rate was greater at T_E than T_A; however, the effect on CO_2 on flooding impact differed between the temperature treatments, with a greater reduction observed at C_ET_E than C_ET_A in both soils (Fig. 6).

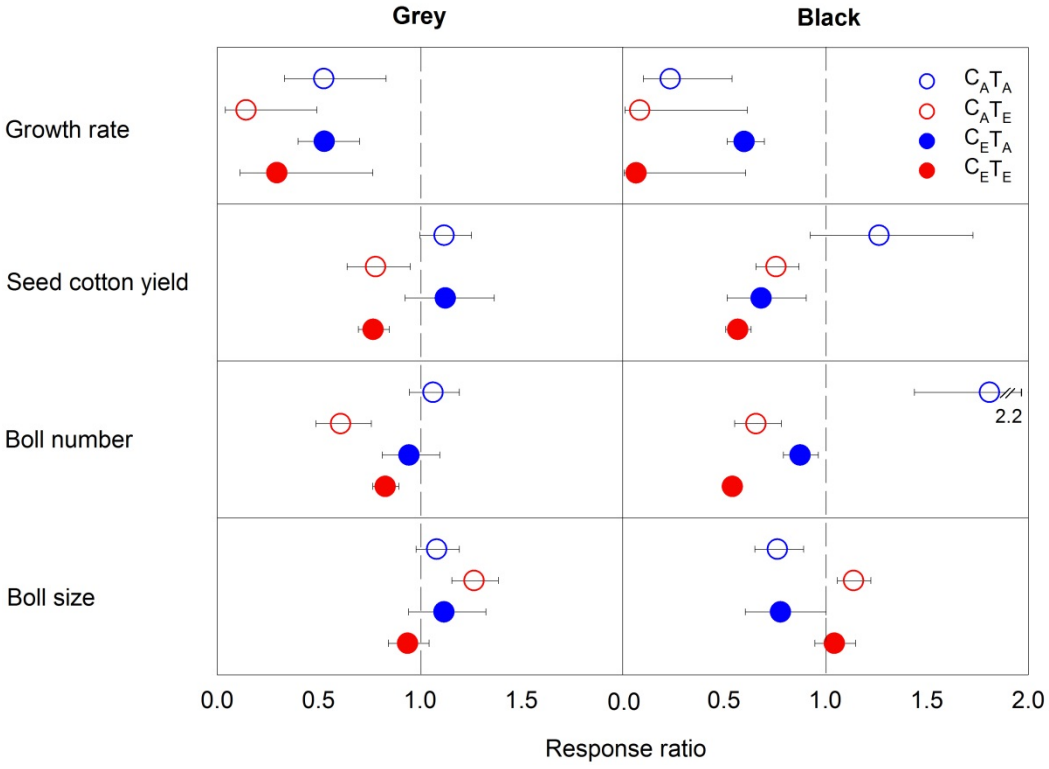


Fig. 6 The impact of flooding on vegetative growth rate, seed cotton yield and yield components of cotton plants grown on grey vertosol and black vertosol under the four climate treatments. Response ratios were calculated from comparisons between well-watered plants and flooded plants. Values are mean response ratios with ± 95% confidence intervals (CI). Values >1 and <1 indicate positive and negative responses, respectively. Effects are considered significant when CI does not overlap 1.

Contrary to its lack of effect on seed cotton yield at the current CO_2 and temperature regime, flooding had a significant impact on seed cotton yield in future CO_2 and temperature regimes (Fig. 6, Table 7). Flooding interacted marginally with CO_2 (flood x CO_2 interaction, P=0.07), but significantly with CO_2 and soil (flood x CO_2 x soil interaction, P=0.03); flooding reduced seed cotton yield by 37% at C_E in black vertosol, while C_E had no impact in grey verosol. Flooding impact was also greater at T_E (flood x temperature interaction, P=0.01) in both soils, with the relative yield reduction of 23% and 34% by flooding at T_E in grey and black vertosol, respectively. Flooding impact on boll number was altered by CO_2 (flood x CO_2 interaction, P=0.09), temperature (flood x temperature interaction, P<0.0001) and their interaction (flood x CO_2 x temperature interaction, P=0.02). Although the magnitude of its effect differed between the CO_2 treatments, flooding at T_E significantly reduced boll number by 28% in grey vertosol and 40% in black vertosol. Flooding impact on boll size was negligible (Fig. 6, Table 7), with only marginal differences between well-watered and flooded plants observed at C_AT_E in which flooding increased boll size by 27% in grey vertosol and 14% in black vertosol (flood x CO_2 x temperature interaction, P=0.07).

While flooding had a significant impact on vegetative growth and reproductive growth, the positive effect of T_E was evident for both well-watered and flooded plants (Table 7, Fig. S4). Seed cotton yield and boll size were significantly increased by T_E particularly in black vertosol (soil x temperature interaction, P<0.0001).

Relationships between immediate flooding responses and yield responses

Correlation analyses were conducted to examine whether immediate physiological and soil response to flooding correlated with vegetative growth and yield response. Response ratio of post-flooding vegetative growth did not correlate with that of stomatal conductance to flooding (Fig. 7a); however, there was a very strong positive correlation between the impact of post-flooding vegetative growth rate and soil nitrate concentrations (Fig. 7b, r=0.93, P=0.001). Response of seed cotton yield to flooding was positively correlated with stomatal conductance (Fig. 7c, r=0.71, P=0.05). Unlike its effect on vegetative growth, flooding impact on soil nitrate did not correlate with seed cotton yield (Fig. 7d). Changes in seed cotton yield under flooding were positively correlated with boll number (Fig. 7e, r=0.83, P=0.01), but not with boll size (Fig. 7f), indicating that reduced boll production induced by flooding at flowering contributed substantially to reduction in yield.

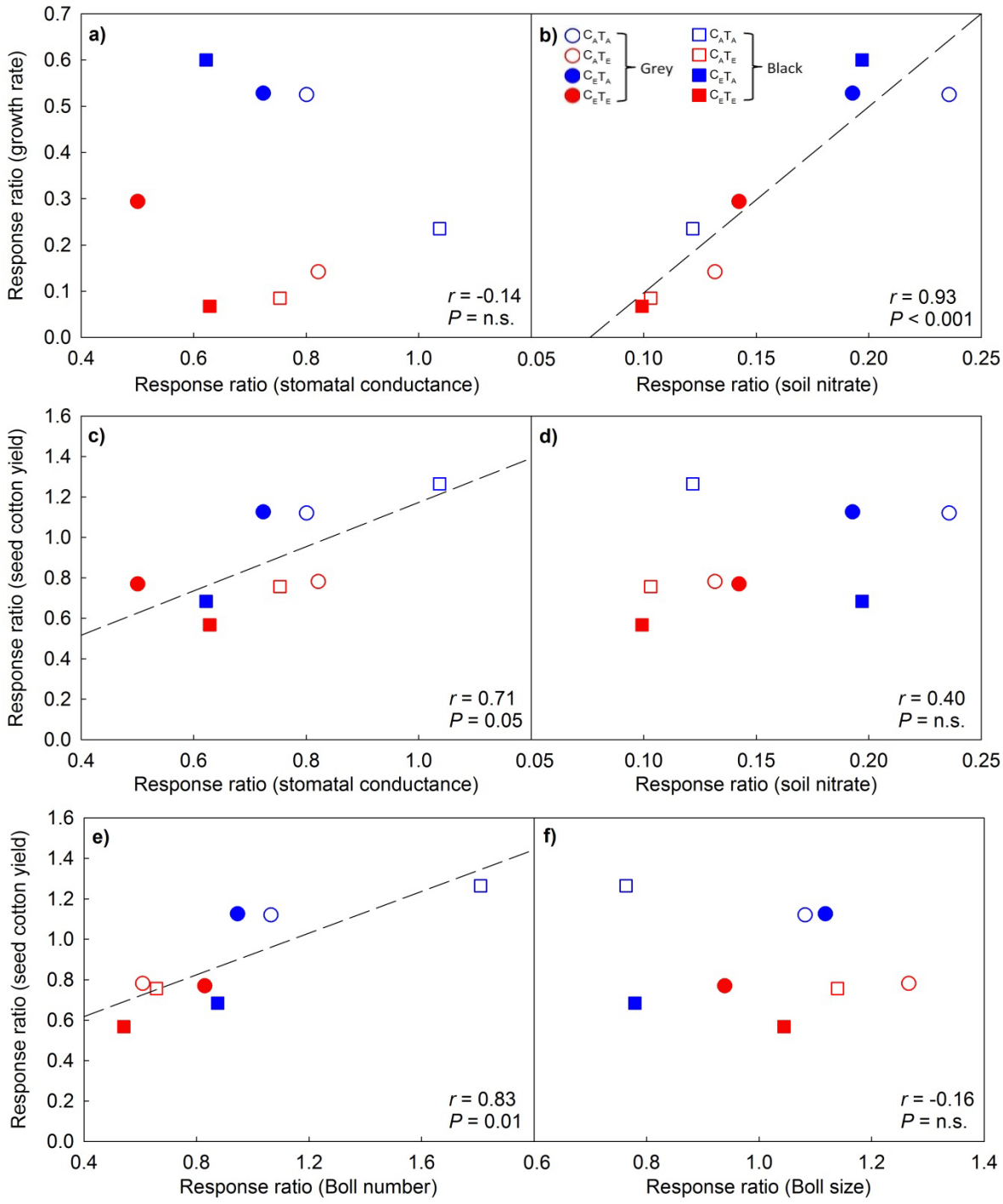


Fig. 7 Relationship between the flooding impact on post-flooding growth rate and stomatal conductance (a) and soil nitrate (b), seed cotton yield and stomatal conductance (c), soil nitrate (d), boll number (e) and boll size of cotton plants grown on grey vertosol and black vertosol under the four climate treatments.

The impact of drought at early flowering on leaf physiology and nutrients under current and future CO_2 and temperature regimes

In the current CO_2 and temperature regime (C_AT_A), the detrimental impact of drought on plant physiology and leaf nitrogen was evident by the sixth day of the drought treatment (Table 6, 8). Drought reduced photosynthetic rate (P<0.0001) by 81% and 94% in grey and black vertosol, respectively, when compared to well-watered control plants. Stomatal conductance was strongly reduced (P<0.0001) by 86% in both soils. Drought reduced leaf N (P=0.0002) by 10% and 18% in grey and black soils, respectively. The drought impact did not differ between the two soils at C_AT_A.

The impact of drought on leaf physiology and nutrient status was significant in the future CO_2 and temperature regimes (Table 8, 9). Drought impact on photosynthetic rate did not interact with CO_2, but interacted with temperature (drought x temperature interaction, P<0.0001) and with CO_2 and temperature (drought x CO_2 x temperature interaction, P<0.0001), which also differed between the two soils (drought x soil x CO_2 x temperature interaction, P<0.04). While the effects of CO_2 and temperature on photosynthetic response to drought varied in magnitude and between the two soils, both C_E and T_E reduced the negative impact of drought on photosynthetic rate in both soils. Drought impact on stomatal conductance was not altered by CO_2 or temperature; however, it was marginally affected by the interaction between CO_2, temperature and soil (drought x soil x CO_2 x temperature interaction, P<0.09), where both C_E and T_E ameliorated the negative impact of drought. Drought impact on leaf N was not altered by the main effect of CO_2 and temperature but by the interaction between CO_2 and temperature (drought x CO_2 x temperature interaction, P=0.04). In both soils, the negative impact of drought on leaf N was reduced at C_AT_E compared to C_AT_A (Table 8).

The leaf physiology of both well-watered and drought plants was significantly influenced by CO_2 and temperature treatment. Although there was a significant drought x soil x CO_2 x temperature interaction, the strong positive effect of CO_2 x temperature was evident in photosynthetic rate (P<0.0001) and stomatal conductance (P<0.0001, Fig. S2).

Table 8 The effect of drought on leaf physiology at six days into the treatment and N concentrations of cotton plants at 13 days into the treatment grown on grey vertosol and black vertosol under the four climate change treatments. The effect is expressed in response ratios calculated from comparisons between well-watered plants and drought plants. Values are mean response ratios. Asterisks indicate significant effect based on ± 95% confidence intervals.

Soil	Grey				Black			
Climate treatment	C_AT_A	C_AT_E	C_ET_A	C_ET_E	C_AT_A	C_AT_E	C_ET_A	C_ET_E
Photosynthetic rate	0.19 *	0.97	0.70 *	0.94	0.06 *	1.03	0.83 *	0.83 *
Stomatal conductance	0.14 *	0.39 *	0.31 *	0.72 *	0.14 *	0.61 *	0.61 *	0.57 *
Leaf N	0.90 *	0.96 *	0.92 *	0.93 *	0.82 *	0.96 *	0.91 *	0.88 *

The consequences of drought on cotton yield, yield components and soil nutrients in the current and future CO_2 and temperature regimes

The drought treatment initiated at early flowering led to a significant reduction in vegetative and reproductive growth compared to that of well-watered plants (Fig. 8). In the current CO_2 and temperature regime, drought significantly reduced vegetative growth (P=0.0001, Table 6) by 95% and 91% in grey and black vertosol, respectively, with its effect marginally different between the two soils (drought x soil interaction, P=0.08). Drought significantly reduced seed cotton yield (P=0.0003) in both soils, with 56% and 54% reduction in seed cotton yield in grey and black vertosol, respectively, compared to well-watered plants. Boll number and size were significantly reduced by drought (P=0.01, P=0.002 respectively) in both soils. Boll number was reduced by drought by 39% and 25% in grey and black vertosol, respectively, while boll size was reduced by 25% and 43%, respectively. Mortality of cotton plants at the end of the drought treatment led to a large amount of residual nitrate in the soil at harvest (P<0.0001, Fig. S4, Table 6) compared to that of well-watered plants. In well-watered plants, soil nitrate concentrations significantly decreased from 49.4 mg N g soil^{-1} at early flowering to 4.1 mg N g soil^{-1} at harvest in grey vertosol and from 62.3 mg N g soil^{-1} to 5.1 mg N g soil^{-1} in black vertosol. In drought plants, however, the amount of nitrate in the drought soil at harvest did not differ from the amount of nitrate at the pre-drought concentrations measured at early flowering (Fig. S5, Table S2).

In future CO_2 and temperature regimes, drought impact on vegetative growth was not altered by CO_2, but altered by temperature (drought x temperature interaction, P<0.0001, Fig. 8, Table 9) and by CO_2 and temperature interaction (drought x CO_2 x temperature interaction, P=0.005). Although their effects varied in magnitude, drought impact on vegetative growth was generally ameliorated by T_E and C_E in both soils. Drought impact on seed cotton yield

was altered by both CO_2 (drought x CO_2 interaction, $P<0.0001$) and temperature (drought x temperature interaction, $P<0.0001$), with its effect marginally greater in black than grey vertosol (drought x soil x temperature interaction, $P=0.07$). The reduction in seed cotton yield by drought was greater at C_E, with 88% reduction at C_E compared to 67% at C_A in both grey and black vertosol. The reduction in seed cotton yield in drought was greater at T_E, (82% and 84% in grey vertosol and black versotol, respectively) compared to T_A (73% and 71%, respectively). Drought impact on boll number was altered by CO_2 (drought x CO_2 interaction, $P=0.002$), temperature (drought x temperature interaction, $P=0.05$) and their interaction (drought x CO_2 x temperature interaction, $P=0.004$) with a marginal difference between the two soils (drought x soil x CO_2 x temperature interaction, $P=0.10$). The negative effect of drought on boll number was greater at T_E (54% reduction on average) than at T_A (44% reduction) in both soils and the largest reduction was observed at C_ET_A in black vertosol (66% reduction), compared to well-watered plants. Drought impact on boll size was altered by CO_2 (drought x CO_2 interaction, $P=0.002$) which differed between the two soils (drought x soil x CO_2 interaction, $P=0.01$), and temperature (drought x temperature interaction, $P<0.0001$). Drought impact on boll size was greater at T_E in both soils, while the impact of drought on boll size at C_ET_A was greater in grey vertosol (80% reduction) than black vertosol (62% reduction). The amount of residual nitrate in the soil was altered by CO_2 (drought x CO_2 interaction, $P=0.02$), and CO_2 and temperature interaction (drought x CO_2 x temperature interaction, $P=0.001$); however, this difference was due to the CO_2 and temperature effect on soil nitrate concentrations at pre-drought (Fig. S5, Table S2) and not at the end of the drought treatment because the eventual mortality of plants due to drought meant that changes in soil nitrate concentrations were minimal during the drought treatment.

Due to the strong impact of drought treatment on reproductive growth, the impacts of CO_2 and temperature were less pronounced by the end of the experiment (Fig. S4), except for residual soil nitrate which was strongly influenced by the positive effect of temperature on soil nitrate concentrations prior to the drought treatment.

Table 9 Results of analysis of variance (ANOVA) showing the immediate impact of drought and its interactions with CO_2, temperature and soil on leaf physiology and leaf nitrogen and their consequences on the post-drought growth rate, seed cotton yield, yield components of cotton plants and residual soil nitrate. Values are probability with significant results (P<0.05) shown in bold, marginally significant results (P<0.1) in italics and non-significant results (P>0.1) as n.s.

	Immediate impact			Consequences				
Parameters	Photosynthetic rate	Stomatal conductance	Leaf N	Post-drought growth rate	Seed cotton yield	Boll number	Boll size	Residual soil nitrate
Drought effects								
Drought	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Drought x Soil	n.s.	n.s.	n.s.	**0.01**	n.s.	n.s.	n.s.	n.s.
Drought x CO_2	n.s.	n.s.	n.s.	n.s.	**<0.0001**	**0.002**	**0.0002**	**0.02**
Drought x Temp	**<0.0001**	n.s.	n.s.	**<0.0001**	**<0.0001**	**0.05**	**<0.0001**	n.s.
Drought x Soil x CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	**0.01**	**0.01**	n.s.
Drought x Soil x Temp	n.s.	n.s.	n.s.	n.s.	*0.07*	n.s.	n.s.	n.s.
Drought x CO_2 x Temp	**<0.0001**	n.s.	**0.04**	**0.005**	n.s.	**0.004**	n.s.	**0.001**
Drought x Soil x CO_2 x Temp	**0.04**	*0.09*	n.s.	n.s.	n.s.	*0.10*	n.s.	n.s.
Non-drought effects								
Soil	**0.02**	**0.01**	n.s.	n.s.	**0.03**	n.s.	**<0.0001**	**0.03**
CO_2	**<0.0001**	n.s.	**<0.0001**	**<0.0001**	*0.08*	**0.003**	n.s.	**0.0003**
Temp	**<0.0001**	**<0.0001**	**<0.0001**	*0.06*	**<0.0001**	**0.0003**	**<0.0001**	**<0.0001**
Soil x CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	**0.001**	n.s.	n.s.
Soil x Temp	**0.02**	**0.005**	n.s.	n.s.	**0.01**	n.s.	**0.01**	
CO_2 x Temp	**<0.0001**	**<0.0001**	**<0.0001**	*0.08*	n.s.	**<0.0001**	**0.001**	**<0.0001**
Soil x CO_2 x Temp	n.s.	n.s.	n.s.	n.s.	n.s.	*0.07*	n.s.	n.s.

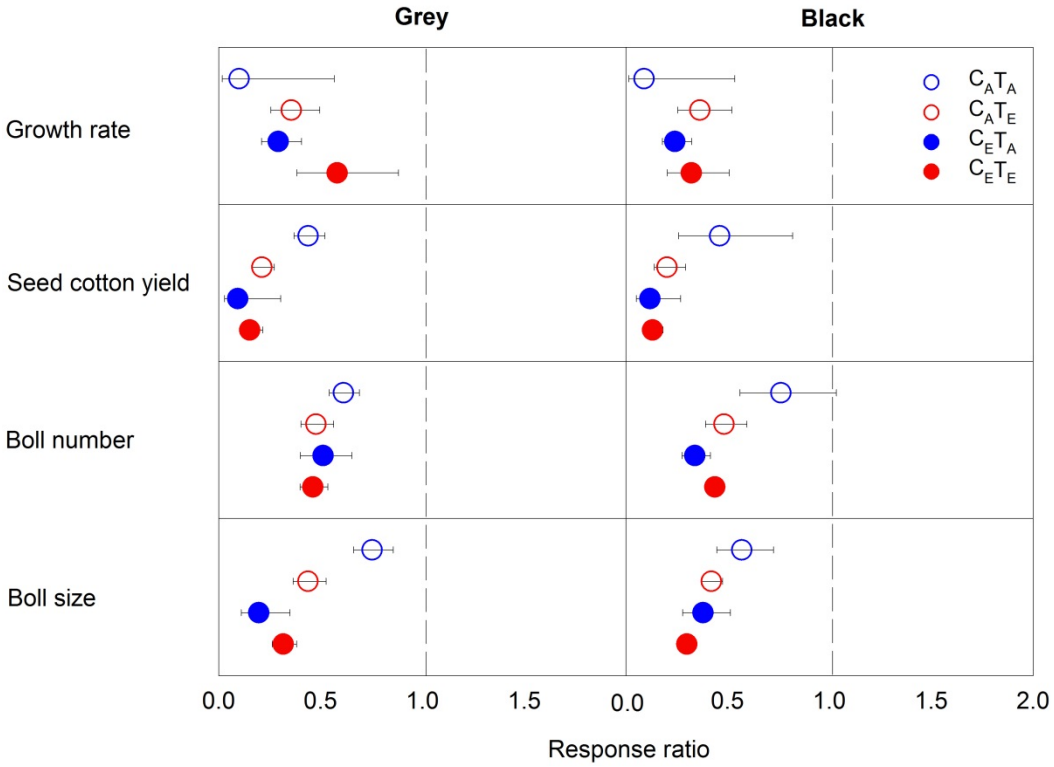


Fig. 8 The impact of drought on vegetative growth rate during the drought treatment, seed cotton yield and yield components of cotton plants grown on grey vertosol and black vertosol under the four climate treatments. Response ratios were calculated from comparisons between well-watered plants and drought plants. Values are mean response ratios with ± 95% confidence intervals (CI). Values >1 and <1 indicate positive and negative responses, respectively. Effects are considered significant when CI does not overlap 1.

Relationships between immediate drought responses and yield responses

Correlation analyses were conducted to examine whether immediate physiological responses to drought were correlated with vegetative growth and yield responses. The response ratio of seed cotton yield to drought was strongly and positively correlated with boll size (Fig. 9a, r=0.91, P=0.002) and boll number (Fig. 9b, r=0.87, P=0.01), reflecting the detrimental impact of drought on boll production, as well as the importance of pre-existing bolls in determining seed cotton yield. In addition, boll development was more strongly affected by drought at C_E compared to C_A relative to well-watered plants, and the two soils differed greatly in their response to drought at T_A.

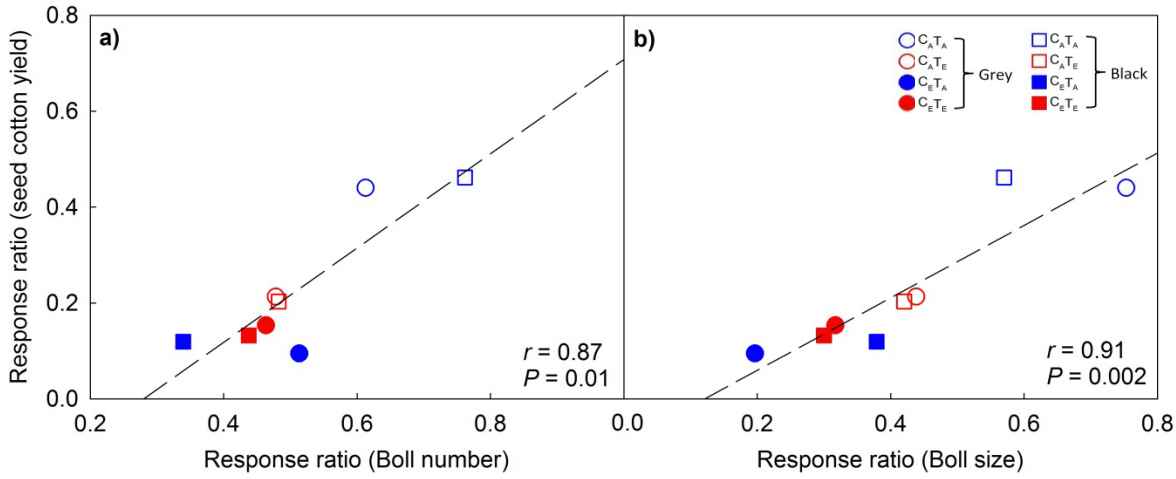


Fig. 9 Relationship between the drought impact on seed cotton yield and boll number (a) and boll size (b) of cotton plants grown on grey vertosol and black vertosol under the four climate treatments.

Discussion

Climate change impacts on cotton productivity under well-watered condition

Our study found that there were complex interactions between C_E, T_E and soil that influenced vegetative and reproductive growth differently as soil N availability and resource allocation changed throughout crop development (Fig. 10). T_E was the dominant factor in cotton productivity, accelerating the rate of plant development and vegetative growth while C_E had a strong impact on leaf physiology. T_E, and C_E to a lesser extent, increased seed cotton yield; however, there was an interaction between C_E and T_E on phenology, vegetative growth, leaf physiology and nutrient status that influenced the crop response during development. T_E impacted soil N availability, with greater soil N at T_E than T_A at flowering contributing to a greater seed cotton yield at T_E than T_A. Changes in soil N availability and leaf N status during crop development and their relationships with seed cotton yield indicate that the differences in soil N availability became an important determinant of reproductive growth and resource allocation within the plant (Fig. 10b). Plant responses to C_E and T_E were generally similar between the two soils during vegetative growth; however, differences in soil properties exerted a stronger impact on the response of yield components to C_E and T_E, suggesting that differences in their ability to continuously provide N impacted reproductive response to C_E and T_E. This study therefore provides some insights into the complex interactions between C_E and T_E on crop productivity through plant-soil interactions and highlights the importance of

incorporating soil responses in understanding the mechanisms by which C_E and T_E impact crop productivity (for further discussion, please see attached manuscripts).

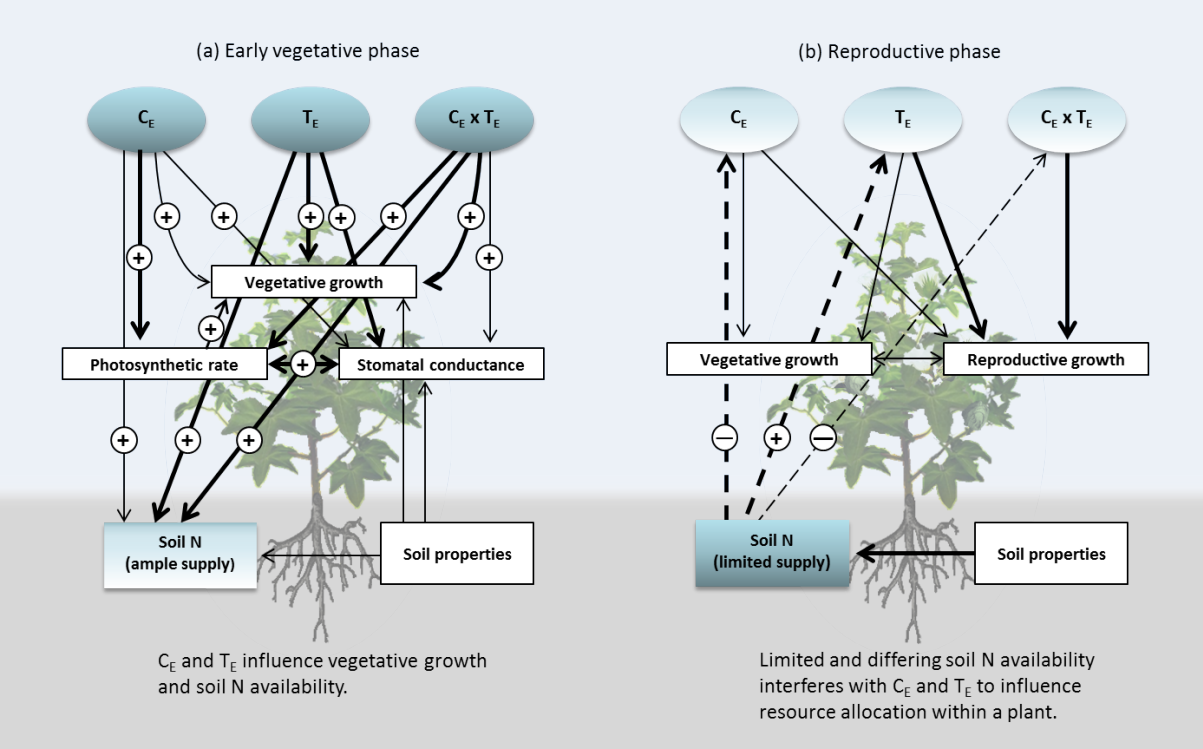


Fig. 10 Conceptual diagrams summarising the main and interactive effects of C_E, T_E and soil on cotton productivity. (a) Early vegetative growth is strongly influenced by T_E which accelerates the growth and development of cotton plants. C_E also increases the rate of growth via its strong positive effect on photosynthetic rate which accompanies increased stomatal conductance in the absence of water limitation. The combined C_E and T_E (i.e. C_ET_E) increases vegetative growth and photosynthetic rate and induces early transition to reproductive growth. T_E increases the amount of N remaining at flowering, while C_E only increases it marginally. Differences in soil properties affect stomatal conductance and vegetative growth, but have little effect on crop responses to C_E and T_E. (b) Response to C_E and T_E during reproductive phase is strongly influenced by the remaining soil N availability (indicated by dotted arrows) and resource allocation within the plant. Differences in soil properties start to exert a stronger influence over reproductive growth via their effect on soil N availability. The effect of C_E on reproductive growth is limited by soil N availability and resource allocation to vegetative growth, while the greater soil N availability at T_E allows plants to allocate more resources to reproductive growth while maintaining some vegetative growth. Despite the greater soil N availability at T_E, plants at C_ET_E allocate all resources to reproductive growth, suggesting that vegetative growth is limited by soil N availability. However, combined with the greater photosynthetic capability at C_E, plants at C_ET_E produce the greatest reproductive output. Thickness of arrows indicates the strength of the relationship/effect.

Extreme weather impact under the current and future CO_2 and temperature regimes

Our results showed that the impact of flooding and drought on cotton productivity differed when plants were grown under future CO_2 and temperature regimes, and both physiological and soil nutrient responses were strongly linked to vegetative and reproductive growth responses to the extreme weather events. At the current CO_2 and temperature regimes, flooding had negative impacts on leaf physiology, vegetative growth, leaf and soil N status but had no effect on seed cotton yield in both soils. Drought had a detrimental impact on leaf physiology, leaf N status, growth and yield and led to a large amount of residual N in the soil. Contrary to expectations, physiological responses to flooding were more pronounced in plants grown at C_E, and C_E did not ameliorate the impact of flooding on cotton yield. T_E did not exacerbate flooding nor drought impact on leaf physiology; however, the impacts of flooding and drought on cotton yield were greater at T_E than T_A. Overall, flooding and drought impacts on cotton yield were greater in the future CO_2 and temperature regimes, largely due to the greater yield potential of cotton in the future CO_2 and temperature regimes than the current regime in the absence of extreme weather events. Therefore, our findings indicate that the instability of crop productivity is likely to increase as more extreme climates are expected under future climate regimes (for further discussion, please see attached manuscripts).

Implications for future cotton productivity

The positive yield response to C_E and T_E we found suggests that cotton productivity may be improved under future climatic conditions. C_E is likely to benefit productivity, provided that soil N and water availability are adequately managed during crop development (Mauney et al. 1978). However, a recent meta-analysis by Feng *et al.* (2015) has indicated that C_E may reduce plant N acquisition regardless of soil N availability, and that different mechanisms and processes such as reduced N mineralisation (Luo et al. 2004), increased microbial N immobilisation (de Graaff et al. 2006), reduced nitrate assimilation (Bloom et al. 2014; Bloom et al. 2010), reduced demand for Rubisco (Long et al. 2004) and reduced transpiration-driven mass flow of soil N (McDonald et al. 2002; McGrath and Lobell 2013) act in parallel to influence plant N acquisition. Thus, identifying the relative importance of these mechanisms in controlling N acquisition in cotton is necessary to develop an adequate N management strategy to maintain enhanced cotton productivity at C_E.

The positive effect of T_E is restricted to moderate temperature increases and will be reversed if temperatures increase beyond the optimal growth temperature (Reddy et al. 1995; Yoon et

al. 2009). A strong response to T_E has also been observed in other crop species (Jumrani and Bhatia 2014; Lobell and Field 2007) as temperature is the main climatic factor that influences the growth of most crop species. Fluctuations in temperature during the growing season are likely to affect crop growth, with both maximum and minimum temperatures outside the optimal growth temperature. Thus, one needs to be cautious when extrapolating the results from this temperature controlled glasshouse study into the highly variable environment of the field. Nonetheless, measuring crop response in a controlled environment is necessary to unravel complex interactions between multiple climatic factors that affect crop productivity. Thus, this study provides some insight into mechanisms that likely regulate plant performance in the field.

The extent to which C_E and T_E may affect yield response is an important aspect in assessing the sustainability of agricultural productivity. It has been suggested that the effect of C_E may be short-lived, due to resource limitations required to support enhanced plant growth (Arp 1991; Luo et al. 2004). Thus, if resource availability is adequately managed, the positive impact of C_E may be sustained (Liberloo et al. 2007). This requires an understanding of plant-soil interactions and whether climate-induced changes have feedback effects on subsequent crop production through changes in soil fertility and function. Changes in belowground C input may induce changes in soil microbial community structure and function, which may impact nutrient cycling and availability (Bardgett et al. 2008; de Graaff et al. 2006; Singh et al. 2010). Such changes in soil processes are likely to be influenced by differences in soil physical, chemical and biological properties. Thus, the understanding of long-term effects of climate change drivers on crop productivity needs to integrate both above- and below-ground responses as well as feedbacks between these responses on crop productivity.

Implications for future cotton productivity under extreme climates

A recent review by Thornton et al (2014) highlighted the importance of including climate variability and extreme weather events when evaluating the impact of climate change on agricultural production. While most research has focused on the impact of C_E and T_E on crop yield, projected increases in extreme weather events could alter our predictions of the impact of climate change on agricultural production (Porter and Semenov 2005). Indeed, our results indicate that future CO_2 and temperature regimes increased yield in cotton under optimal water conditions; however, this may not be true with projected increases in extreme weather events. Relative yield loss caused by flooding and drought is likely to be greater when yield potential is higher under future CO_2 and temperature regimes. This may lead to a large year-

to-year variability in yield, increasing unpredictability and instability of agricultural production. A recent meta-analysis by Challinor et al (2014) found that inter-annual variability in agricultural yield is likely to increase under climate change, yet such variability remains largely unassessed. Thus, our study provides empirical evidence and rationale for assessing the effect of extreme weather events in the context of climate change.

Appropriate adaptation has potential to avoid or even reverse yield loss expected under climate change (Howden et al. 2007; Challinor et al. 2014). For example, the negative impact of flooding on crop yield may be reduced by additional N supply (Hodgson 1982; Swarup and Sharma 1993; Singh et al. 2002) or the impact of T_E may be avoided by shifting the planting date (Lobell and Field 2007). Irrigation may be the only management option for drought-affected crops; however, adequate measures to retain residual soil N in the system may benefit subsequent crop production and contribute to climate mitigation by reducing the risk of nitrous oxide emission upon re-wetting. Our results indicate that yield response depends on both plant and soil nutrient responses to extreme weather events and suggest that adaptation and management practices become increasingly important as climatic variability increases under future climate regimes. Recovery of both plant and soil systems is clearly important to reduce the yield loss of the affected crop; however, the management practice should also consider long-term implications of extreme weather events and future CO_2 and temperature regimes on soils that differ in their physical and chemical characteristics. Our study therefore provides a framework for future research and management guidelines to reduce the vulnerability and instability of agricultural production under projected future climates.

Conclusions

Climate change impact

Our study showed that:

- T_E was the dominant factor in cotton productivity, accelerating the rate of plant development and vegetative growth while C_E had a strong impact on leaf physiology.
- Vegetative growth was dominated by the interactive effects of T_E and C_E on phenology, physiology and soil nutrients, and crop responses were similar in the two soils. However, during reproductive growth, the effects of T_E and C_E were limited by soil N availability, inducing changes in resource allocation between vegetative and reproductive growth.

- The reduction in soil N availability during reproductive growth also caused differences in soil properties to exert stronger influence over the crop response to C_E and T_E.
- Together, these results highlight the interactions between C_E, T_E and soil on crop productivity that are highly dynamic in their impacts depending on soil resource availability and resource allocation within the plants during crop development.
- This study demonstrated the importance of examining both plant and soil responses in understanding the mechanisms by which C_E, T_E and soil impact crop productivity, and provides evidence that differences in soil properties can modulate crop responses to C_E and T_E by their ability to continuously provide resources as per the crop's demand.
- Such knowledge is crucial in developing an effective adaptation strategy (e.g. additional N fertiliser application or optimisation to prevent N limitation at C_E) for crop production under future climate, and future research should explicitly consider plant-soil interactions in assessing the impact of climate change on crop production.

Extreme weather impacts

We found that:

- The magnitude of flooding and drought impact on cotton productivity was greater at future CO_2 and temperature regimes due to the greater yield potential under these conditions in the absence of extreme weather events.
- C_E did not ameliorate the impact of flooding on cotton yield, while flooding caused a rapid loss of N from the soil, contributing to the reduced vegetative growth and yield, particularly at T_E.
- Flooding and drought had contrasting consequences for soil N availability, with drought-induced loss of biological activity resulting in a large amount of residual N in the soil.
- Our study demonstrated that differences in soil characteristics can influence crop responses to extreme weather events, suggesting that adaptation strategies should explicitly consider soil characteristics in mediating such responses.
- This study highlights the importance of an integrated approach, considering both plant and soil systems, to assist the recovery of the affected crops but also to recover the soil system for the subsequent crop production.

- This study provides a framework for effective management (e.g. adequate fertiliser management following flooding and drought events) to ensure the long-term resilience of agricultural production under projected future climates.

Key knowledge for extension/ farmers

- Following the flooding event, more fertiliser (particularly N) will be required to re-build soil nutrients lost through leaching and gaseous losses for the subsequent crop production.
- In case of a drought event, fertiliser application should be optimised depending on the residual N from the previous season.
- Soil types play important role in above response and should be explicitly considered in the development of adaptation/ management practices

Expt. 2 – Glasshouse experiment (season 2)

The legacy of climate change and extreme weather events on cotton productivity and soil nutrients.

Background and aims

Plant-soil feedbacks play a central role in nutrient cycling. Changes in crop productivity, resource allocation and nutrient uptake can impact soil nutrient availability in both the short- and long-term, through changes in organic matter input into the soil. Projected changes in atmospheric concentrations of CO_2, temperature and extreme weather events have been shown to impact crop productivity. However, the short-term nature of most studies makes it difficult to assess the full extent of altered climate on crop productivity through plant-soil feedback, thus potentially limiting our ability to predict the long-term implications of these changes. In particular, it is unknown as to whether an extreme weather event in a previous season has a legacy effect on the subsequent crop production and how such effect may differ under current and future CO_2 and temperature regimes. Therefore, we examined the main and interactive effects of elevated CO_2 (C_E) and temperature (T_E) on cotton productivity in a controlled environment over two seasons to assess whether crop response was affected by the legacy of these treatments through plant-soil feedback, or remained consistent. We also imposed flooding and drought treatments in the first season and assessed the legacy of these treatments in the second season under current and future CO_2 regimes.

Materials and methods

Soil and plant materials and climate conditions in the glasshouse

Following the end of the previous experiment (see above for experimental design, set up and treatments), aboveground plant materials were turned into fresh mulch and incorporated back into each pot. Pots were stored for approximately seven months at two temperatures; 24 °C (day) for T_E pots and 20 °C (day) for T_A pots to maintain the temperature treatment during the simulated winter fallow. We did not apply CO_2 treatment to the soils during the fallow, as CO_2 effects on soils are plant-derived. Pots (well-watered and flooded pots) were watered on a monthly basis to simulate the winter rainfall in Narrabri based on the climate record from the last 30 years (from Bureau of Meteorology website). For drought pots, the amount of

water added was reduced to simulate the winter rainfall of Narrabri during the millennium drought (from Bureau of Meteorology website). After the fallow period, all pots were placed in the same CO_2 and temperature conditions as their previous season (see above) and watered to field-capacity and allowed to drain for two weeks prior to planting cotton seeds, as described previously.

The legacy effect of CO_2 and temperature were examined by growing cotton plants under the four climate change treatments (C_AT_A, C_AT_E, C_ET_A, C_ET_E) on the soils previously subjected to those climate change treatments and have plant litter produced in those climate change treatments incorporated back into them. The legacy of extreme weather events (flooding and drought) was assessed by growing cotton plants on the previously flood-subjected and drought-subjected soils under the four climate change treatment under well-watered conditions. Pots were fertilised at a rate of 190 kg N ha^{-1}, as previously described, regardless of the existing soil inorganic N to examine the legacy of previous treatments on subsequent cotton production.

Legacy effects – plant responses

Plant development and growth, leaf physiology and leaf nutrients were measured periodically as previously described. Additionally, we measured vegetative biomass (leaf, stem, root biomass) as well as reproductive biomass at the end of the experiment by oven-drying at 70 °C.

Legacy effects – soil responses

Soil inorganic N (NH_4^+ and NO_3^-), total C and N were measured as previously described. In addition to soil total C, we also measure the amount of dissolved organic C using an extraction method described in Harrison and Bardgett (2003). The amount of dissolved organic C in the extracts were analysed using a total organic C analyser (TOC-L CPH/CPN, Shimadzu Scientific Instruments, Rydalmere, NSW, Australia).

Potential nitrification rate (PNR) was measured periodically during the experiment using a method described by Kandeler (1996) with some modifications. Briefly, 5 g of fresh sieved soil was added to 20 mL of buffer solution (containing NaCl, 8.0 g L^{-1}, KCl, 0.2 g L^{-1}, Na_2HPO_4, 0.2 g L^{-1}, NaH_2PO_4, 0.2 g L^{-1}, $(NH_4)_2SO_4$, 0.13214 g L^{-1}, adjusted to pH 7.1), shaken at 120 rpm for 10 min, and then stored at -20 °C, which served as the control sample (t0). Another 5 g of fresh sieved soil was also added to 20 mL of the same buffer solution with $KClO_3$ (at 50 mg L^{-1}), shaken at 120 rpm for 10 min, and then further shaken for 5 h at

25 °C (t1). Then, both the control (t0) and incubated (t1) samples were shaken with 5 mL of 2M KCl for 15 min, then filtered through Whatman 42 filter papers. The concentrations of nitrite were measured colourimetrically. PNR was calculated by the difference between the amount of nitrite at t1 and t0.

Net N mineralisation was measured using a 28-day laboratory incubation. 10 g of fresh sieved soil was split into two subsamples, with one of the subsamples being immediately extracted with 2M KCl to measure the initial inorganic N (NH_4^+ and NO_3^-) concentrations (as described earlier). The remaining subsample was adjusted with water (~60% of field capacity) and incubated in the dark at 25 °C for 28 days. Net N mineralisation was calculated by the difference in inorganic N concentrations between the pre- and post-incubated samples.

In situ soil respiration (CO_2) and nitrous oxide (N_2O) fluxes were measured periodically during the experiment using a static chamber method. For each pot, a polyvinyl chloride chamber collar (10 cm in diameter, 15 cm in height) were inserted 8 cm into the soil in each pot for the entire duration of the experiment. Using a chamber top (10 cm in diameter, 5 cm in height) to create a closed chamber, air samples (15 ml) were taken from the headspace (headspace volume = 1194 cm^3) after 0, 15, 30 and 45 min using a syringe through a septum equipped on each chamber top. The gas sample was immediately injected into a pre-evacuated 10-mL glass vial (Agilent Technologies, USA) sealed with a butyl rubber stopper and aluminium seal (Sigma-Aldrich, USA). Measurements were taken between 10 am and 2 pm to minimise diurnal temperature variations. Gas samples were analysed for CO_2 and N_2O concentration on a 7890A gas chromatograph with a G1888 network headspace sampler (Agilent Technologies, USA) equipped with a methanizer to convert CO_2 to CH_4 for detection by a flame ionization detector and a micro electron capture detector (μECD) for N_2O. This system uses two 1/8″ stainless steel packed columns (80/100 HayeSep Q®, Supelco, USA) and has a minimum detection limit of 61.41 ppm and 0.02 ppm for CO_2 and N_2O, respectively. Fluxes were calculated as the slope of the linear regression from the measured headspace gas concentrations with time (Matthias et al., 1980) and expressed as μg CO_2-C/N_2O–N cm^2 h^{-1}. To avoid bias against low fluxes, fluxes below minimum detectable flux were not discarded (De Klein and Harvey, 2012). Fluxes presented as negative values represent net sink while positive values represent net source taking place in the soil.

Legacy effects – microbial responses

DNA was extracted from 0.25g soil using MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. The quantity

and quality of the extracted DNA were examined using NanoDrop® ND-2000c UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

The abundance of the bacterial 16S rRNA gene and fungal 18S rRNA gene were quantified by quantitative PCR (qPCR) on an iCycler iQ 5 thermocycler (BioRad Laboratories, Hercules, CA, USA) using the primer pairs 338f and 518r for bacteria and ITSf and 5.8s for fungi. The 10µl reaction mixture contained 5µl SensiFast SYBR No-ROX reagent (Bioline, Sydney, NSW, Australia), 0.25µl of each primer (20µM), 0.25µl of BSA (20mM) and 1µl of diluted DNA template (1–10 ng). Amplification conditions were as follows: 95°C for 3min, 40 cycles of 10s at 95°C, 10s at 60°C and 20s at 72°C, followed by melt curve from 65°C to 95°C at 0.5°C increment. Standard curves were developed using ten-fold serial dilutions of plasmid containing the correct insert of the bacterial 16S rRNA gene or fungal 18S rRNA gene. Triplicate reactions were produced for each DNA sample. Melt curve analyses were conducted to verify the specificity of the amplification products, and the PCR efficiency for different assays ranged between 110% and 120%.

Statistical analyses

Climate change legacy under well-watered condition

Data from well-watered control treatments were analysed by four-way analysis of variance (ANOVA) in R statistical software to test the main and interactive effects of soil, CO_2, temperature and season, as previously described. To compare the magnitude of climate change effects on plant and soil responses between the two seasons, we calculated the response ratio between the first season responses and the second season responses under each climate change treatment in each soil, to be used as an effect-size metric. We calculated the natural logarithm of the response ratio, as described previously. Biomass and soil C and N data from the second season's well-watered control treatment were analysed by three-way ANOVA to test the main and interactive effects of soil, CO_2 and temperature, and were further analysed by two-way ANOVA for each soil to compare means using the Tukey's *post hoc* comparison. All data were checked for normality and heteroscedasticity, and log-transformed where necessary.

Extreme weather legacy under climate change treatments

We used structural equation modelling (SEM) to identify the relative importance and effects of soil legacy variables and microbial variables on cotton productivity in the subsequent season. Unlike regression or analysis of variance, SEM offers the ability to separate multiple pathways of influence and view them as a system (Shipley, 2002; Grace, 2006; Delgado-Baquerizo et al., 2015). Another important capability of SEM is its ability to partition direct and indirect effects that one variable may have on another and estimate the strengths of these multiple effects (Shipley, 2002; Grace, 2006; Delgado-Baquerizo et al., 2015). SEM was generated based on the known effects and relationships among the treatments, soil and microbial properties and the response variable (cotton productivity, Fig. S7). In this study, we were interested in how the legacies of CO_2, temperature, flooding and drought treatments impact the subsequent cotton productivity through their impact on soil and microbial properties. Soil and microbial properties data were normalised (log-transformed) prior to analyses when needed. When these data manipulations were complete, we parameterised our model using our data set and tested its overall goodness of fit. The overall goodness of fit in our models was tested, as explained in Schermelleh-Engel et al. (2003). There is no single universally accepted test of overall goodness of fit for structural equation models applicable in all situations regardless of sample size or data distribution. Most modellers circumvent this problem by using multiple goodness of fit criteria. We used the Chi-square test (χ^2; the model has a good fit when $0 \leq \chi^2 \leq 2$ and $0.05 < P \leq .00$ and acceptable fit when $2 \leq \chi^2 \leq 3$ and $0.01 < P \leq 0.05$) and the root mean square error of approximation (RMSEA; the model has a good fit when RMSEA $0 \leq$ RMSEA ≤ 0.05 and $0.10 < P \leq 1.00$ and acceptable fit when RMSEA $0.05 \leq$ RMSEA ≤ 0.08 and $0.05 < P \leq 1.00$). Additionally, and because some variables were not normal, we confirmed the fit of the model using the Bollen– Stine bootstrap test (the model has a good fit when 0.10 < bootstrap $P \leq 1.00$ and acceptable fit when 0.05 < bootstrap $P \leq 0.10$). Finally, we calculated the standardised total effects of all predictor variables. The net influence that one variable has upon another is calculated by summing all direct and indirect pathways between the two variables. If the model fits the data well, the total effect should approximately be the bivariate correlation coefficient for that pair of variables (Grace, 2006).

Results

Climate change legacy (well-watered plants only)

Plant and soil responses to CO_2 and temperature between the two seasons

Responses of cotton productivity, physiology and nutrient status to C_E and T_E significantly differed between the two seasons (Table 10, Fig. 11). Seed cotton yield at C_AT_A more than doubled in the second season in both soils; however, the magnitude of positive T_E effect was substantially reduced in the second season compared to the first season, particularly in black vertosol (Soil x Temp x Season interaction, P=0.03), and the direction of C_E effect at T_A changed from positive in the first season to negative in the second season in both soils (CO_2 x Temp x Season interaction, P<0.0001, Fig. 11). Vegetative growth rate was also greater in the second season than the first season, and the responses to C_E and T_E interacted with soil and season (Table 10, Fig. 11). The positive effects of both T_E and C_E on vegetative growth rate were reduced in the second season, particularly in grey vertosol (Soil x Temp x Season interaction, P=0.02, Soil x CO_2 x Season interaction, P=0.03).

Leaf physiology responses to C_E and T_E also differed significantly between the seasons (Table 10, Fig. 11). Photosynthetic rate at C_AT_A was significantly greater (by 49%) in the second season than the first season in both soils, and stomatal conductance also doubled in the second season compared to the first season in both soils. The response of photosynthetic rate to C_E and T_E differed between the seasons, particularly the C_E effect at T_A, in both soils (CO_2 x Temp x Season interaction, P<0.0001, Fig. 11). In the first season, photosynthetic rate was increased by 109% in C_ET_A compared to C_AT_A in both soils; however, this strong positive effect was reduced in the second season to 14% (Fig, 11). The response of stomatal conductance to C_E and T_E also differed significantly between the two seasons (CO_2 x Temp x Season interaction, P<0.0001, Fig. 11). In the first season, C_E and T_E interactively increased stomatal conductance; however, such an increase was only observed at C_AT_E in the second season, and a large decrease (by 52%) was observed at C_ET_A in both soils (Fig. 11).

Table 10 Results of analysis of variance (ANOVA) showing the effects of soil, CO_2, temperature and season on crop productivity, leaf physiology, leaf N and soil nitrate status of cotton plants at flowering stage. Values are probability with significant results (P<0.05) shown in bold, marginally significant results (P<0.1) in italic and non-significant results (P>0.1) as n.s.

	Productivity		Physiology		Nutrient	
Factor	Yield	GR	*A*	*gs*	Leaf N	Soil nitrate
Soil	n.s.	n.s.	**0.0004**	**0.02**	**0.04**	**0.04**
CO_2	**<0.0001**	*0.06*	**<0.0001**	**<0.0001**	**<0.0001**	*0.09*
Temp	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0003**
Season	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.01**	**<0.0001**
Soil x CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Soil x Temp	n.s.	*0.06*	**0.05**	n.s.	**0.02**	n.s.
CO_2 x Temp	**<0.0001**	**<0.0001**	**0.0001**	**0.001**	**0.003**	*0.10*
Soil x Season	n.s.	**<0.0001**	n.s.	n.s.	n.s.	**0.001**
CO_2 x Season	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	n.s.
Temp x Season	n.s.	*0.05*	**<0.0001**	n.s.	**0.04**	*0.07*
Soil x CO_2 x Temp	*0.07*	n.s.	*0.06*	n.s.	n.s.	n.s.
Soil x CO_2 x Season	n.s.	**0.03**	n.s.	n.s.	n.s.	n.s.
Soil x Temp x Season	**0.03**	**0.02**	n.s.	n.s.	**0.003**	n.s.
CO_2 x Temp x Season	**<0.0001**	n.s.	**<0.0001**	**<0.0001**	**<0.0001**	*0.05*
Soil x CO_2 x Temp x Season	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Yield=seed cotton yield, GR=vegetative growth rate, *A*=photosynthetic rate, *gs*=stomatal conductance.

Leaf and soil N responses to C_E and T_E also significantly differed between the two seasons (CO_2 x Temp x Season interaction, P<0.0001, Fig. 11). In the first season, both C_E and T_E interactively increased leaf N in both soils; however, these positive effects disappeared in the second season and were even reversed at C_ET_A in both soils. In black vertosol, the positive effect of T_E in the first season was also reversed in the second season (Soil x Temp x Season interaction, P=0.003). Soil nitrate availability at flowering was marginally altered by CO_2 x Temp x Season interaction (P=0.05, Table 10), where the positive effect of T_E at C_A was particularly evident in the first season (Fig. 11). However, the strongest difference was observed between the two seasons where soil nitrate availability at flowering was significantly lower in the second season than the first season (P<0.0001), particularly in black vertosol (Soil x Season interaction, P<0.001).

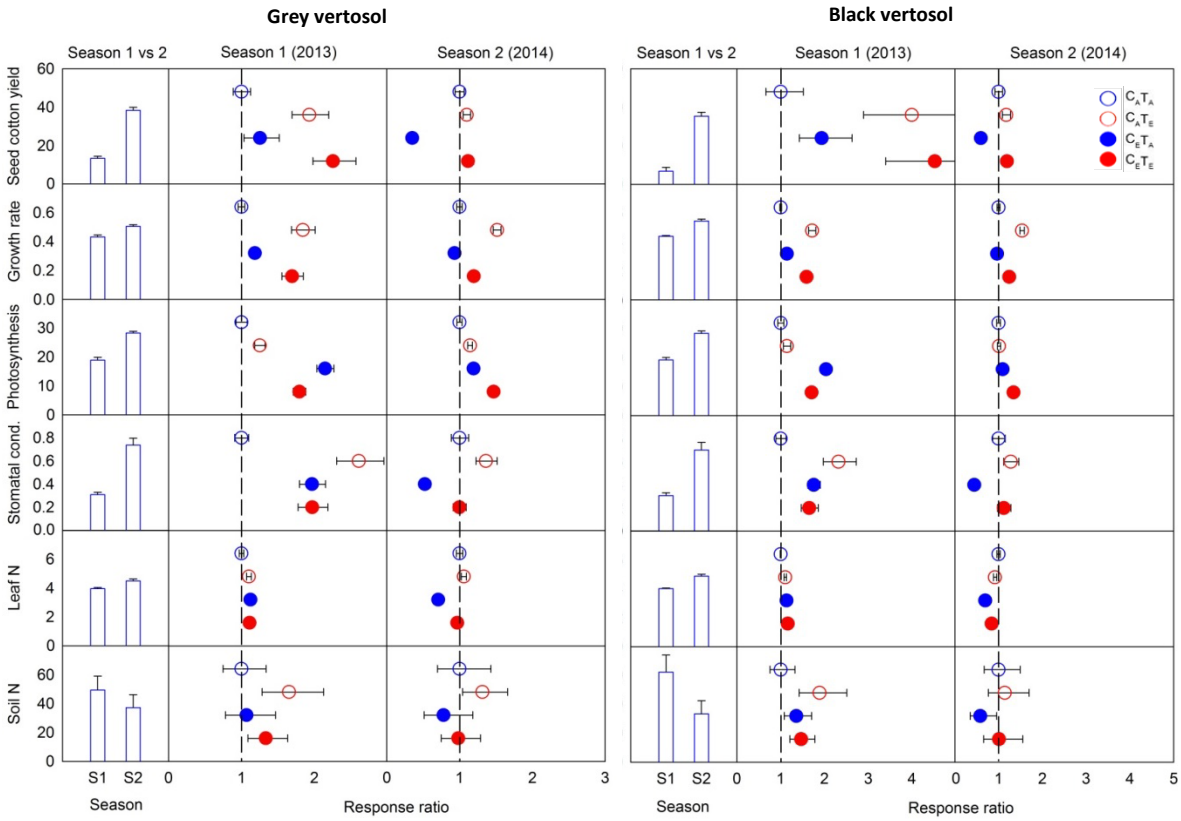


Fig. 11 The comparisons between the two seasons in the effect of CO_2 and temperature on crop productivity, leaf physiology and leaf and soil nitrogen of cotton plants grown on grey and black vertosols.

Biomass allocation in the second season

Biomass production was significantly altered by C_E and T_E in both soils (Table 11, Fig. 12). Seed cotton yield was significantly increased by T_E (by 15%) but strongly decreased by C_E at T_A (by 53%) in both soils (CO_2 x Temp interaction, P<0.0001), although the responses marginally differed between the two soils (CO_2 x Temp x Soil interaction, P=0.08). Leaf biomass was increased by C_E (P=0.001), and marginally interacted with temperature (CO_2 x Temp interaction, P=0.08) and soil (CO_2 x Temp x Soil interaction, P<0.1). Stem biomass was strongly altered by CO_2 x Temp interaction (P<0.0001), where C_E at T_A significantly increased stem biomass by 25% while C_E at T_E reduced it by 31% (Fig. 12). Root biomass was significantly decreased by T_E (P<0.0001); however, there was a significant CO_2 x Temp x Soil interaction effect (P<0.0001) where C_E at T_A increased root biomass in grey vertosol. In total, C_E reduced the total biomass by 14% in both soils, and the total biomass was also marginally reduced by T_E in black vertosol (P=0.07).

Table 11 The results of analysis of variance showing the effects of CO_2, temperature and soil on biomass allocation of cotton plants in the second season. Values are probability with significant results (P<0.05) shown in bold, marginally significant results (P<0.1) in italic and non-significant results (P>0.1) as n.s.

Factor	Leaf	Stem	Root	Reproductive remains	Seed cotton	Dead mass	Total biomass
CO_2	**0.001**	n.s.	**0.01**	n.s.	**<0.0001**	n.s.	**0.002**
Temp	**0.03**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	n.s.	n.s.
Soil	**<0.0001**	**<0.0001**	n.s.	**0.004**	n.s.	n.s.	**<0.0001**
CO_2 x Temp	*0.08*	**<0.0001**	**<0.0001**	n.s.	**<0.0001**	n.s.	n.s.
CO_2 x Soil	n.s.	n.s.	**<0.0001**	*0.06*	*0.09*	n.s.	n.s.
Temp x Soil	n.s.	n.s.	n.s.	*0.09*	n.s.	n.s.	*0.07*
CO_2 x Temp x Soil	*0.10*	n.s.	**<0.0001**	n.s.	*0.08*	n.s.	n.s.

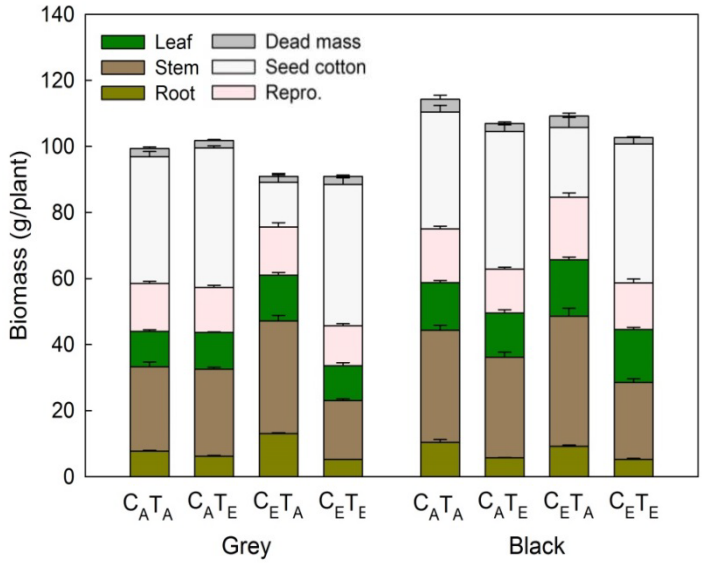


Fig. 12 Biomass allocation (leaf mass, stem mass, root mass, seed cotton mass, other reproductive mass and dead mass) of cotton plants grown on grey and black vertosol under four climate change treatment in the second season.

Soil N availability and processes in the second season

Following the seven-month fallow period at pre-planting, there was a large difference in the amounts of soil nitrate between the four climate change treatments in both soils (Table 12, Fig. 13a). Soil nitrate availability was the highest in C_AT_A in both soils, and was significantly greater in black vertosol than grey vertosol (P=0.003). Both C_E and T_E significantly reduced soil nitrate availability in both soils (P<0.0001, P=0.02 respectively); however, the reduction was particularly large at C_ET_A (CO_2 x Temp interaction, P<0.0001) in black vertosol (CO_2 x Temp x Soil interaction, P=0.02).

Some soil processes that are related to N cycling were also affected by C_E or T_E (Table 12, Fig. 13). Potential nitrification rate (PNR) was increased by C_E in both soils (P=0.01). Black vertosol had a significantly higher PNR than grey vertosol (P<0.0001), although the response to C_E did not differ between the two soils. Net N mineralisation was also higher in black vertosol than grey vertosol (P<0.0001), and the two soils showed an opposite response to T_E (Temp x Soil interaction, P=0.003). T_E increased net N mineralisation in black vertosol and decreased it in grey vertosol. There were no significant effects of C_E or T_E on nitrous oxide (N_2O) fluxes, although N_2O at C_ET_A tended to be higher (or less negative) than other treatments in both soils.

Table 12 The results of analysis of variance showing the effects of CO_2, temperature and soil on soil nitrate concentrations at pre-planting, potential nitrification rate (PNR), net N mineralisation and N_2O flux of soils planted with cotton plants in the second season. Values are probability with significant results (P<0.05) shown in bold, marginally significant results (P<0.1) in italic and non-significant results (P>0.1) as n.s.

Factor	Soil nitrate	PNR	Net N min	N_2O flux
Soil	**0.003**	**<0.0001**	**<0.0001**	n.s.
CO_2	**<0.0001**	**0.01**	n.s.	n.s.
Temp	**0.02**	n.s.	n.s.	n.s.
Soil x CO_2	**0.01**	n.s.	n.s.	n.s.
Soil x Temp	**0.02**	n.s.	**0.003**	n.s.
CO_2 x Temp	**<0.0001**	n.s.	n.s.	n.s.
Soil x CO_2 x Temp	**0.02**	n.s.	n.s.	n.s.

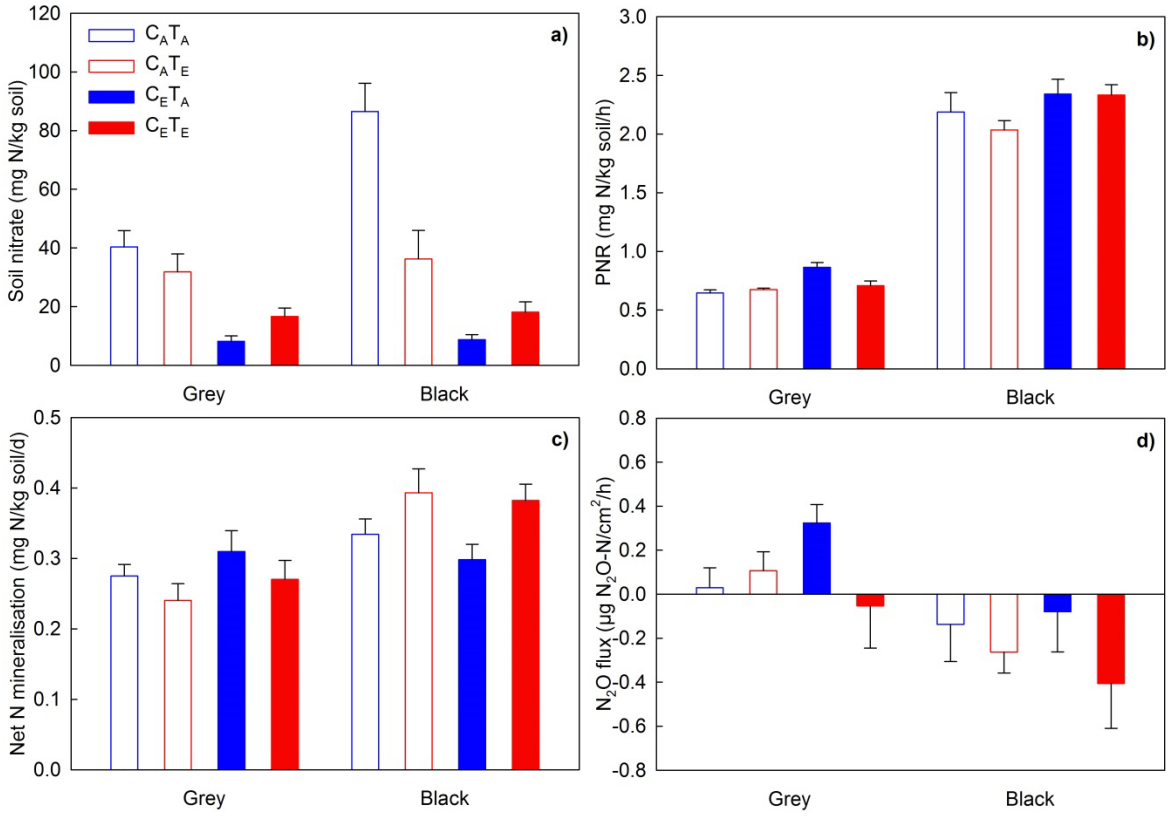


Fig. 13 Soil nitrate concentrations at pre-planting, potential nitrification rate (PNR), net N mineralisation and N_2O flux of soils planted with cotton plants grown on grey and black vertosol under four climate change treatment in the second season.

Belowground C allocation in the second season

Belowground C allocation was significantly influenced by C_E and T_E (Table 13, Fig. 14 and 15). Root C concentration was significantly increased by C_E (P<0.0001) and T_E (P<0.0001) in both soils, which interacted with each other (P=0.02) to significantly enhance root C concentrations at C_ET_E (Fig. 14a); however, when root biomass was taken into account, T_E decreased the amount of root C (P<0.0001) while C_E had a positive effect on the amount of root C but only at T_A in grey vertosol (CO_2 x Temp x Soil interaction, P<0.0001, Fig. 14b). Soil total C was significantly increased by C_E (P=0.01) while it was decreased by T_E (P=0.01) in both soils (Fig. 15a). Soil C was significantly greater in black vertosol than grey vertosol (P<0.0001), however, the response to C_E and T_E did not differ between the soils. C_E and T_E interactively altered soil respiration (P=0.02), which also differed between the two soils (CO_2 x Temp x Soil interaction, P=0.02). In grey vertosol, C_E at T_A increased soil respiration by 56% while C_E at T_E had no effect on soil respiration (Fig. 15b). In black vertosol, C_E increased soil respiration by 25% at both T_A and T_E (Fig. 15b). The amount of dissolved organic C (DOC) differed between the soils, CO_2 and temperature treatments (CO_2 x Temp x Soil interaction, P=0.01, Fig. 14c), largely driven by the large difference in the

amount of DOC at C_AT_A between the two soils, as the amount found at C_AT_E, C_ET_A and C_ET_E were similar between the two soils.

Table 13 The results of analysis of variance showing the effects of soil, CO_2 and temperature on root C (%), root C mass, soil C (%), soil respiration and the amount of dissolved organic C (DOC) of soils planted with cotton plants in the second season. Values are probability with significant results (P<0.05) shown in bold, marginally significant results (P<0.1) in italics and non-significant results (P>0.1) as n.s.

Factor	Root C (%)	Root C mass	Soil C (%)	Soil respiration	DOC
Soil	n.s.	n.s.	**<0.0001**	**<0.0001**	**0.001**
CO_2	**<0.0001**	**0.01**	**0.01**	**0.05**	**<0.0001**
Temp	**<0.0001**	**<0.0001**	**0.01**	*0.07*	n.s.
Soil x CO_2	n.s.	**<0.0001**	n.s.	n.s.	n.s.
Soil x Temp	n.s.	n.s.	n.s.	n.s.	*0.06*
CO_2 x Temp	**0.02**	**<0.0001**	n.s.	**0.02**	**0.0001**
Soil x CO_2 x Temp	n.s.	**<0.0001**	n.s.	**0.02**	**0.01**

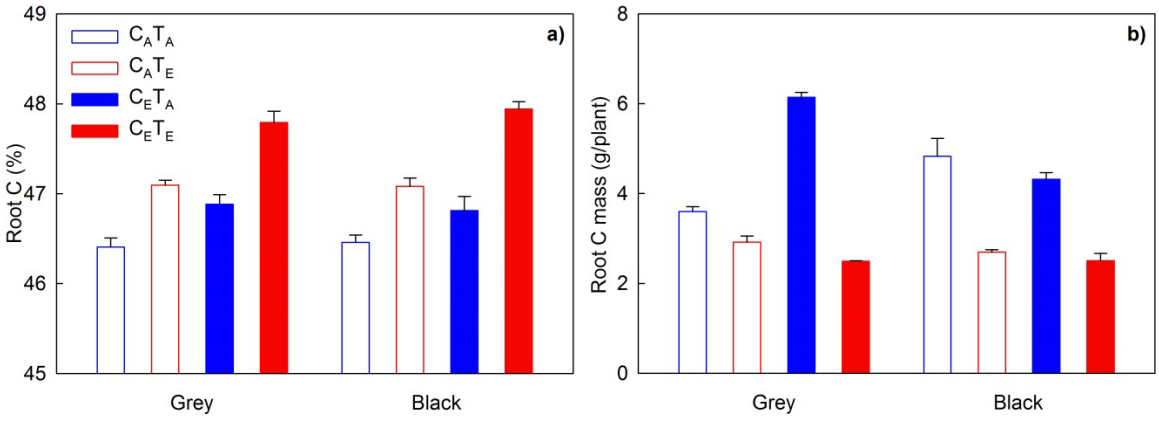


Fig. 14 Root C concentrations (a) and root C mass (b) of cotton plants grown on grey vertosol and black vertosol under the four climate change treatments in the second season.

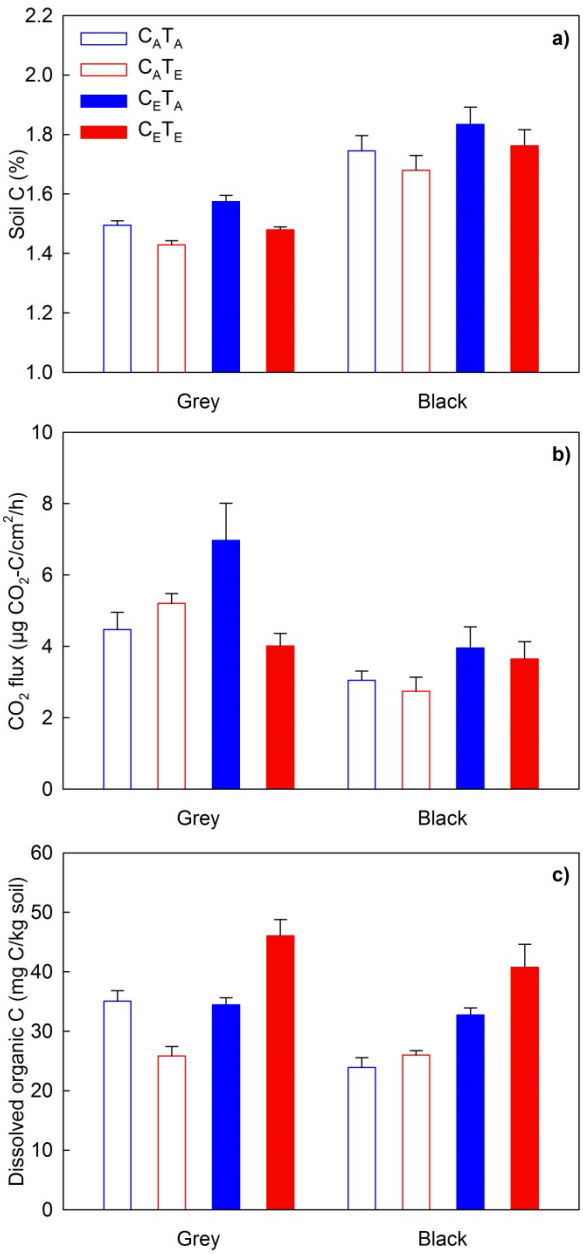


Fig. 15 Soil total C (a), soil respiration (b) and dissolved organic C (c) of grey vertosol and black vertosol planted with cotton under the four climate change treatments in the second season.

Changes in microbial abundance

The abundance of bacteria and fungi were the highest at C_ET_A in both soils (CO_2 x Temp interaction, P=0.08 and P=0.01 for bacteria and fungi, respectively). The abundance of bacteria and fungi were significantly higher in grey vertosol than black vertosol (P<0.0001 for both bacteria and fungi, Fig 16, Table 14).

Table 14 The results of analysis of variance showing the effects of soil, CO_2 and temperature on the abundance of bacteria and fungi of soils planted with cotton plants in the second season. Values are probability with significant results (P<0.05) shown in bold, marginally significant results (P<0.1) in italics and non-significant results (P>0.1) as n.s.

Factor	Bacteria	Fungi
Soil	**<0.0001**	**<0.0001**
CO_2	n.s.	n.s.
Temp	*0.09*	**0.003**
Soil x CO_2	n.s.	n.s.
Soil x Temp	n.s.	*0.09*
CO_2 x Temp	*0.08*	**0.01**
Soil x CO_2 x Temp	n.s.	n.s.

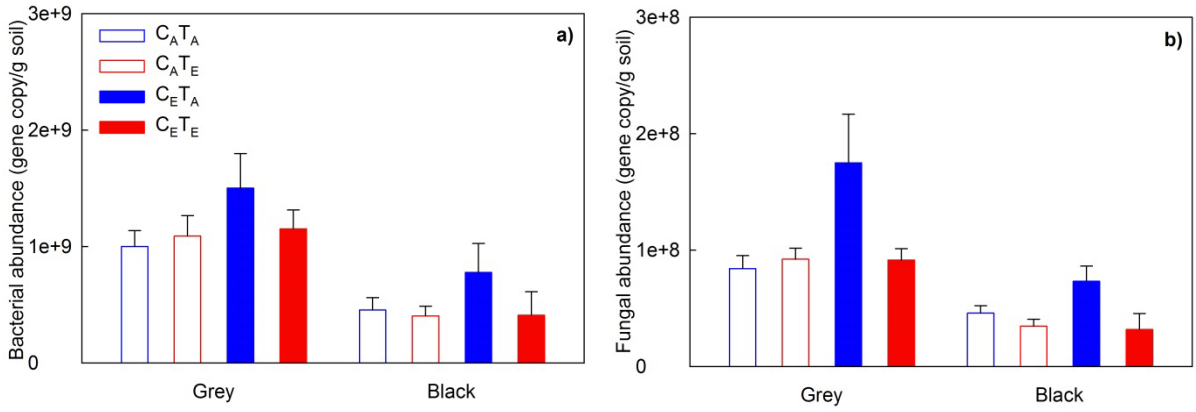


Fig. 16 The abundance of bacteria (a) and fungi (b) of grey vertosol and black vertosol planted with cotton under the four climate change treatments in the second season.

Correlations between plant variables and soil variables

We expected that the second season's cotton productivity would be driven by the interactions between the climate change effects on plant and soil processes during the second season and the legacy of climate change effects in the previous season on soil processes, in particular, changes in soil N availability following the litter decomposition process. We therefore explored the relationships between residue quality, soil N availability, leaf physiology and biomass production. We found a strong positive correlation (r=0.78, P<0.0001, Fig. 17a) between litter N concentrations and the amount of soil nitrate at pre-planting in the second season. Soil nitrate availability at pre-planting was also correlated with leaf N measured at flowering (r=0.63, P<0.0001, Fig. 17b) of the second season; however, the changes in leaf N was not reflected in the differences in photosynthetic rate (Fig. 17c). There was, however, a positive correlation between leaf N and seed cotton yield (r=0.73, P<0.0001, Fig. 17d).

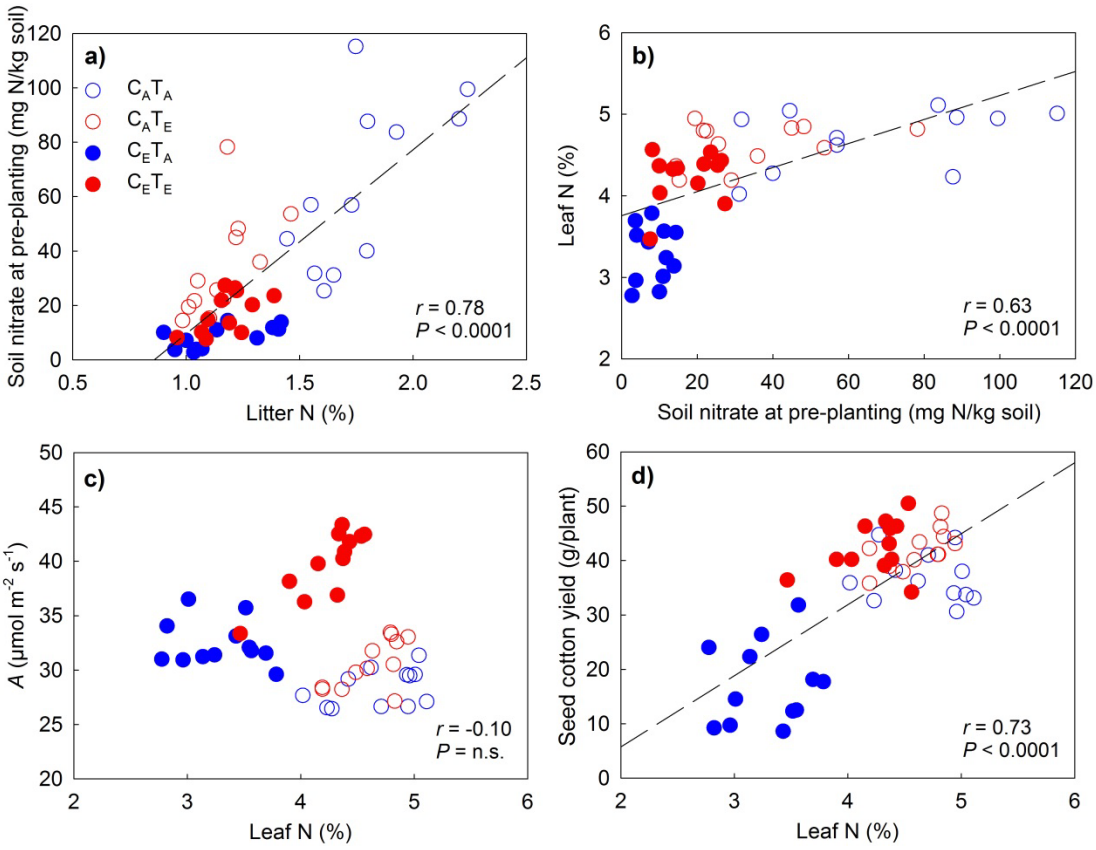


Fig. 17 Relationships between litter N concentrations, soil nitrate, photosynthetic rate (*A*) and seed cotton yield.

To explore the reasons behind the lack of correlations between photosynthetic rate and seed cotton yield, we further examined the relationships between leaf physiology, biomass production and soil processes. First of all, we found a strong relationship between photosynthetic rate and stomatal conductance as expected; however, this relationship was only observed at each CO_2 levels (Fig. S7). We found a negative relationship between photosynthetic rate and total biomass production (r=−0.40, P=0.01, Fig. 18a) and that total biomass was more strongly correlated with the total leaf area (r=0.71, P<0.0001, Fig. 18b). Furthermore, we found that there was a strong negative correlation between vegetative biomass and seed cotton yield (r=−0.71, P<0.0001, Fig. 18c), indicating that seed cotton yield was also influenced by a shift in resource allocation within the plant. Interestingly, while we found limited relationships between photosynthetic rate and plant growth variables, we found a positive correlation between photosynthetic rate and the amount of dissolved organic C in the soil (r=0.61, P<0.0001, Fig. 18d), further suggesting that C_E and T_E may have resulted in changes in plant C allocation patterns.

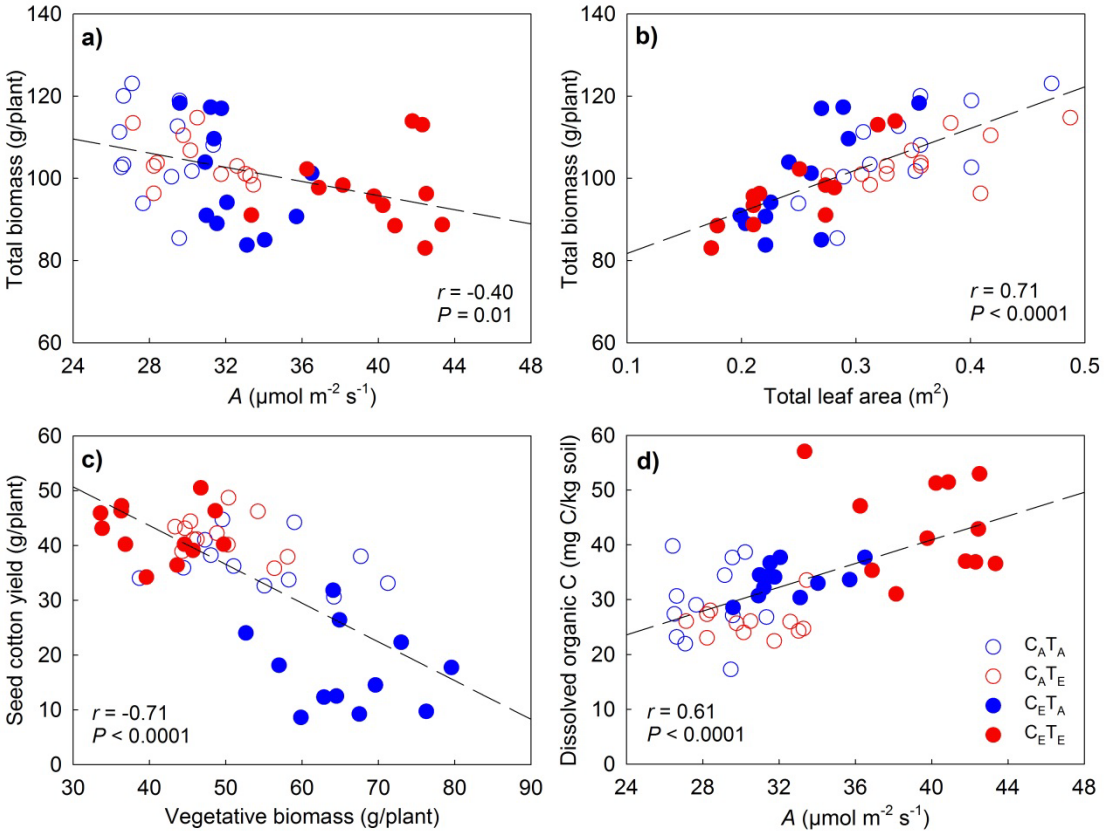


Fig. 18 Relationships between photosynthetic rate (*A*) and total biomass (a), total leaf area and total biomass (b), vegetative biomass and seed cotton yield (c) and photosynthetic rate and soil dissolved organic C (d) from cotton plants grown on grey and black vertosols under the four climate change treatments.

Extreme weather event legacy

We examined how cotton productivity in the second season was influenced by flooding and drought events in the previous season under the current and future climatic conditions, using structural equation modelling. First of all, we created a prior model based on the known effects and relationships among the treatments, soil and microbial properties and response variable (i.e. seed cotton yield, Fig. S7). In this study, we were interested in how the legacies of CO_2, temperature, flooding and drought treatments impact the subsequent crop productivity through their impact on soil and microbial properties, thus we incorporated the soil and microbial properties measured at the pre-planting of the second season to assess the legacy effects.

Seed cotton productivity in the second season was influenced by both flooding and drought events in the previous season through their impacts on soil and microbial properties (Fig. 19). Drought had a strong positive effect on soil nitrate availability, owing to the large amount of residual nitrate due to the death of the previous crop. The death of the previous crop also led to a small amount of plant biomass to be re-incorporated back into the soil, thereby affecting total soil C. These changes together decreased soil C:N ratios of soil subjected to drought in the previous season. Drought also influenced soil microbial community by shifting the community towards more bacteria than fungi (i.e. decreased fungal to bacterial ratios). The impact of flooding on soil properties was less evident, with only a small, yet significant increase in soil nitrate. Flooding impact on soil microbial community mirrored that of drought, with a shift in community towards more bacteria than fungi.

The legacy of elevated CO_2 and temperature treatments on cotton productivity through soil and microbial properties were also evident. Elevated CO_2 strongly reduced soil nitrate availability at pre-planting and shifted the microbial community towards more fungi than bacteria. Elevated temperature, on the other hand, increased soil nitrate availability, decreased soil total C and had no effect on soil microbial community at pre-planting.

These legacy effects of extreme weather events, elevated CO_2 and temperature on soil and microbial properties influenced cotton productivity of the second season, alongside with the direct impacts of elevated CO_2 and temperature on cotton productivity. While cotton productivity was strongly correlated with temperature treatment of the second season, it was also strongly correlated with soil nitrate availability and soil C:N ratios at pre-planting, suggesting that the legacy effects through changes in soil and microbial properties can influence the cotton productivity of the subsequent season. When both indirect and direct

effects were considered, cotton productivity of the second season was influenced the most by temperature treatment, followed by drought, CO_2 and flooding treatments.

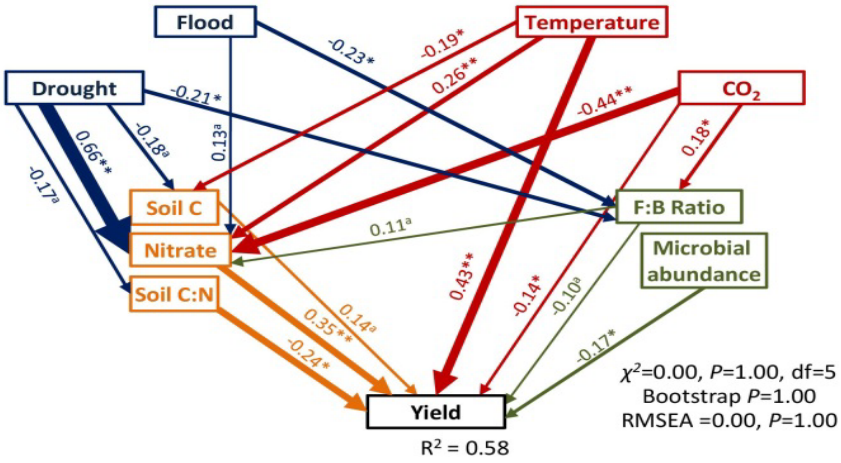


Figure 19 Structural equation model based on the legacy effects of CO_2, temperature, flooding and drought treatments through soil and microbial properties on subsequent cotton productivity (yield). Numbers adjacent to arrows are standardized path coefficients, analogous to partial regression weights and indicative of the effect size of the relationship. Arrow width is proportional to the strength of path coefficients. As in other linear models, R^2 indicates the proportion of variance explained and appears below the response variable in the model. Model fitness details (χ^2 vs RMSEA and non-parametric Bootstrap parameters) are also presented. Significance levels are as follows: *P<0.05 and **P<0.01.

Discussion/conclusions

Climate change legacy

We found that:

- Cotton productivity was influenced by legacies of elevated CO_2 and temperature on soil and microbial properties that induced changes in the allocation of C resources between aboveground and belowground as well as between vegetative and reproductive growth.
- Overall, elevated temperature had a positive effect on cotton productivity both directly and indirectly through its legacy effect.
- The initial positive effect of elevated CO_2 disappeared in the second season where its legacy through crop residue on soil and microbial properties strongly reduced soil N availability, limiting the enhancement of photosynthetic rate previously seen at

elevated CO_2 and altering resource allocation towards belowground and less towards reproductive growth.

- This negative legacy of elevated CO_2 was only evident at ambient temperature, and the large difference in biomass allocation pattern suggests that the positive effect of elevated temperature on soil N availability may have played a role in shifting resource allocation towards more reproductive growth to produce the highest yield.
- This study also suggests that the response of non-harvestable biomass to these environmental changes should also be considered when assessing the impacts of climate change on cotton productivity and developing effective residue management strategies to ensure sustainable cotton production.

Extreme weather legacy

We also found that:

- Flooding and drought events occurred in the previous season can affect the soil and microbial properties and that those changes can indirectly influence cotton productivity of the subsequent season.
- Therefore, this study demonstrates that cotton productivity is influenced by the complex interactions and feedbacks between plant growth, physiology and soil nutrient availability that are altered directly or indirectly through legacies by elevated CO_2, temperature and extreme weather events.

Key knowledge for extension/ farmers

- For climate change adaptation strategy, the results from this study highlights the need for N fertiliser to be optimised (more fertiliser in the case of C_E) in order to ensure future crop productivity.
- For extreme weather adaptation strategies, the new management practice for fertiliser application should reflect the climatic history of the field and soil nutrient status to maximise the productivity.

Expt. 3 – Glasshouse experiment (season 1&2) – soil microbial community

The impact and legacy of climate change and extreme weather events on soil microbial community and soil processes.

Background and aims

Elevated CO_2, temperature and extreme weather events such as flooding and drought has the potential to drastically impact crop productivity directly or indirectly through changes in soil processes that affect nutrient availability to crops. Soil microbial communities mediate those soil processes, thus changes in soil microbial community composition and activity induced by elevated CO_2, temperature and extreme weather events may significantly impact crop responses to these factors or vice versa, potentially leading to cascading changes and feedbacks that could impact the functioning of the cropping system as a whole. Therefore, we examined the impact of elevated CO_2, temperature and extreme weather events on soil microbial community of grey vertosol and black vertosol planted with cotton over two seasons.

Materials and methods

Experimental setup in the glasshouse

Please see the materials and methods in the previous sections.

Soil collection

Soil samples were collected from the top 10cm of each pot using a corer (3 cm in diameter) at flowering and harvest of the first season and pre-planting and flowering of the second season. Soil samples were passed through a 4 mm- sieve, and subsamples were immediately stored at −20 °C until further processing.

16S rRNA Gene Sequencing and bioinformatics processing

DNA was extracted from each sample as described previously. PCR amplification of 16S rRNA gene fragments was conducted using the primers 341F (5’ CCTACGGGNGGCWGCAG) and 805R (5’ GACTACHVGGGTATCTAATCC). This PCR

primer produced paired reads of ~301bp, each targeting the V4 region of the 16S rRNA. Primer sequences were modified by adding Illumina overhang adapters: forward 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG and reverse 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG. PCR products were sequenced on the MiSeq platform (Illumina, San Diego, CA, USA) at Western Sydney University, Australia.

Raw sequences were processed following the Quantitative Insights into Microbial Ecology (QIIME) 1.7.0 (Caporaso, Kuczynski et al. 2010) to join the paired ends. After removing chimeras, uclust was used to pick operational taxonomic units (OTUs) at a 97% identity (Edgar 2010). Representative sequences from individual OTUs were then aligned using PyNAST (Caporaso, Bittinger et al. 2010) and assigned using ribosomal database project (RDP) Classifier (Wang, Garrity et al. 2007) based on the Greengenes database (DeSantis, Hugenholtz et al. 2006). The community composition of bacteria was described by the relative abundance of sequences on the phylum level. Taxa that accounted for a minor fraction of total bacteria (<1%) was not included in the downstream analyses. Alpha diversity was characterised by Shannon's diversity index.

Soil chemical analyses

Soil samples were analysed for the concentrations of ammonium, nitrate and phosphate, soil pH and relative water content. Please see the materials and methods in the previous sections for more detail.

Data analyses

Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarity matrices was used to visualise shifts in the bacterial community compositions based on the 97% OTU level across different treatments (Caporaso, Bittinger et al. 2010). The effects of CO_2, temperature, flooding/ drought and sampling time on the bacterial community composition were assessed by permutational ANOVA/MANOVA (PERMANOVA). All multivariate analyses were performed in Primer 6 PERMANOVA+ (PRIMER-E, Plymouth). The relationship between soil chemical properties and the relative abundance and community composition of the major bacterial phyla were explored using a Spearman's correlation analysis.

Results

Climate change impacts

Bacterial diversity

The diversity of the bacterial community assessed by Shannon's diversity index showed significant changes over time in both soils (*P*<0.0001 for both soils, Table 15). In grey vertosol, CO_2 and temperature interactively influenced the diversity (CO_2 x Temp interaction, *P*=0.03) where T_E generally increased the diversity, except at flowering in 2014 when T_E at C_E had the lowest diversity (CO_2 x Temp x Sampling interaction, *P*=0.07). In black vertosol, C_E significantly decreased the bacterial diversity (*P*<0.0001), and T_E decreased the diversity in 2013 but increased it in 2014 sampling (Temp x Sampling interaction, *P*=0.002).

Table 15 Changes in soil bacterial diversity (Shannon's diversity index) of grey vertosol and black vertosol planted with cotton under the four climate change treatments. Values are means of Shannon's diversity index with standard errors in parentheses. Results of analysis of variance (ANOVA) are also presented, showing the effects of CO_2, temperature and sampling time on the diversity index. Significant results (*P*<0.05) are shown in bold, marginally significant results (*P*<0.1) in italic and non-significant results (*P*>0.1) as n.s. The initial sampling data were excluded from this analysis, as CO_2 and temperature treatments were not applied to those samples.

			2013		2014	
Soil	Initial	Climate	Flowering	Harvest	Pre-planting	Flowering
Grey	9.6 (0.01)	C_AT_A	8.8 (0.16)	9.2 (0.07)	9.1 (0.05)	8.7 (0.22)
		C_AT_E	9.1 (0.08)	9.3 (0.08)	9.2 (0.05)	9.1 (0.04)
		C_ET_A	9.0 (0.04)	9.3 (0.08)	9.1 (0.06)	9.0 (0.10)
		C_ET_E	9.3 (0.07)	9.3 (0.06)	9.2 (0.07)	8.7 (0.10)
Black	9.4 (0.02)	C_AT_A	9.5 (0.03)	9.5 (0.09)	9.2 (0.07)	9.2 (0.04)
		C_AT_E	9.4 (0.05)	9.4 (0.06)	9.3 (0.04)	9.4 (0.05)
		C_ET_A	9.3 (0.09)	9.3 (0.10)	8.7 (0.13)	9.2 (0.03)
		C_ET_E	9.2 (0.09)	9.4 (0.05)	9.1 (0.06)	9.2 (0.05)

ANOVA results	Grey	Black
CO_2	n.s.	**<0.0001**
Temp	**0.02**	*0.09*
Sampling	**<0.0001**	**<0.0001**
CO_2 x Temp	**0.03**	n.s.
CO_2 x Sampling	n.s.	n.s.
Temp x Sampling	n.s.	**0.002**
CO_2 x Temp x Sampling	*0.07*	n.s.

Bacterial community composition

Bacterial community composition differed significantly between the two soils (PERMANOVA, *P*=0.0001), thus the impact of CO_2 and temperature were assessed separately.

In grey vertosol, the composition of the bacterial community was strongly influenced by sampling time (PERMANOVA, *P*=0.0001, Fig. 20, Table 16) and its effect magnified with time. While forming a clearly distinct group from the initial sampling, the bacterial composition at 2013 flowering and harvest were similar to each other. In 2014 though, the bacterial compositions at pre-planting and flowering were clearly separated from each other and from those of 2013 and the initial sampling.

The impacts of CO_2 and temperature treatments interacted with sampling time to affect the bacterial composition (CO_2 x temperature x sampling interaction, *P*=0.002). At flowering in 2013, the bacterial composition was strongly influenced by temperature (*P*=0.02) which also interacted with CO_2 (CO_2 x temperature interaction, *P*=0.03) in a way that the temperature effect on the bacterial composition was only evident at ambient temperature (T_A). This difference disappeared at harvest in 2013, as there were no differences in the bacterial compositions between the treatments. At pre-planting in 2014, the bacterial community composition was strongly influenced by temperature (*P*=0.001) and CO_2 (*P*=0.01) which also marginally interacted with each other (CO_2 x temperature interaction, *P*=0.05). The nature of this interaction however differed from that of flowering in 2013, and a stronger temperature effect was observed at T_E than T_A. At flowering in 2014, the bacterial community composition was again influenced by temperature (*P*=0.003) which also interacted with CO_2 (CO_2 x temperature interaction, *P*=0.02) in a similar way as flowering in 2013 where the temperature effect was greater at T_A than T_E.

In black vertosol, the composition of the bacterial community was also strongly influenced by sampling time (*P*=0.0001, Fig. 20) with the bacterial composition clearly separating between 2013 and 2014.

The impacts of CO_2 and temperature treatments interacted with sampling time to affect the bacterial composition (CO_2 x sampling interaction, *P*=0.01, temperature x sampling interaction, *P*=0.001). At flowering in 2013, CO_2 and temperature significantly influenced bacterial community composition (*P*=0.001 and *P*=0.03, respectively), with the bacterial composition clearly separating between C_E and T_E. At harvest in 2013, the CO_2 effect on bacterial composition was still evident (*P*=0.02), although it interacted with temperature

(CO_2 x temperature interaction, *P*=0.02) in a way that the effect of CO_2 was only evident at T_A and not at T_E. At pre-planting in 2014, the bacterial community composition differed considerably from that of flowering and harvest in 2013; however, the bacterial composition was still consistently influenced by CO_2 (*P*=0.001) and also by temperature (*P*=0.04). At flowering in 2014, however, the bacterial composition was influenced by CO_2 (*P*=0.001) and temperature (*P*=0.002) which marginally interacted with each other (*P*=0.10) with a stronger temperature effect observed in C_E than C_A.

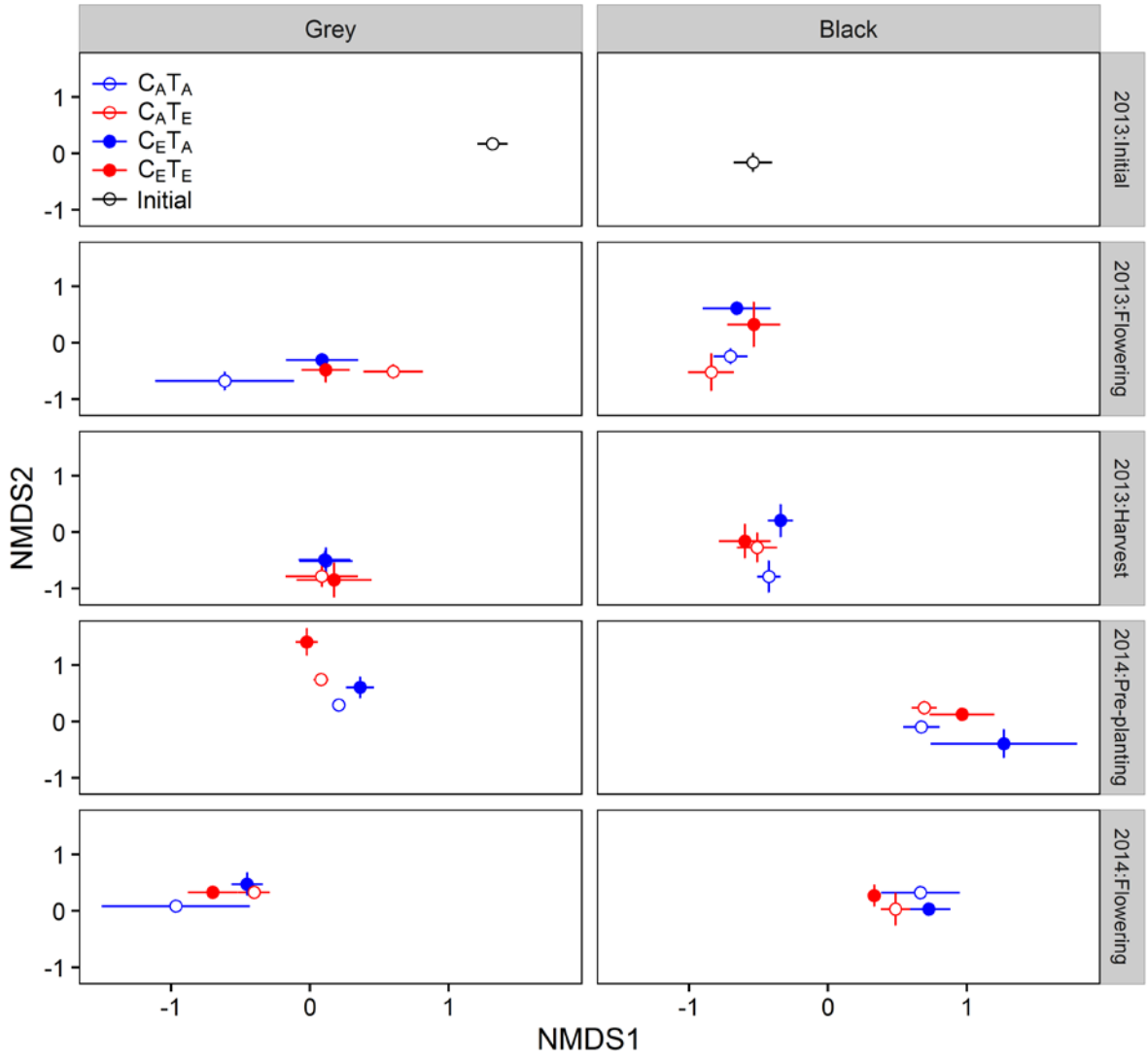


Fig. 20 Soil bacterial community composition of grey vertosol and black vertosol planted with cotton under the four climate change treatments measured at five sampling times over the two growing seasons.

Table 16 The results of permutational ANOVA/MANOVA (PERMANOVA), showing the effects of CO_2 and temperature on the bacterial community composition of grey and black vertosols planted with cotton plants. Significant results (P<0.05) are shown in bold, marginally significant results (P<0.1) in italic and non-significant results (P>0.1) as n.s. The initial sampling data were excluded from this analysis, as CO_2 and temperature treatments were not applied to those samples.

			2013		2014	
Soil	Source	Overall	Flowering	Harvest	Pre-planting	Flowering
Grey	CO_2	n.s.	n.s.	n.s.	**0.01**	n.s.
	Temp	**0.001**	**0.02**	n.s.	**0.001**	**0.003**
	CO_2 x Temp	n.s.	**0.03**	n.s.	*0.05*	**0.02**
	Sampling	**0.001**				
	CO_2 x Sampling	*0.06*				
	Temp x Sampling	**0.001**				
	CO_2 x Temp x Sampling	**0.002**				
Black	CO_2	*0.08*	**0.001**	**0.02**	**0.001**	**0.001**
	Temp	**0.02**	**0.03**	n.s.	**0.04**	**0.002**
	CO_2 x Temp	n.s.	n.s.	**0.02**	n.s.	*0.10*
	Sampling	**0.001**				
	CO_2 x Sampling	**0.01**				
	Temp x Sampling	**0.001**				
	CO_2 x Temp x Sampling	n.s.				

Relative abundance of the major bacterial phyla

The relative abundance of major bacterial phyla found in the initial soils of grey and black vertosols showed that both soils were dominated by *Actinobacteria,* followed by *Proteobacteria, Acidobacteria* and *Chloroflexi,* which together comprised more than 75% of bacterial abundance in these soils (Fig. 21).

The relative abundance of major bacterial phyla changed significantly over time in both soils, except for the abundance of *Verrucomicrobia* in both soils and *Acidobacteria* in black vertosol (Table 17, Fig. 22). In grey vertosol, the relative abundance of phyla such as *Nitrospirae*, *Planctomycetes, Gemmatimonadetes*, *Firmicutes* and *Acidobactetria* showed some responses to CO_2 and temperature treatments, however, the strong sampling effect and its interaction with CO_2 and/or temperature treatment meant that the direction and the magnitude of those responses were highly variable with time. In black vertosol, the effects of CO_2 and temperature treatments were generally observed in different phyla to those of grey vertosol; *Actinobacteria*, *Proteobacteria*, *Fimicutes* and *Bacteroidetes* responded to CO_2 and temperature treatments in black vertosol, as well as *Planctomycetes* and *Gemmatimonadetes* that also responded in grey vertosol.

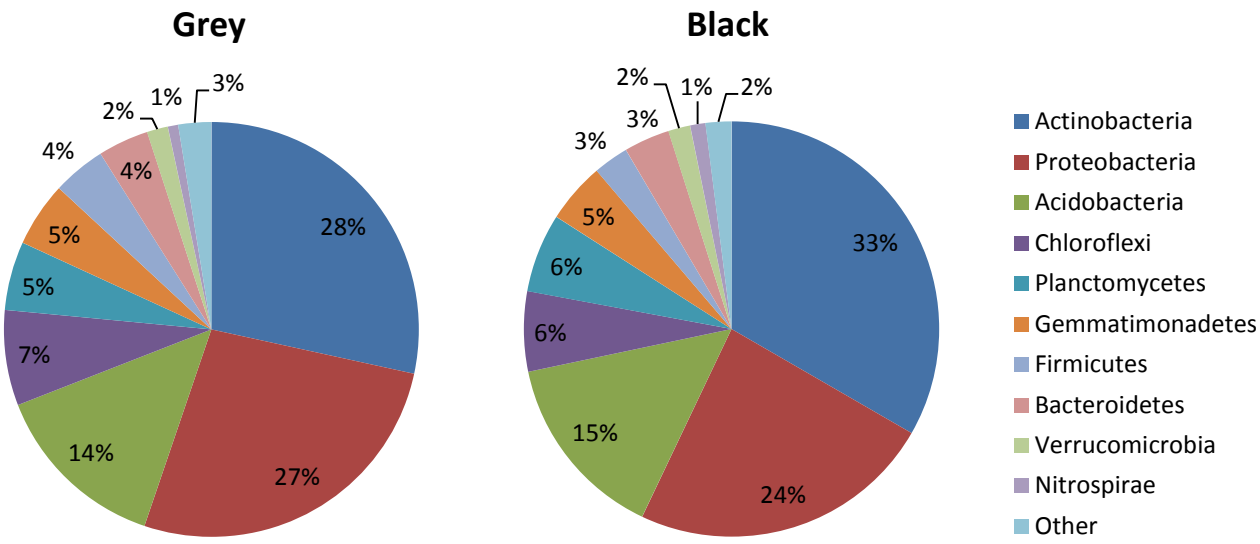


Fig. 21 The relative abundance of the major bacterial phyla found in the initial grey and black vertosols prior to the application of CO_2, temperature and extreme weather treatments.

Table 17 The results of analysis of variance showing the effects of CO_2, temperature and sampling time on the relative abundance of the major bacterial phyla of grey and black vertosols planted with cotton plants. Values are probability with significant results (P<0.05) shown in bold, marginally significant results (P<0.1) in italics and non-significant results (P>0.1) as n.s.

	CO_2	Temp	Sampling	CO_2 x Temp	CO_2 x Sampling	Temp x Sampling	CO_2 x Temp x Sampling
Grey							
Actinobacteria	n.s.	n.s.	**<0.0001**	n.s.	n.s.	**0.04**	n.s.
Proteobacteria	n.s.	n.s.	**<0.0001**	n.s.	n.s.	n.s.	n.s.
Acidobacteria	*0.05*	n.s.	**<0.0001**	**0.01**	n.s.	n.s.	n.s.
Chloroflexi	n.s.	n.s.	**<0.0001**	n.s.	**0.03**	**0.03**	n.s.
Planctomycetes	n.s.	**0.02**	**<0.0001**	**0.04**	n.s.	**<0.0001**	n.s.
Gemmatimonadetes	**0.04**	n.s.	**<0.0001**	**0.01**	n.s.	n.s.	n.s.
Firmicutes	n.s.	n.s.	**0.0001**	**0.03**	n.s.	n.s.	n.s.
Bacteroidetes	n.s.	n.s.	**<0.0001**	n.s.	**0.04**	n.s.	*0.09*
Verrucomicrobia	n.s.	n.s.	n.s.	*0.07*	n.s.	*0.08*	n.s.
Nitrospirae	**0.03**	**0.001**	**<0.0001**	n.s.	n.s.	n.s.	n.s.
Other	n.s.	n.s.	**<0.0001**	**0.01**	**0.03**	**0.002**	**0.01**
Black							
Actinobacteria	**0.01**	n.s.	**<0.0001**	n.s.	n.s.	n.s.	*0.08*
Proteobacteria	**0.01**	n.s.	**0.04**	n.s.	n.s.	n.s.	n.s.
Acidobacteria	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Chloroflexi	n.s.	n.s.	**<0.0001**	n.s.	*0.08*	n.s.	**0.02**
Planctomycetes	n.s.	**0.003**	**<0.0001**	n.s.	n.s.	*0.06*	n.s.
Gemmatimonadetes	n.s.	*0.08*	**0.0002**	**0.003**	n.s.	n.s.	*0.07*
Firmicutes	**0.02**	n.s.	**<0.0001**	n.s.	n.s.	n.s.	n.s.
Bacteroidetes	**0.03**	n.s.	**<0.0001**	n.s.	n.s.	n.s.	n.s.
Verrucomicrobia	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Nitrospirae	n.s.	n.s.	**0.001**	n.s.	n.s.	n.s.	n.s.
Other	*0.07*	n.s.	**<0.0001**	n.s.	n.s.	n.s.	n.s.

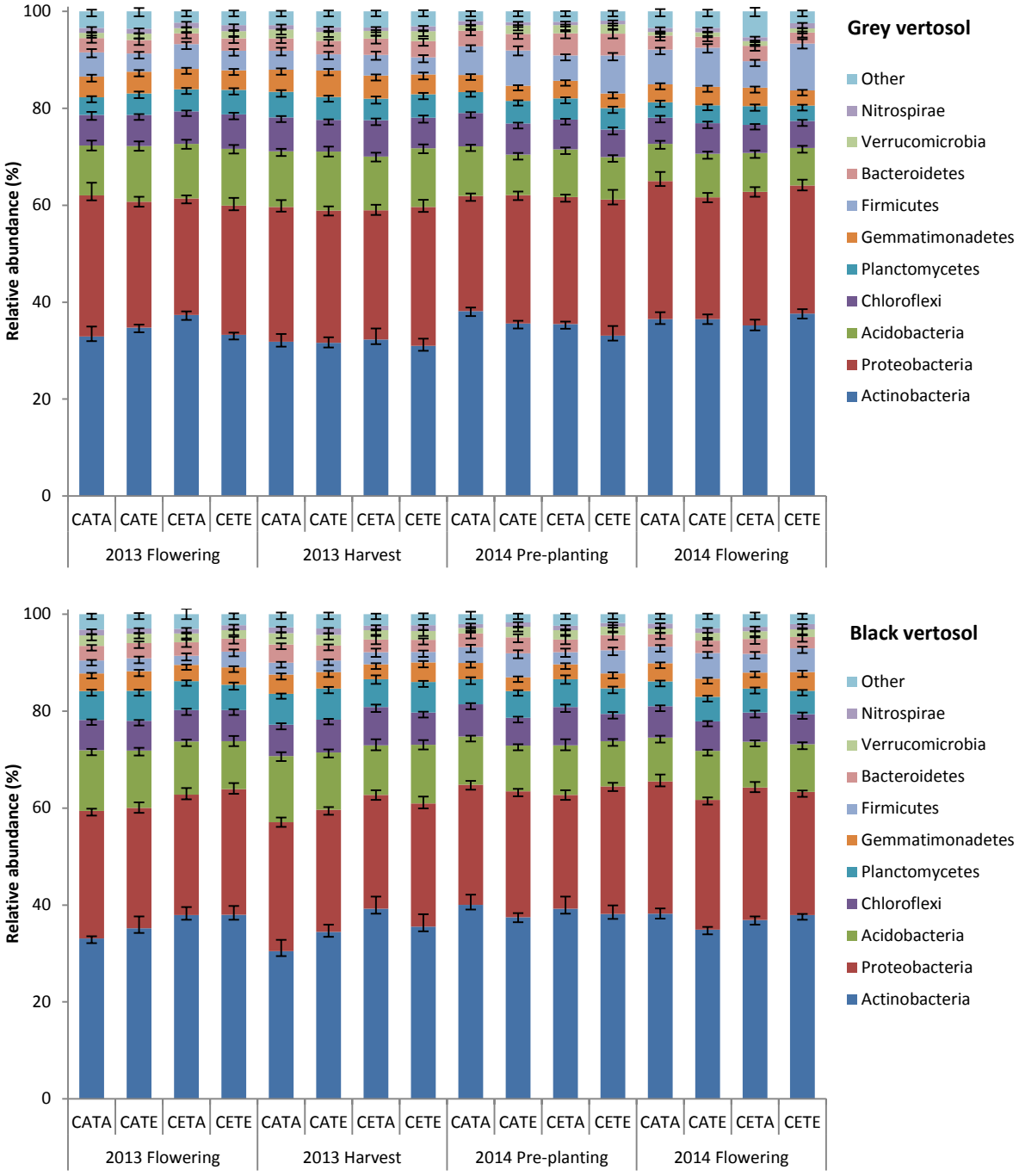


Fig. 22 The relative abundance of the major phyla in grey vertosol (top) and black vertosol (bottom) planted with cotton under the four climate change treatments measured at four sampling times over the two growing seasons.

Correlations between bacterial phyla and soil properties

Correlation analyses between soil chemical properties and the relative abundance of the major bacterial phyla, the composition of the overall bacterial community and the major bacterial phyla (based on NMDS analyses) showed that soil chemical properties strongly correlated with both the abundance and community composition of the major bacterial phyla in both soils (Table 18). In grey vertosol, soil pH correlated with the abundance and composition of bacterial phyla, but ammonium, phosphate and nitrate also correlated with the bacterial phyla. In black vertosol, however, soil ammonium dominantly correlated the most with the abundance and composition of the bacterial phyla, and only a few correlations were observed with other soil properties.

Table 18 Spearman's correlation between soil chemical properties and the relative abundance of the major bacterial phyla, the composition of the overall bacterial community and the major bacterial phyla (based on NMDS analyses). Significant levels are: **** $P<0.0001$, *** $P<0.001$, ** $P<0.01$, * $P<0.05$.

Spearman's rho	Grey vertosol					Black vertosol				
	Soil pH	RWC	Nitrate	Ammonium	Phosphate	Soil pH	RWC	Nitrate	Ammonium	Phosphate
Relative abundance										
Actinobacteria	0.32 **	0.06	-0.06	-0.36 ***	-0.32 **	0.10	0.07	-0.06	-0.52 ****	-0.05
Proteobacteria	-0.25 *	-0.13	0.30 **	0.21 *	-0.01	-0.03	-0.09	0.04	0.39 ***	0.07
Acidobacteria	0.27 *	-0.22 *	-0.24 *	0.20	0.10	0.07	-0.06	-0.06	-0.11	-0.13
Chloroflexi	0.42 ****	0.07	-0.12	-0.24 *	-0.18	0.19	0.09	-0.26 *	-0.33 **	0.26 *
Planctomycetes	-0.32 **	-0.22 *	0.19	0.39 ***	0.27 **	-0.13	-0.24 *	0.16	0.46 ****	-0.17
Gemmatimonadetes	0.14	0.44 ****	-0.21 *	-0.43 ****	-0.07	-0.04	0.21	0.14	-0.27 *	0.19
Firmicutes	-0.31 **	0.11	0.23 *	-0.16	-0.14	0.21	0.13	-0.15	-0.47 ****	0.00
Bacteroidetes	0.53 ****	-0.07	-0.16	-0.17	-0.32 **	0.33 **	0.09	-0.21	-0.56 ****	0.09
Verrucomicrobia	0.01	0.12	-0.23 *	0.02	0.24 *	-0.20	-0.02	0.07	0.08	-0.07
Nitrospirae	0.64 ****	-0.22 *	-0.43 ****	-0.11	-0.28 **	0.24 *	0.04	-0.17	-0.34 **	-0.06
Other	-0.18	0.55 ****	-0.06	-0.27 *	0.08	-0.10	0.32 **	0.06	-0.44 ****	-0.01
Community composition										
Overall	-0.29 **	-0.27 **	0.11	0.46 ****	0.36 ***	0.03	-0.30 **	-0.04	0.57 ****	-0.02
Actinobacteria	0.28 **	-0.26 *	-0.33 **	0.36 ***	0.25 *	0.00	-0.30 **	0.00	0.56 ****	-0.09
Proteobacteria	-0.22 *	0.23 *	-0.21 *	-0.14	0.23 *	0.01	0.26 *	-0.05	-0.54 ****	0.03
Acidobacteria	-0.29 **	-0.02	0.02	0.29 **	0.35 ***	-0.14	-0.14	0.11	0.45 ****	0.13
Chloroflexi	-0.33 **	-0.01	-0.02	0.26 *	0.33 **	0.05	0.01	0.02	0.26 *	-0.13
Planctomycetes	0.42 ****	-0.21	0.01	-0.04	-0.39 ***	0.17	0.07	-0.22	-0.56 ****	0.14
Gemmatimonadetes	0.10	0.27 *	-0.33 **	-0.35 ***	0.15	-0.22 *	-0.16	0.17	0.36 ***	0.11
Firmicutes	0.27 *	0.24 *	-0.15	-0.38 ***	-0.27 **	-0.12	-0.33 **	0.14	0.55 ****	-0.20
Bacteroidetes	0.47 ****	-0.22 *	0.00	0.16	-0.28 **	-0.02	0.23 *	-0.04	-0.54 ****	-0.04
Verrucomicrobia	-0.42 ****	0.17	0.17	0.21	0.31 **	0.12	0.20	-0.10	-0.48 ****	0.00
Nitrospirae	0.37 ***	-0.14	-0.19	0.17	0.05	-0.20	-0.13	0.13	-0.06	0.03

Extreme weather impacts

Bacterial diversity

Flooding impact on the bacterial diversity was generally small (Table 19). In grey vertosol, flooding interacted with temperature and sampling (Water x Temp x Sampling interaction, *P*=0.07) to marginally influence the diversity, although a highly interactive nature of their effects makes it difficult to see a clear pattern in the response. In black vertosol, flooding interacted with CO_2, temperature and sampling (Water x CO_2 x Temp x Sampling interaction, *P*=0.02), therefore no consistent pattern of flooding effect were observed.

Table 19 The impact of flooding and drought on the bacterial diversity (Shannon's diversity index) of grey vertosol and black vertosol planted with cotton under the four climate change treatments over two seasons. Values are relative changes in the Shannon's diversity index calculated against well-watered control treatments. Results of analysis of variance (ANOVA) are also presented, showing the effects of water (flooding/drought), CO_2, temperature and sampling time on the diversity index. Significant results (*P*<0.05) are shown in bold, marginally significant results (*P*<0.1) in italic and non-significant results (*P*>0.1) as n.s.

		Flooding effect (%)				Drought effect (%)			
		2013		2014		2013		2014	
Soil	Climate	Flowering	Harvest	Pre-planting	Flowering	Flowering	Harvest	Pre-planting	Flowering
Grey	C_AT_A	5.5	0.9	-1.1	0.9	3.8	-0.4	-6.1	0.9
	C_AT_E	-0.3	1.9	2.3	0.9	0.7	-0.5	-4.6	-2.1
	C_ET_A	-0.2	-0.9	0.6	0.7	1.3	-1.6	-5.5	-3.0
	C_ET_E	-3.1	2.5	1.8	3.1	-0.6	-1.4	-5.5	-0.2
Black	C_AT_A	-2.7	-0.7	0.2	0.7	-1.2	-1.8	-4.0	-0.5
	C_AT_E	-1.3	-0.9	1.1	-0.2	0.7	-1.3	-4.1	-3.2
	C_ET_A	-2.7	1.7	5.2	0.7	1.4	1.6	0.2	-2.7
	C_ET_E	1.5	1.0	0.8	0.4	1.3	-1.1	-1.7	-1.1

ANOVA results	Well-watered vs Flooded		Well-watered vs Drought	
	Grey	Black	Grey	Black
Water	*0.06*	n.s.	**0.0001**	**0.001**
Water x CO_2	n.s.	**0.02**	n.s.	**0.02**
Water x Temp	n.s.	n.s.	n.s.	n.s.
Water x Sampling	n.s.	**0.01**	**0.01**	**0.004**
Water x CO_2 x Temp	n.s.	n.s.	n.s.	n.s.
Water x CO_2 x Sampling	n.s.	n.s.	n.s.	n.s.
Water x Temp x Sampling	*0.07*	n.s.	n.s.	n.s.
Water x CO_2 x Temp x Sampling	n.s.	**0.02**	n.s.	n.s.
CO_2	**0.05**	**0.0002**	n.s.	**0.02**
Temp	**<0.0001**	**0.05**	n.s.	n.s.
Sampling	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
CO_2 x Temp	**0.002**	n.s.	**0.05**	n.s.
CO_2 x Sampling	n.s.	*0.09*	n.s.	n.s.
Temp x Sampling	n.s.	**0.01**	*0.07*	n.s.
CO_2 x Temp x Sampling	**0.01**	**0.05**	**0.05**	n.s.

Unlike the flooding impact, drought had a stronger effect on the bacterial diversity in both soils (*P*=0.0001 and *P*=0.001 in grey and black vertosols respectively), where drought reduced the bacterial diversity particularly from harvest in 2013 onwards (Water x Sampling interaction, *P*=0.01 and *P*=0.004 for grey and black vertosols respectively).

Bacterial community composition

The impacts of flooding were also evident in the bacterial community composition in both soils (Fig. 23, 24, Table 20). In grey vertosol, flooding significantly influenced the bacterial community composition (*P*=0.002), which also interacted with sampling time (Flooding x Sampling interaction, *P*=0.001) with the magnitude of flooding effects slightly reduced at flowering in 2014. Flooding effect did not interact with CO_2 or temperature, except at pre-planting in 2014, flooding effect interacted with temperature to significantly impact the bacterial community composition (Flooding x Temp x Sampling interaction, *P*=0.02). Flooding also significantly influenced the bacterial community composition in black vertosol (*P*=0.002), which also interacted with sampling time (Flooding x Sampling interaction, *P*=0.001) with the magnitude of flooding effects increased in 2014 sampling. Flooding effect significantly interacted with CO_2, temperature and sampling (*P*=0.05) where flooding marginally interacted with CO_2 (Flooding x CO_2 interaction, *P*=0.06) and temperature (Flooding x Temp interaction, *P*=0.08) at pre-planting in 2014 to influence the bacterial community composition.

The impacts of drought were also evident in the bacterial community composition in both soils (Fig. 23, 24, Table 21) with its effect generally stronger than that of flooding effect. Drought effects were particularly small at flowering in 2013 in both soils, suggesting that the drought treatment had not been applied long enough to significantly influence the bacterial community composition. In grey vertosol, the drought effect interacted with CO_2, temperature and sampling (*P*=0.01), and particularly strong interactions with CO_2 and temperature were observed at pre-planting in 2014. At flowering in 2014, the drought impact on bacterial community was still evident (*P*=0.001), particularly at T_A (Drought x Temp interaction, *P*=0.001). In black vertosol, drought effect also interacted with CO_2, temperature and sampling (*P*=0.001), with the bacterial community composition being most influenced by drought and its interactions with CO_2 and temperature at pre-planting and flowering in 2014. Similar to the grey vertosol, drought impact on bacterial community was still evident at flowering in 2014 (*P*=0.001), particularly at T_A (Drought x Temp interaction, *P*=0.01).

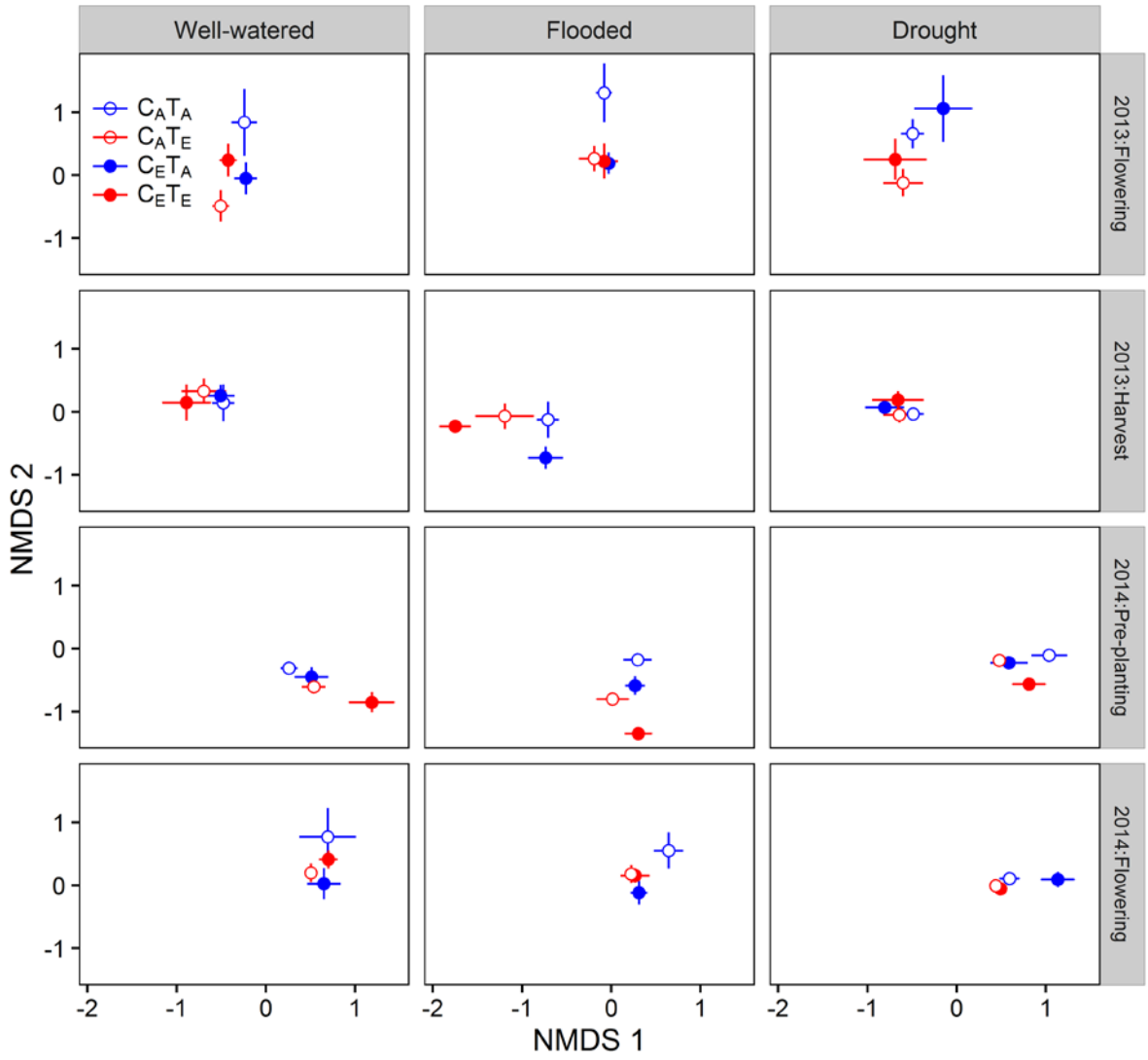


Fig. 23 The impact of flooding and drought on soil bacterial community composition of grey vertosol planted with cotton under the four climate change treatments measured at four sampling times over the two growing seasons.

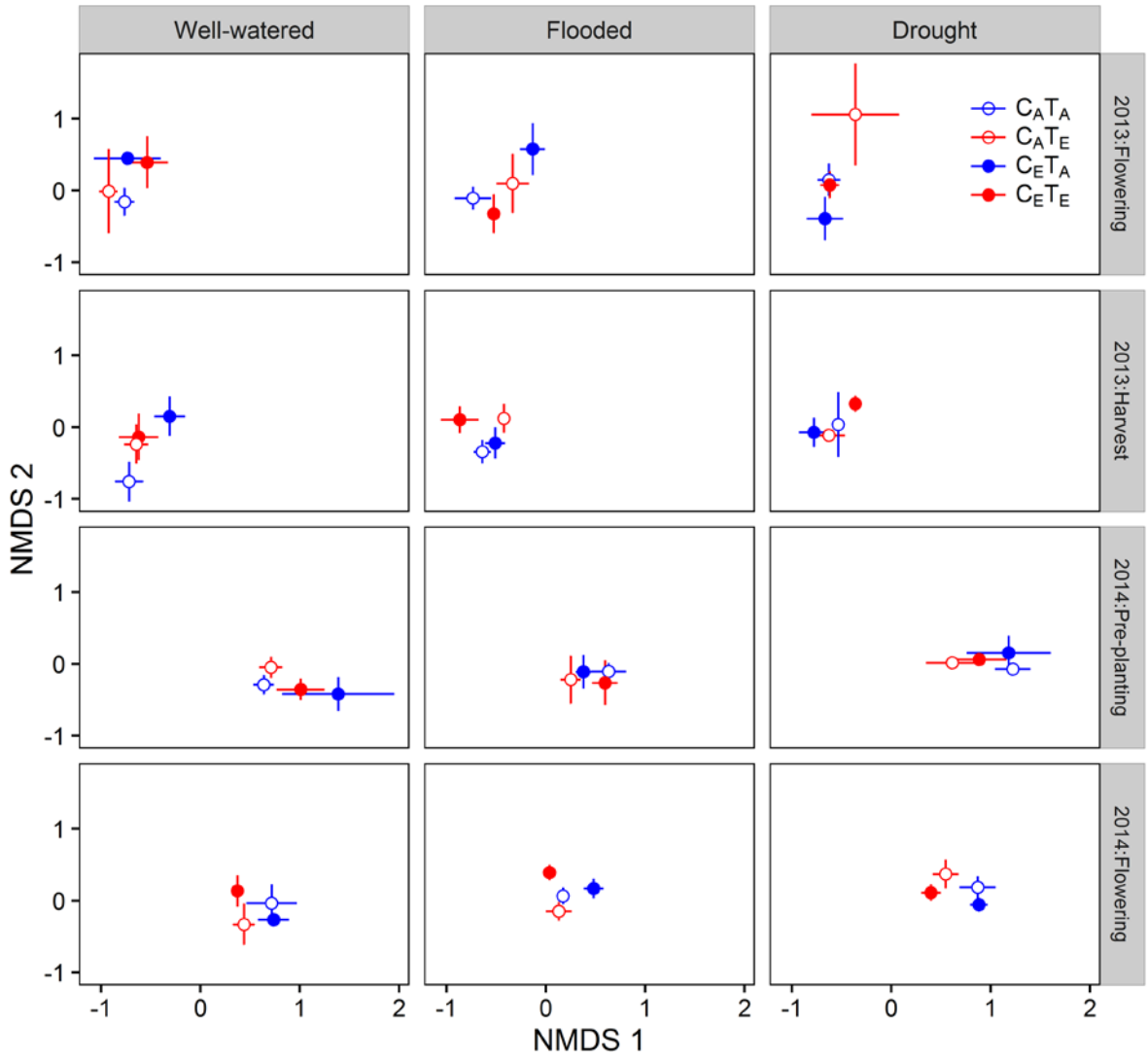


Fig. 24 The impact of flooding and drought on soil bacterial community composition of black vertosol planted with cotton under the four climate change treatments measured at four sampling times over the two growing seasons.

Table 20 The results of permutational ANOVA/MANOVA (PERMANOVA), showing the main and interactive effects of flooding, CO_2 and temperature on the bacterial community composition of grey and black vertosols planted with cotton plants. Significant results ($P<0.05$) are shown in bold, marginally significant results ($P<0.1$) in italic and non-significant results ($P>0.1$) as n.s. The initial sampling data were excluded from this analysis, as CO_2 and temperature treatments were not applied to those samples.

	Grey vertosol					Black vertosol				
		2013		2014			2013		2014	
Source	Overall	Flowering	Harvest	Pre-planting	Flowering	Overall	Flowering	Harvest	Pre-planting	Flowering
Flooding effect										
Flooding	**0.002**	**0.004**	**0.003**	**0.001**	**0.03**	**0.002**	**0.01**	**0.05**	**0.001**	**0.002**
Flooding x CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*0.06*	n.s.
Flooding x Temp	*0.06*	n.s.	n.s.	**0.01**	n.s.	n.s.	n.s.	n.s.	*0.08*	n.s.
Flooding x CO_2 x Temp	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Flooding x Sampling	**0.001**					**0.001**				
Flooding x CO_2 x Sampling	n.s.					*0.05*				
Flooding x Temp x Sampling	**0.02**					*0.07*				
Flooding x CO_2 x Temp x Sampling	n.s.					**0.05**				
Non-flooding effect										
CO_2	**0.01**	**0.01**	*0.09*	**0.001**	**0.03**	**0.01**	**0.01**	n.s.	**0.001**	*0.10*
Temp	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.004**	n.s.	*0.06*	**0.001**	**0.001**
CO_2 x Temp	**0.002**	**0.002**	**0.04**	**0.04**	**0.002**	n.s.	**0.02**	*0.07*	n.s.	**0.03**
Sampling	**0.001**					**0.001**				
CO_2 x Sampling	**0.001**					**0.002**				
Temp x Sampling	**0.001**					**0.001**				
CO_2 x Temp x Sampling	**0.001**					**0.003**				

Table 21 The results of permutational ANOVA/MANOVA (PERMANOVA), showing the main and interactive effects of drought, CO_2 and temperature on the bacterial community composition of grey and black vertosols planted with cotton plants. Significant results ($P<0.05$) are shown in bold, marginally significant results ($P<0.1$) in italic and non-significant results ($P>0.1$) as n.s. The initial sampling data were excluded from this analysis, as CO_2 and temperature treatments were not applied to those samples.

	Grey vertosol					Black vertosol				
		2013		2014			2013		2014	
Source	Overall	Flowering	Harvest	Pre-planting	Flowering	Overall	Flowering	Harvest	Pre-planting	Flowering
Drought effect										
Drought	**0.001**	n.s.	**0.001**	**0.001**	**0.001**	**0.001**	n.s.	**0.01**	**0.001**	**0.001**
Drought x CO_2	n.s.	n.s.	n.s.	**0.01**	n.s.	n.s.	**0.05**	n.s.	*0.09*	n.s.
Drought x Temp	**0.001**	**0.03**	n.s.	**0.001**	**0.001**	*0.08*	n.s.	n.s.	**0.03**	**0.01**
Drought x CO_2 x Temp	n.s.	n.s.	n.s.	**0.001**	*0.09*	n.s.	**0.02**	*0.08*	n.s.	n.s.
Drought x Sampling	**0.001**					**0.001**				
Drought x CO_2 x Sampling	**0.002**					*0.06*				
Drought x Temp x Sampling	**0.001**					**0.02**				
Drought x CO_2 x Temp x Sampling	**0.01**					**0.001**				
Non-Drought effect										
CO_2	*0.06*	*0.09*	n.s.	**0.01**	*0.08*	n.s.	n.s.	**0.03**	**0.03**	**0.05**
Temp	**0.001**	**0.02**	n.s.	**0.001**	**0.001**	**0.03**	n.s.	n.s.	**0.001**	**0.004**
CO_2 x Temp	*0.06*	**0.01**	n.s.	**0.01**	**0.004**	n.s.	n.s.	n.s.	n.s.	n.s.
Sampling	**0.001**					**0.001**				
CO_2 x Sampling	**0.01**					**0.001**				
Temp x Sampling	**0.001**					**0.001**				
CO_2 x Temp x Sampling	**0.001**					n.s.				

Correlations between bacterial phyla and soil properties

Correlation analyses between soil chemical properties and the relative abundance of the major bacterial phyla and the composition of the overall bacterial community (based on NMDS analyses) showed that soil chemical properties strongly correlated with both the abundance and community composition of the bacterial community (Table 22). While the strong correlations with soil pH and ammonium that were observed in well-watered control treatments (see Table 18) were still evident, soil nitrate and relative water content, which did not show strong correlations in the well-watered control subset, also correlated with the relative abundance and the overall bacteria composition. This provides further evidence that the relative abundance of the major bacterial phyla and the overall composition were influenced by flooding and drought treatments that have been shown to significantly impact soil water content and soil nitrate availability (Fig. S3, S5).

Table 22 Spearman's correlation between soil chemical properties and the relative abundance of the major bacterial phyla and the composition of the overall bacterial community (based on NMDS analyses) of well-watered, flooded and drought soils planted with cotton under the four climate change treatments over two seasons. Significant levels are: **** $P<0.0001$, *** $P<0.001$, ** $P<0.01$, * $P<0.05$.

Spearman's rho	Grey vertosol					Black vertosol				
	Soil pH	RWC	Nitrate	Ammonium	Phosphate	Soil pH	RWC	Nitrate	Ammonium	Phosphate
Relative abundance										
Actinobacteria	0.27 ****	0.01	-0.11	-0.27 ****	-0.29 ****	0.18 **	0.16 **	-0.17 **	-0.21 ***	-0.09
Proteobacteria	-0.13 *	-0.24 ****	0.26 ****	0.37 ****	0.03	-0.13 *	-0.26 ****	0.12 *	0.31 ****	0.05
Acidobacteria	0.16 **	0.01	-0.13 *	-0.02	0.07	0.15 *	0.05	-0.05	-0.11	-0.07
Chloroflexi	0.22 ***	0.01	-0.07	-0.15 *	-0.09	0.06	0.05	-0.12 *	-0.20 **	0.19 **
Planctomycetes	-0.27 ****	-0.08	0.15 *	0.34 ****	0.22 ***	-0.15 *	-0.06	0.15 *	0.12	-0.17 **
Gemmatimonadetes	0.19 **	0.50 ****	-0.37 ****	-0.52 ****	-0.22 ***	0.11	0.42 ****	-0.19 **	-0.32 ****	0.06
Firmicutes	-0.21 ***	0.17 **	0.08	-0.21 ***	-0.12	0.09	0.22 ***	-0.13 *	-0.21 ***	0.04
Bacteroidetes	0.33 ****	-0.10	-0.05	-0.16 **	-0.17 **	0.22 ***	0.09	-0.16 *	-0.27 ****	0.09
Verrucomicrobia	-0.03	0.18 **	-0.19 **	-0.20 ***	0.12 *	-0.07	0.07	0.01	-0.07	0.02
Nitrospirae	0.38 ****	-0.14 *	-0.15 *	-0.17 **	-0.13 *	0.20 **	0.07	-0.17 **	-0.15 *	0.03
Other	-0.09	0.40 ****	-0.18 **	-0.39 ****	0.00	0.10	0.33 ****	-0.10	-0.43 ****	0.07
Community composition										
Overall	-0.25 ****	-0.14 *	0.18 **	0.43 ****	0.28 ****	-0.12 *	-0.29 ****	0.14 *	0.38 ****	-0.03

Discussion / conclusions

Climate change impact

We found that:

- Soil bacterial communities of grey and black vertosols planted with cotton over the two growing seasons showed temporal changes in their diversity, overall community composition and the relative abundance of the major bacterial phyla, most likely reflecting their interactions with plants and their activity at the time of sampling.
- We found that the bacterial community composition was different between the two soils, and their responses to CO_2 and temperature treatments also differed in their direction, magnitude and timing.
- The response of the bacterial community to CO_2 and temperature treatments was particularly strong at pre-planting in the second season.
- As the non-harvestable plant biomass was incorporated into the soil after the harvest in 2013, this suggests that this response may have been driven by changes in plant residue quality induced by CO_2 and temperature treatments in the previous season.
- Furthermore, relatively small effects of CO_2 and temperature treatments on the bacterial composition at harvest when plants were relatively inactive further suggest that the effects of CO_2 and temperature on soil bacterial community are strongly influenced by plant responses to CO_2 and temperature treatments.

Extreme weather impact

We found that:

- Flooding and drought events applied in the first season showed both immediate and long-lasting effects on the bacterial diversity and community composition.
- While the correlation analyses revealed that changes in soil nutrients associated with flooding and drought impacts were linked to changes in the abundance and composition of the bacterial community, the long-term effects of flooding and drought events on the bacterial community suggest that the changes in the bacterial community in turn will affect soil functions including nutrient availability, as evident in the strong correlations between soil nutrient availability and the abundance and composition of the bacterial community.

New Knowledge for extension and Farmers

- Together with the changes in cotton productivity response to CO_2 and temperature observed in the second season (see Expt. 2), our data suggest that the response of the soil microbial community to elevated CO_2, temperature and extreme weather events plays a key role in determining soil nutrient availability that ultimately influence cotton productivity.
- Soil microbial communities play an important role in minimising impact of climate change and extreme weather events on cotton productivity. Thus, the management practice which promotes soil health and microbes should be adopted for improved and sustainable crop production.

*** The following two experiments were conducted as a part of Ms Nguyen's PhD thesis.**

Expt. 4 – Field waterlogging experiments (PhD thesis)

Impacts of water logging on soil nitrification and ammonia-oxidizing communities in cotton farming.

Background and aims

Cotton, a valuable industrial crop contributing to the world fiber production, is immensely dependent on nitrogen supply to maintain high productivity. A large amount of nitrogen fertilizer ranging from 240-270 kg N ha^{-1} has been estimated to optimize the productivity of Australian cotton crops (Rochester & Constable, 2015). In the context of increasing frequency and intensity of extreme weather events such as flooding, cotton industry is expected to face the risk of reduced crop productivity as a consequence of soil nitrogen depletion and altered plant physiological processes including declined photosynthesis, transpiration and radiation-use efficiency (Sahay, 1989; Bange *et al*., 2004). Upland cotton plants (*Gossypium Hirsutum* L.) are mainly grown on heavy clay soils (vertosol) with very low rates of drainage (Hodgson & Chan, 1982) under furrow irrigation systems in Australia. Such conditions may promote waterlogging events in the case of poor irrigation management following substantial precipitation (Bange *et al*., 2004).

Waterlogging alters soil O_2 concentration as a factor controlling nitrification process. In particular, excessive water content in the soil upon waterlogging decreases O_2 diffusion capacity, leading to hypoxic or even anoxic environments that inhibit the activity of nitrifying communities, resulting in depleted soil nitrogen availability that will negatively affect nitrogen-dependent cotton crop productivity. The rate of nitrification process has been reported to decrease in the response to waterlogging conditions (Patrick & Reddy, 1975); however, the underlying mechanism of whether and how ammonia-oxidizing communities responding to waterlogging stress still remains unclear. Therefore, in this study, cotton was used as a model crop to explore the response of ammonia-oxidizing communities and nitrification rate to waterlogging. I hypothesized that waterlogging will create unfavorable conditions affecting ammonia-oxidizing communities, thereby changing nitrification rate.

Materials and Methods

Field site

This field experiment was conducted at the Australian Cotton Research Institute (ACRI) at Narrabri (30.31^{o}S, 149.78^{o}E) in north-west New South Wales, Australia. This region, a semiarid ecosystem, holds hot summers with maximum and minimum daily temperature of 35^{o}C and 18^{o}C, respectively. Annual rainfall is about 644 mm, of which one third falls in summer months (Bureau of Meteorology, NSW). The soil is cracking grey clay soil (vertosols) and alkaline with pH 7.5-8.0, sorted Ug 5.25 as Northcote classification.

Cotton cultivation

The CSIRO cultivar Sicot BRF71 was used in this experiment. Cotton was planted on ridges spaced 1 meter apart separated by a furrow in which water was applied as furrow-flood irrigation. There was 4 times of irrigation in total and each time of irrigation provided approximately 90-100 mm water to follow normal agronomic practices. Nitrogen was applied as anhydrous ammonia before sowing at a rate of 180 kg N/ha.

Experimental design

The experimental field was divided into 4 blocks, with each block consists of three plots. Each plot was 160 meter long and contained 8 rows; each row was 1 meter (Fig. 25). Waterlogging was simulated by running furrow irrigation for 120 hours (Fig. 26). There were two different times of water treatment. The first waterlogging event (WL1) was applied when cotton plants were in the early flowering stage, whereas the second event (WL2) was applied at the final stage of flowering. In particular, WL1 began on 16 January 2014 and ended 21 January 2014, and WL2 began on 7 February 2014 and ended 11 February 2014.

During the season, there were 4 times of regular furrow irrigation and each run for 8 hours. Such irrigations were applied on 16 and 29 January, 7 and 21 February, 2014.

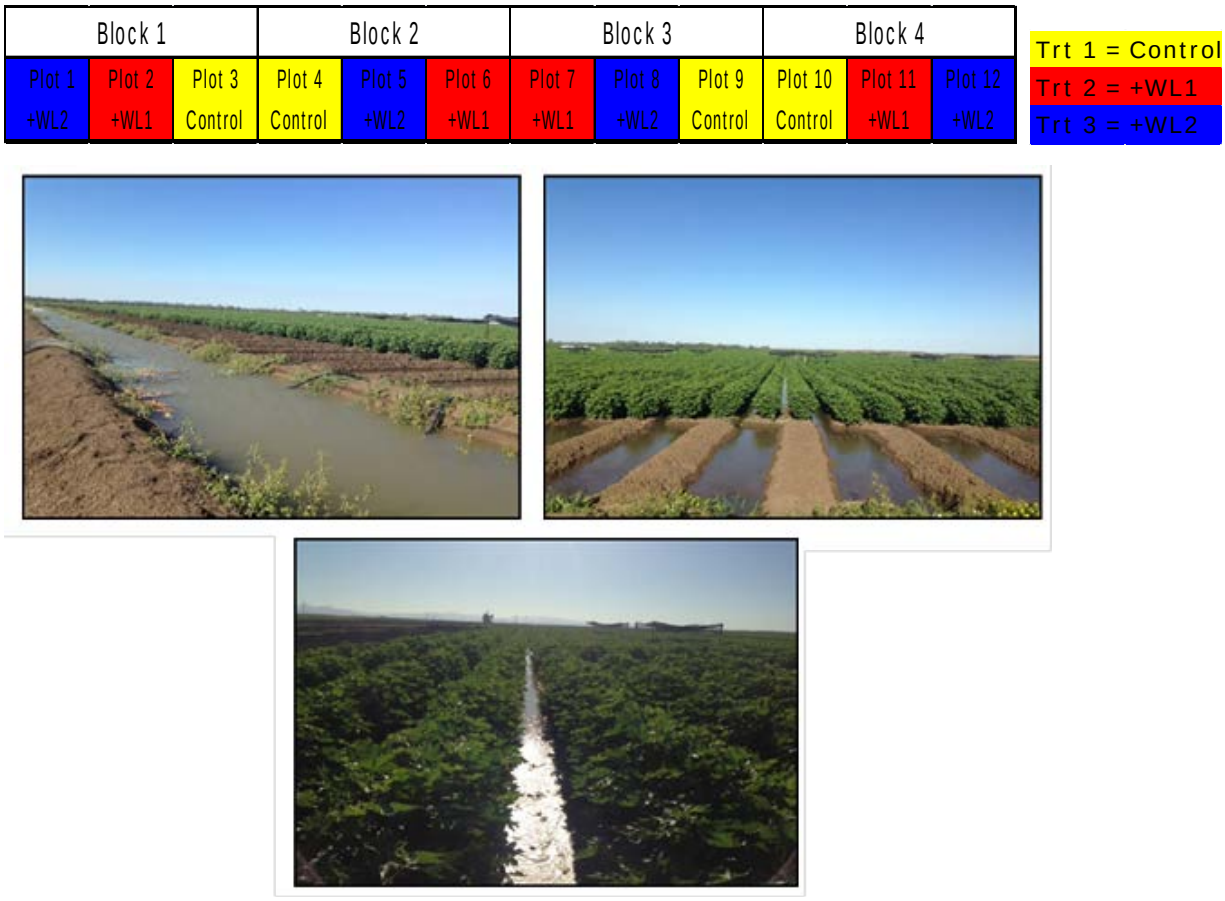


Fig. 25 Detailed experimental design: the field layout of treatments in Narrabri, NSW, and photo of cotton field at Narrabri when soil was subjected to the first waterlogging event by running furrow irrigation for 120 hours.

Soil sampling

Soil cores were taken from the field at the depth of about 10 cm. Samples were collected before and after treatment applied, then transferred to Soil laboratory, Hawkesbury Institute for the Environment (HIE), Western Sydney University for further analysis. In HIE, soil samples were homogenized and sieved through 4 mm mesh, and then kept in cold room (4^{o}C) for chemical analyses. Subsamples of soil were kept at -20^{o}C for molecular analyses.

Soil physicochemical analyses

Soil gravimetric water content was determined by drying 3 grams of fresh soil samples in oven at 105^{o}C for 24 hours. For soil pH, a suspension of fresh soil and mili-Q water with the ratio 1:5 was shaking for 1 hour, and then measured by a pH meter (Seve-nEasy pH, Metler,

Toledo, Switzerland). Soil NH_4^+ and NO_3^- were measured by taking 5 gram of fresh soil samples and then mix with 50 ml of 2M KCl. After that, the mixture was shaken at 180 rpm for 1 hour, and then filtered by Whatman no 42. The NH_4^+ and NO_3^-concentration of filtered solution were analyzed by a SEAL AQ2 discrete analyzer (SEAL analytical Inc., USA). In order to determine soil total N and C, fresh soil samples were dried at 40^{o}C for at least 72 hours, then ground prior to submit to analyze % C and N by a LECO macro - CN analyzer (LECO, USA).

Potential nitrification rate

Potential nitrification rate was determined according to the chlorate inhibition method from Kandeler (1996). Five grams of fresh sieved soil was placed into 125 mL bottle, before 20 mL of 1mM PBS containing 1mM $(NH4)_2SO_4$ and 50 mgL^{-1} of $KClO_3$ was added into each sample. The sample then was incubated on shaker at 150 rpm, 25^{o}C for 5 hours. For the control, the same amount of sieved soil was placed into a similar bottle. Continuously, 20 mL of 1mM PBS containing 1mM $(NH_4)_2SO_4$ was added and then frozen at -20^{o}C for 5 hours. After incubation, control was thawed to room temperature. All samples had 5 mL of 2M KCl added, and then were securely placed on a shaker for 10 min at 150 rpm. Finally, samples were filtered immediately through Whatman no. 42 filter paper. Extracts were analyzed by adding color reagents comprised of sulfonic acid ($NH_2C_6H_4SO_3H$) in 12% CH_3COOH and naphthylamine in 20% CH_3COOH, subsequently measuring solution by spectrophotometer at 520 nm. Absorbance values were converted into the amount of nitrogen using a standard curve formulated from a series of different concentration of $NaNO_2$.

Microbial community analyses

DNA extraction

Total soil genomic DNA was extracted using the MoBio PowerSoil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA) according to manufacturer's instruction, with slight modifications at the initial cell-lysis step as a FastPrep bead beating system (Bio-101, Vista, CA, USA) at a speed of 5.5 m s-1 for 60 s was used. Amount of 0.25 gram soil kept at -80^{o}C was used to isolate total DNA. The quantity and quality of extracted DNA were checked photometrically using NanoDrop® ND-2000c UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Extracted DNA was kept at -20^{o}C for further manipulations.

Quantitative PCR (qPCR)

The abundances of bacterial and archaeal *amoA* gene (AOB and AOA) were quantified by BioRad C1000 Touch thermal cycler CFX96 Real-Time system (Bio-Rad laboratory, USA), using two pairs of primer CrenamoA23f/CrenamoA616r (Tourna *et al*., 2008) and amoA-1F/amoA-2R (Rotthauwe *et al*., 1997) for AOA and AOB *amoA* genes, respectively. Each sample was quantified in 10 µl reaction including 5 µl GoTaq® qPCR Master Mix (2X), 20µM each primer, 0.1 µl CXR reference dye and 10 ng of template. Details of sequences of the primers and PCR thermal conditions are presented in Table 23.

Standard plasmids were constructed by cloning isolated AOA and AOB *amoA* genes into the pCR®4–TOPO vector (Invitrogen, Carlsbad, CA). A 10-fold serial dilution of plasmid was prepared to generate standard curves. Melt curve analyses were conducted following each assays to verify the specificity of the amplification products. PCR efficiency for different assays ranged between 86% and 97%.

Table 23 Specific primers and thermal conditions used for real-time PCR amplification targeting AOA and AOB *amoA* genes

Primer	**Specificity**	**Sequence**	**Real-time PCR condition**	**Reference**
amoA-1F amoA-2R	AOB	GGGG TTTCTACTGGTGGT CCCCTCKGSAAAGCC TTCTTC	95°C, 10 min, 1 cycle 94°C for 45 s, 58°C for 45 s, 72°C for 45 s, 39 cycles 95°C for 15 s, 60°C for 30 s, to 95°C for 15 s, 1 cycle	Hallin *et al*., 2009
CrenamoA23f CrenamoA616r	AOA	ATGGTCTGGCTWAGA CG GCCATCCATATGTAT GTCCA	95°C, 10 min, 1 cycle 94°C for 45 s, 55°C for 45 s, 72°C for 45 s, 39 cycles 95°C for 15 s, 60°C for 30 s, to 95°C for 15 s, 1 cycle	Hallin et al., 2009

Terminal restriction fragment length polymorphism (TRFLP)

The fragments of AOB and AOA *amoA* genes were amplified using fluorescently labelled primers FAM-CrenamoA23f/CrenamoA616r and VIC-amoA-1F/amoA-2R respectively. PCR was conducted by a Dyad Peltier Thermal cycler (Biorad, Australia). A mixture of 25 µl

including 2.5 µl of 10 x NH_4 reaction buffer (10x, Bioline, Australia), 0.5 µl of 20 mM deoxynucleoside triphosphate (dNTP mix, Bioline, Australia), 0.25 µl of each primer with the concentration of 20 µM (Sigma Aldrich, Australia), 1 µl of BSA (20 mg/ml, NewEngland Biolabs, USA), 1 µl of 50 mM $MgCl_2$ (Bioline, Australia) and 10 ng of DNA template. After denaturing the PCR mixture at 95^{o}C for 5 min, DNA template was amplified with 35 cycles of denaturing at 95^{o}C for 30 s, annealing at 56^{o}C for 30s, and extension at 72^{o}C for 1 min, and finally extension at 72^{o}C for 10 min. PCR amplicons were then visualized on 1% (w/v) agarose gel under UV radiation to check whether successful amplification achieved.

Quadruplicate PCR products of each sample were pooled, and then subjected to purify by Wizard SV Gel and PCR clean-up Systems (Promega, San Luis Obispo, CA, USA) according to instructions of manufacturer. After that, the concentration of purified PCR products was measured by NanoDrop® ND-2000c UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The concentration of purified DNA ranged from 40 to 100 ng/µl. The ratio of $A_{260/280}$ and $A_{260/230}$ varied in the range of 1.8-2.1 and 0.7-1.7, respectively.

Purified PCR products were then subjected to digestion by commercial restriction enzymes. A reaction was composed of 200 ng of DNA, 1 µl of 10x NEB buffer, 0.1 µl of BSA and 5U of restriction enzyme. Two types of restriction enzymes used here were MspI and HpyCH4V (NewEngland BioLabs, USA) for AOB and AOA, respectively. Digests were incubated at 37^{o}C for 3 hours, followed by 95^{o}C for 10 minutes to deactivate the restriction enzymes. TRF were resolved on an ABI PRISM 3500 Genetic analyzer (Applied Biosystems, CA, USA). A geneScan 600-LIZ internal size standard (Applied Biosystems) was applied to each sample. Genemapper version 4.0 (Applied Biosystems) was used to analyze TRFLP profile. TRFs with peak height comprising less than 2% of the total peak height were removed from downstream analyses.

Statistical analysis

One-way analysis of variance was conducted to examine the difference of soil physicochemical properties and potential nitrification rate for before and after treatments applied. The *amoA* gene copy numbers were log –transformed prior to statistical analysis to meet normality assumptions. Spearman's rank test was used to evaluate the degree of correlations among AOB and AOA *amoA* gene copy numbers, AOB and AOA community composition, and potential nitrification rate and soil moisture. $P<0.05$ was considered to be statistically significant. Non-metric multidimensional scaling (NDMS) was used to visualize the Bray-Curtis dissimilarity matrices based on the relative abundance of AOA and AOB

TRFs by using Primer v6 (PRIMER-E Ltd, Plymouth, UK). Significance of Bray-Curtis dissimilarity was examined by PERMANOVA.

Results

Treatment effects on soil physicochemical properties

Soil moisture content

There is a clear difference in soil moisture upon waterlogging treatments applied to cotton crops. Soil moisture content increased by approximately 10% after WL1 and WL2 treatments were applied (Fig. 26). Before WL1, no significant difference in soil moisture content was observed for soil samples from control plots and WL1 plots (P= 0.878). Similarly, the moisture content of soil samples from control and WL2 plots before WL2 applied were quite similar (P= 0.089). After 3 days of WL1 event, there was a significant difference in soil moisture content between control and WL1 plot (P=0.001). The moisture content between WL2 and control plots were also significantly different (P<0.001) after 5 days of WL2 treatment had been applied. At the end of the experiment, there was no big difference of soil moisture among different treatment (P=0.165).

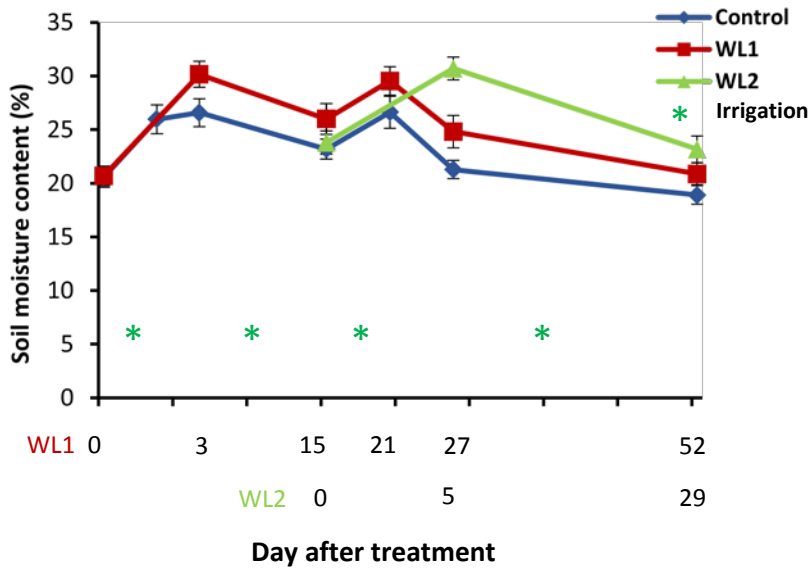


Fig. 26 Changes in soil moisture before and after treatments applied.

Soil pH

Soil pH considerably increased after waterlogging treatments were applied. For the first waterlogging event, soil pH increased from 7.9 to 8.2 whereas it increased from 7.8 to 8.0 for the second waterlogging event (Fig. 27). Soil pH in control plots slightly increased after irrigation and then started to decrease gradually. Statistical analysis showed that there were significant differences in soil pH between control and plots applied treatments (P<0.001). At the end of the experiment, there was difference of soil pH among control, WL1 and WL2 treatments. In particular, pH values of control plot was 7.5 whereas pH of 7.95 and 7.84 belonged to WL1 and WL2, respectively.

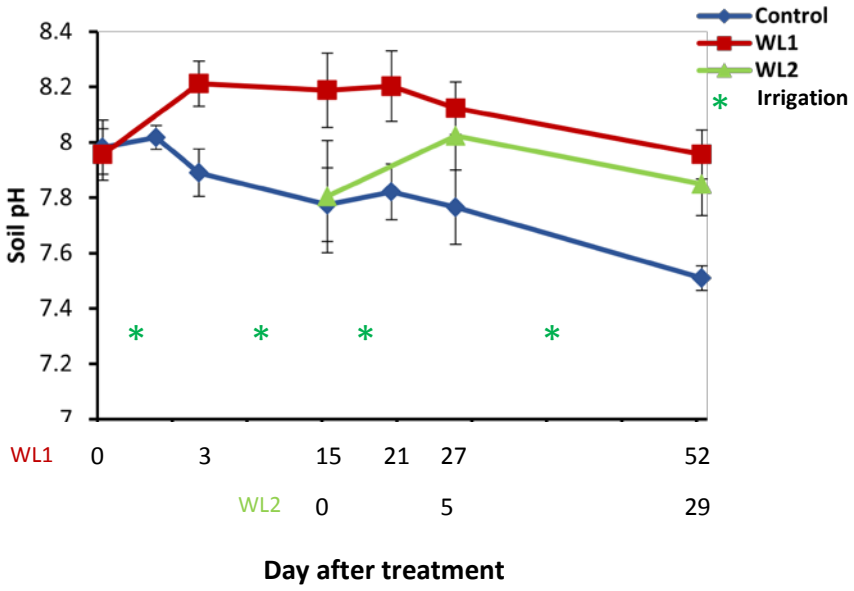


Fig. 27 Changes in soil pH before and after treatments applied.

Soil nutrients

Soil total nitrogen started from 0.86 g/kg soil dry weight (dwt) for samples collected from WL1 and control plots. Afterwards, it started to decrease gradually during the experiment (Fig. 28). Statistical analysis showed a significant difference in soil total N between WL1 and control plots after treatment applied (P<0.001). In particular, WL1 decreased soil total N by 15%. In contrast to this, soil total N between the control and WL2 plots were not significantly different after WL2 (P= 0.192).

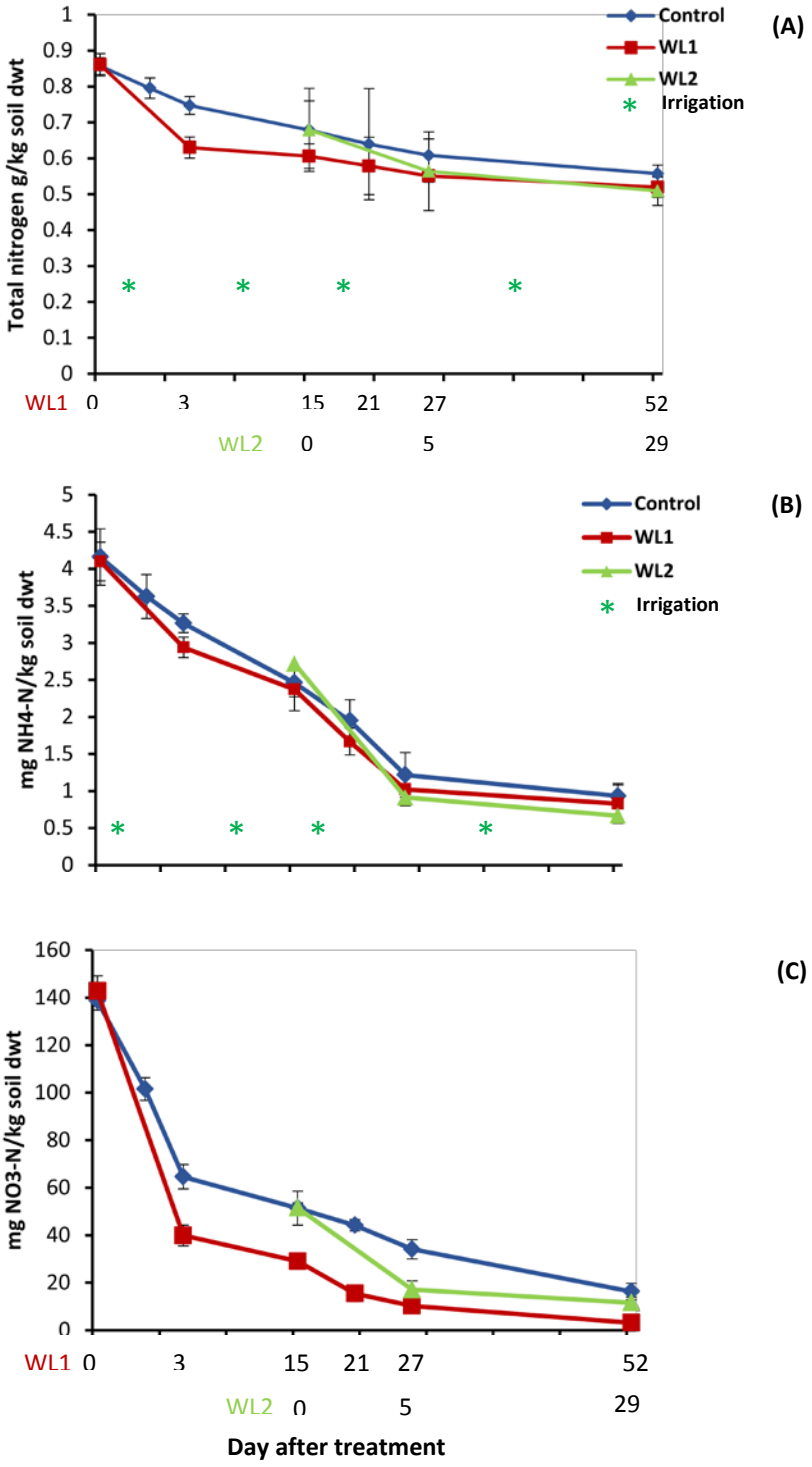


Fig. 28 Changes in total N and inorganic N before and after treatments applied. (**A**) Total nitrogen, (**B**) NH_4^+ concentration, (**C**) NO_3^- concentration

A downward trend was obtained for the concentration of NH_4 and NO_3 in the soil samples collected from control and waterlogging treatments (Fig. 28). Before WL1 was applied, the concentration of NH_4 was similar for control and WL1 plots (P= 0.634), whereas it was

significantly different (P=0.003) after 3 days of treatment. The amount of NH_4^+ decreased about 9.5% after WL1. However, no significant difference in NH_4 concentration was observed either before or after WL2 event (P=0.582 and P=0.403). The concentration of NO_3 before WL1 simulation was not different (P=0.326). After 3 days of treatment, the amount of NO_3 was significantly different between control and WL1 plots (P<0.001). The same result was obtained for WL2 event. No significant difference in NO_3 concentration was observed before WL2 treatment (P=0.999), but NO_3 concentration between control and WL2 was significantly different (P<0.001). Particularly, soil NO_3^- concentration decreased approximately 40% and 50% for WL1 and WL2, respectively. At the end of the experiment, the amount of inorganic nitrogen in the soil dropped to very low amounts. This indicates that reduction of NO_3 availability in soil can be due to decreased nitrification rate caused by waterlogging.

Treatment effects on potential nitrification rate

Potential nitrification rate (PNR) decreased after treatments applied. PNR reduced from 1.2 mg N/kg soil/h to 0.9 mg N/kg soil/h after WL1 event and from 1.1 mg N/kg soil /h to 0.9 mg N/kg soil/h after WL2 event (Fig. 29). PNR of control and WL1 plots were the same before treatment (P=0.797), whereas they were significantly different after treatment 3 days (P=0.041). Before WL2 applied, no significant difference was found for samples collected from control and WL2 plots (P= 0.7171). However, PNR was significantly different after 5 days of WL2 applied (P <0.001). The lower PNR measured and the statistically significant difference in PNR after WL1 and WL2 treatment suggest that waterlogging negatively affects the nitrification rate.

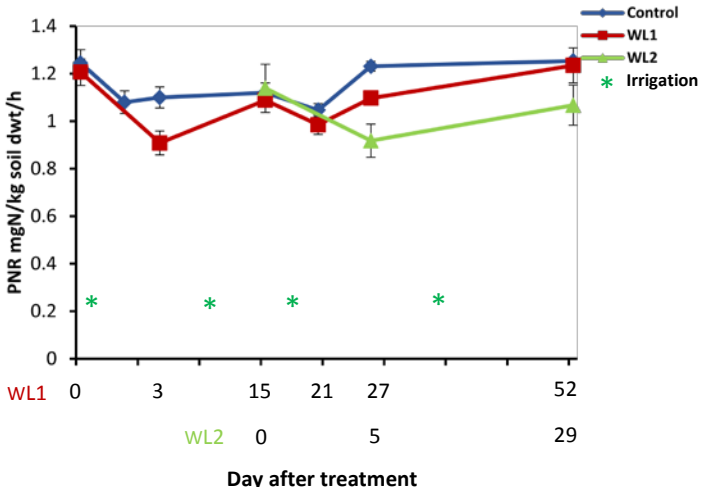


Fig. 29 Changes in potential nitrification rate (PNR) before and after treatments applied.

Treatment effects on ammonia- oxidizer community abundance and composition

Ammonia-oxidizer community abundance

The copy numbers of AOB *amoA* gene varied from 1.18 x 10^6 to 1.42 x 10^7 copies g^{-1} soil. The abundance of AOB *amoA* gene decreased about 10 times after either WL1 or WL2 events. The copy numbers of AOA *amoA* gene varied from 2.29 x 10^8 to 6.27 x 10^8 copies g^{-1} soil (Fig. 30). The abundance of AOA *amoA* genes from WL1 and WL2 plots slightly decreased after treatments applied.

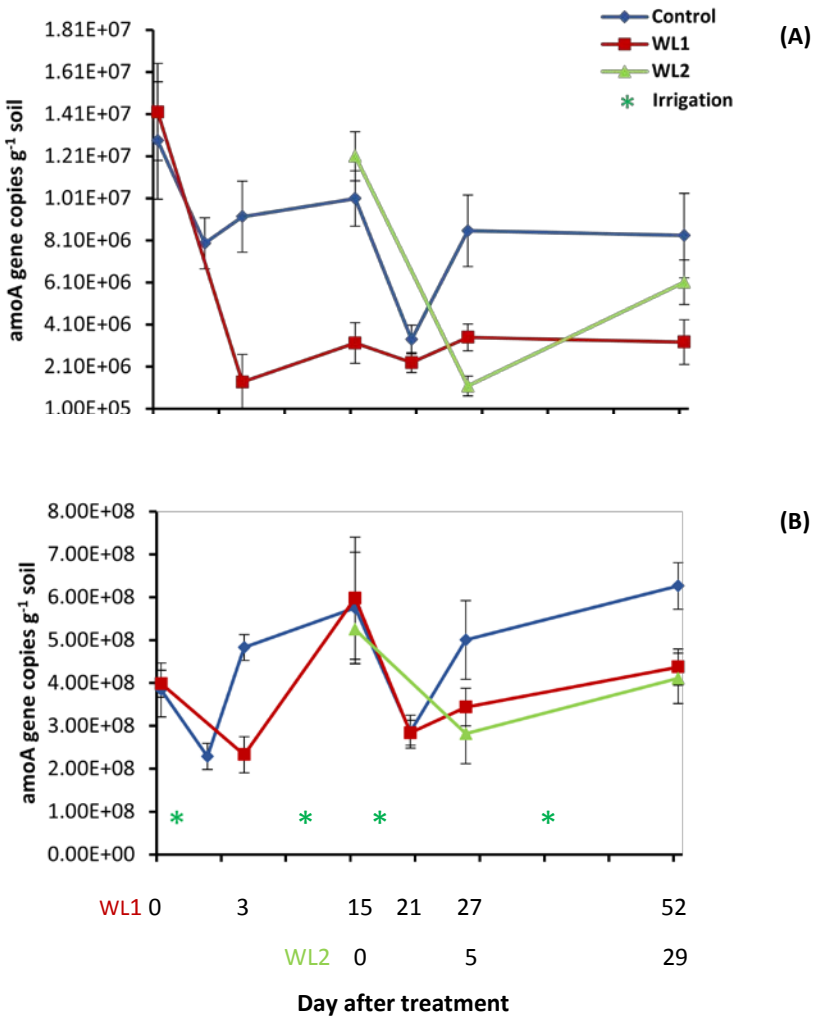


Figure 30 Changes in abundance of (**A**) AOB and (**B**) AOA *amoA* genes before and after treatments applied.

Ammonia-oxidizing community composition

The analysis of TRFLP for AOB *amoA* gene generated 4 different TRFs for all treatments after digestion of PCR products by the restriction enzyme, MspI (Fig. 31). AOB community structures changed for samples collected before and after treatments. In particular, dominant TRF-55 decreased while TRF-251 increased upon WL1 event. For the second waterlogging event, dominant TRF-55 also decreased whereas TRF-251 slightly increased at the sampling time point after the treatment was applied. TRF-149 increased after WL2 event. In contrast to WL treatment, the relative abundance of all TRFs from control plots slightly changed after irrigation. The low number of TRFs yielded from TRFLP suggested a low diversity of ammonia-oxidizing bacterial communities in these soils.

TRFLP of AOA *amoA* gene generated 8 different TRFs for all the treatments (Fig. 31). Among these, TRF-54, TRF-74 and TRF-251 were the three most dominant genotypes. AOA

community structures changed after treatments applied. There was an increase in the relative abundance of TRF-54 and TRF- 251 after WL1 and WL2 events. The TRF-74 decreased upon treatments applied. AOA community structures of control plots slightly changed after irrigation before remaining constant.

Both TRFLP and qPCR data showed a decline in some bacterial and archaeal genotypes upon waterlogging. In TRFLP profile, TRF-55 and TRF-74 decreased after treatment for AOB and AOA, respectively. In agreement with TRFLP fingerprint, the abundance of *amoA* genes also decreased for AOB and AOA. However, the abundance of bacterial *amoA* genes dropped more than that of archaea.

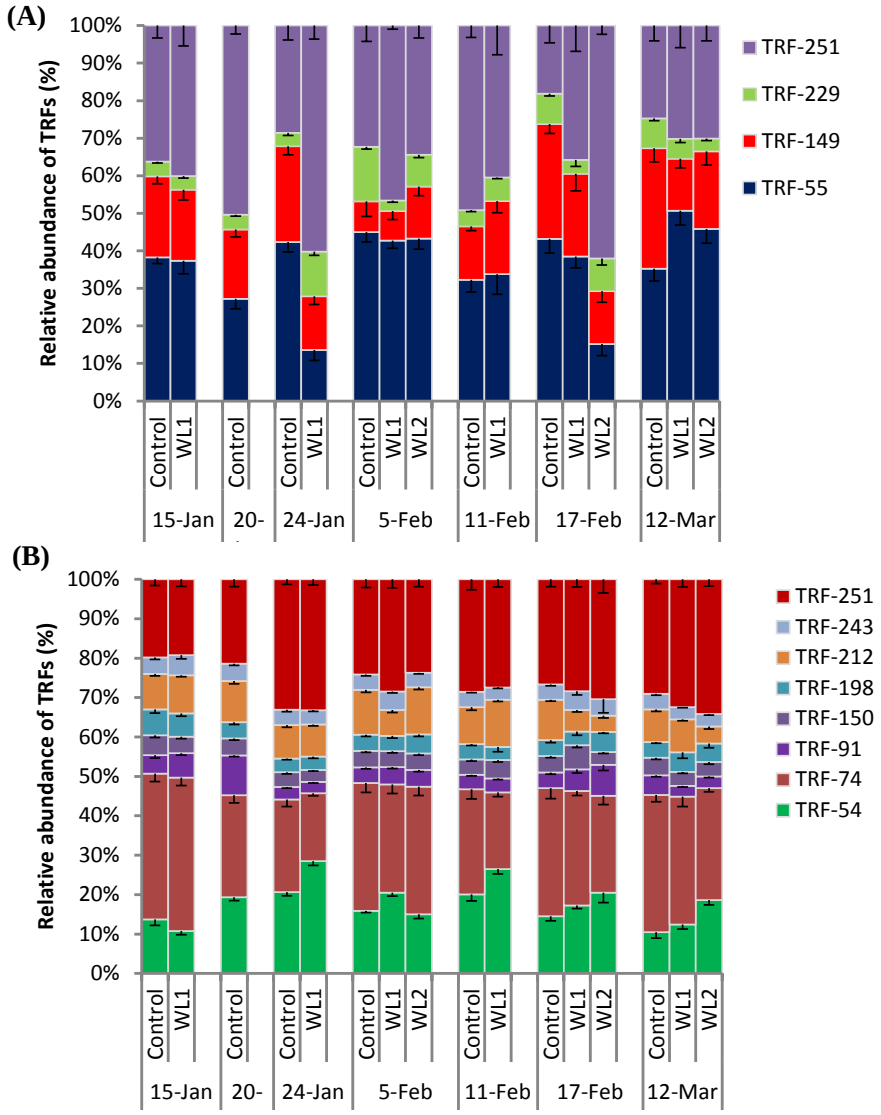


Fig. 31 TRFLP fingerprints of *amoA* gene fragments before and after treatments applied (**A**) Bacterial *amoA* gene. (**B**) Archaeal *amoA* gene. WL1= the first waterlogging event. WL2= the second waterlogging event.

The ammonia-oxidizing community compositions including ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) were plotted according to non-metric multidimensional scaling (NMDS) ordination derived from the Bray-Curtis dissimilarity matrices for the relative abundance of TRFs (Fig. 32). PERMANOVA tests showed significant treatment effects on AOB and AOA community compositions (Table 24, 25).

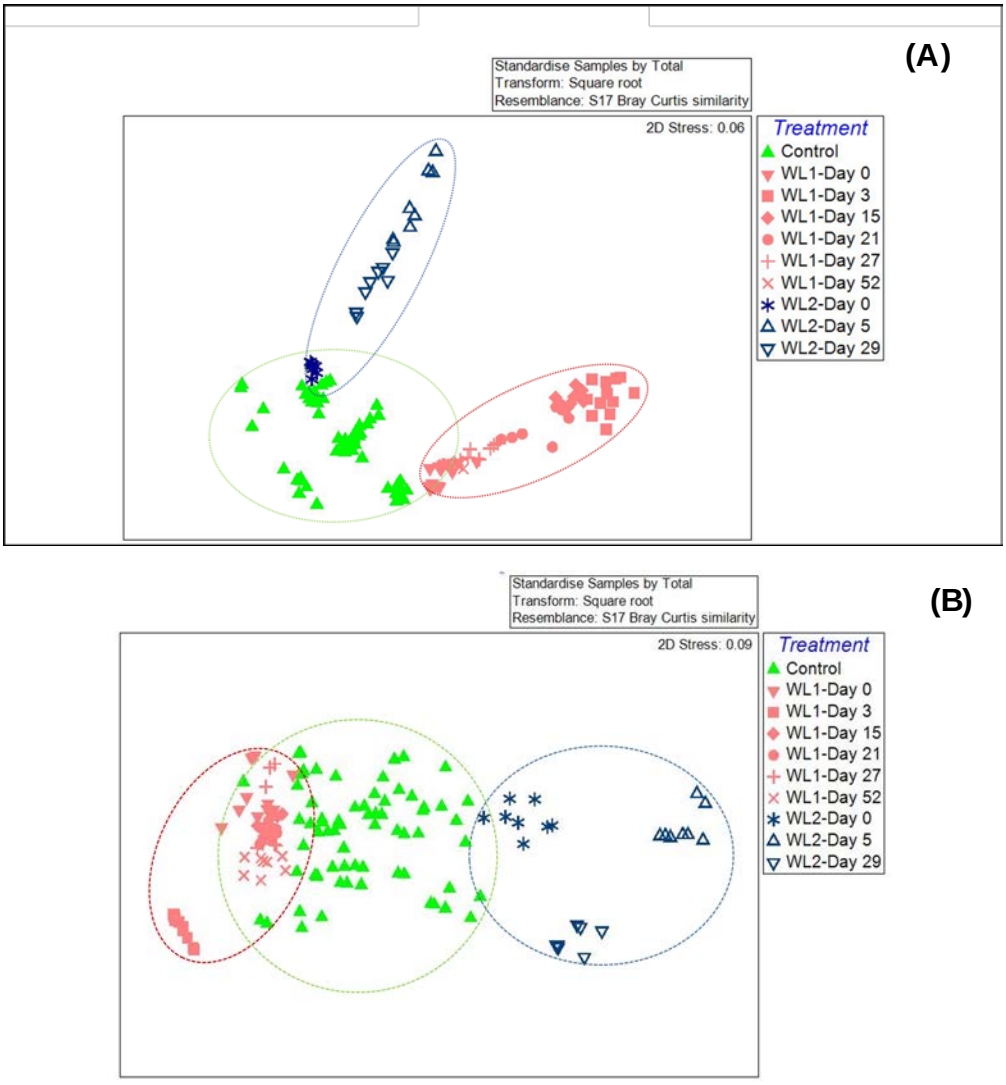


Fig. 32 NMDS ordination derived from the Bray-Curtis dissimilarity matrices are based on the relative abundance of (**A**) AOB and (**B**) AOA TRFs. The stress values for all NMDS plots are lower than 0.11 indicating that the differences are well-presented by the two-dimensional ordinations. WL1= the first waterlogging event, WL2= the second waterlogging event.

Table 24 Outputs of PERMANOVA test for treatment effects on AOB community composition, using Type III sums of squares based 999 permutations of residuals under a reduced model. Significant effects are in boldface (P<0.05). WL1= the first waterlogging event, WL2= the second waterlogging event.

AOB	**Day after treatments**								
	WL1						**WL2**		
	0	**3**	**15**	**21**	**27**	**52**	**0**	**5**	**29**
df	1	1	1	1	1	1	1	1	1
SS	90.56	2768.4	1987.5	871.18	813.92	310.49	15.232	1304.9	744.83
MS	90.56	2768.4	1987.5	871.18	813.92	310.49	15.232	1304.9	744.83
F	10.653	572.07	591.01	129.71	310.77	297.74	8.5938	200.06	244.96
P	**0.001**	**0.001**	**0.002**	**0.001**	**0.001**	**0.001**	**0.004**	**0.001**	**0.001**

Table 25 Outputs of PERMANOVA test for treatment effects on AOA community composition, using Type III sums of squares based 999 permutations of residuals under a reduced model. Significant effects are in boldface (P<0.05). WL1= the first waterlogging event, WL2= the second waterlogging event.

AOA	**Day after treatments**								
	WL1						**WL2**		
	0	**3**	**15**	**21**	**27**	**52**	**0**	**5**	**29**
df	1	1	1	1	1	1	1	1	1
SS	48.87	548.85	390.09	188.58	194.25	368.71	65.667	741.13	203.62
MS	48.87	548.85	390.09	188.58	194.25	368.71	65.667	741.13	203.62
F	3.6167	126.94	70.702	38.365	43.236	62.284	5.179	127.86	53.085
P	**0.004**	**0.001**	**0.001**	**0.001**	**0.002**	**0.001**	**0.001**	**0.001**	**0.001**

Before treatment applied (Day 0), the ammonia-oxidizer communities were expected to similar between control and WL1; control and WL2; however, PERMANOVA test showed P < 0.05 (Table 24, 25). Although such P values obtained, the similarity of AOB, AOA compositions and control composition at Day 0 can be acceptable since NMDS ordination plots showed close distances (Fig. 32). The ordination plots also showed big changes in AOB and AOA community compositions after 3 and 5 following days of WL1 and WL2, respectively. At Day 15, 21, 27 and 52 after WL1, and Day 9 and 29 after WL2, the AOB and AOA communities showed their gradual resilience (Fig. 32).

Correlation analyses

Correlation between potential nitrification rate and soil moisture; AOA, AOB amoA genes and soil moisture

The relationship of potential nitrification rate (PNR) and soil moisture as well as the abundance of AOB, AOA *amoA* gene and soil moisture were examined by Spearman's rank correlation analysis. There was a strong, negative correlation between PNR and soil moisture content (Fig. 33), which was statistically significant (r_S = -0.666, p< 0.001). In terms of the linkage between AOB, AOA *amoA* gene copy number and soil moisture, the same trends were observed. In particular, the abundance of AOB *amoA* genes was in a strong, negative relationship with soil moisture (Fig. 33). Statistical analysis showed that they were significantly correlated (r_S= -0.559, p < 0.001). A strong, negative correlation between AOA amoA gene copy number and soil moisture was also found (Fig. 33), and it is statistically significant (r_S= -0.517, p< 0.001).

Correlation between potential nitrification rate (PNR) and the abundance of AOB and AOA amoA genes

The relationship of potential nitrification rate (PNR) and the abundance of AOB and AOA *amoA* genes were determined by Spearman's rank correlation analysis. There was strong, positive correlation between PNR and AOB *amoA* gene copy number (Fig. 34), which was statistically significant (r_S= 0.620, p < 0.001). Similarly, the abundance of AOA *amoA* gene was strongly, positively correlated with PNR (Fig. 34). Statistical analysis indicated a significant correlation (r_S= 0.58, p< 0.001).

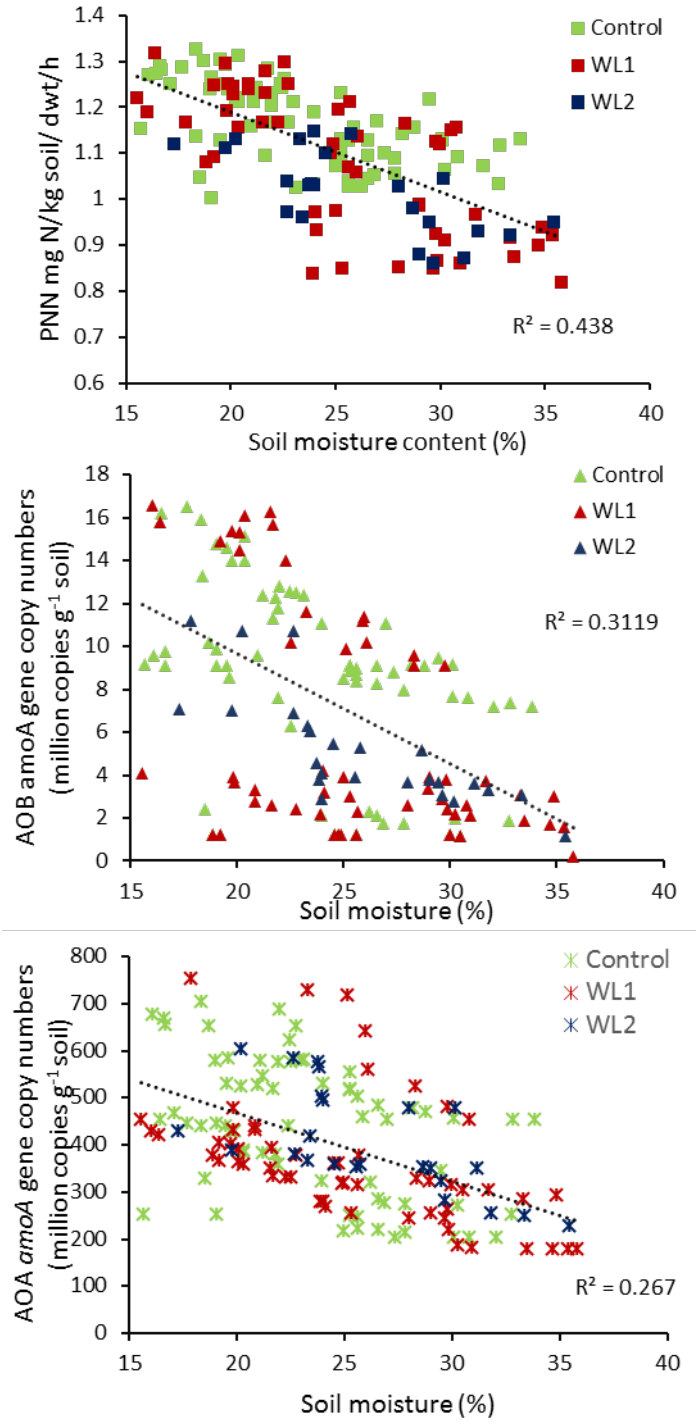


Fig. 33 Relationship of potential nitrification rate (**A**), AOB (**B**), AOA (**C**) *amoA* gene copy numbers and soil moisture. WL1= the first waterlogging event; WL2= the second waterlogging event.

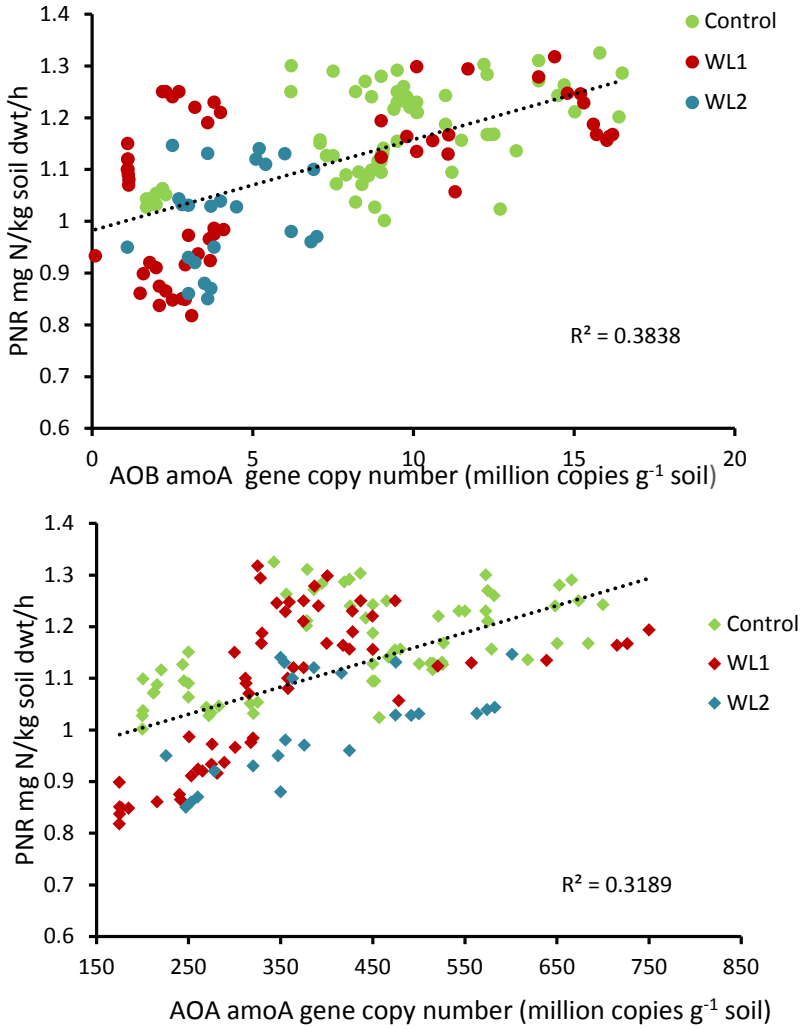


Fig. 34 Relationship between potential nitrification rate and the abundance of (**A**) AOB, (**B**) AOA *amoA* genes. WL1= the first waterlogging event; WL2= the second waterlogging event.

Correlation between potential nitrification rate and ammonia-oxidizing community compositions

The correlations between potential nitrification rate (PNR) and ammonia-oxidizing community compositions were examined by using the RELATE function in Primer v6, which showed strong correlation between AOB and AOA compositions and PNR via Spearman rank correlation. In particular, AOB community composition was statistically significantly correlated to PNR, with Spearman coefficients $r_S = 0.517$, $p = 0.001$. For AOA community composition, there was the same trend observed, but less strongly correlated. Statistical, significant correlation between AOA composition and PNR was determined with Spearman coefficient $r_S = 0.261$ and $p = 0.001$.

Discussion / conclusions

Waterlogging created unfavorable conditions such as increased soil water content leading to hypoxic or even anoxic environment that would affect nitrification process. We found a decline of AOB, AOA abundances and alterations of community composition upon waterlogging treatments. Changes in potential nitrification rate were correlated with ammonia-oxidizing communities, suggesting a strong linkage between ammonia-oxidizers and their functioning in the furrow-irrigated cotton soil. We also found AOB had higher magnitude than AOA in response to waterlogging, supporting the theory of different physiology and ecological niches between AOB and AOA. In terms of the relative contribution of AOB and AOA to nitrification, we did not observe the functional redundancy since both AOB and AOA were strongly correlated with their functioning.

Regarding soil nutrients in response to waterlogging, our results showed significant drop of soil nitrate (NO_3^-) which serves for plant growth and development. Such decrease could be a consequence of reduced nitrification plus with stimulated NO_3^- leaching and denitrification when anoxic condition was created due to waterlogging. However, we did not examine the responses of NO_3^- leaching and denitrification to waterlogging in this study. Soil nitrogen availability depletion resulting from waterlogging is expected to negatively affect cotton crop productivity.

Overall, our findings give more insights into the relationship between soil microbial community and their functioning that may allow us to better predict the response of agro-ecosystems such as cotton farming to global changes including projected higher frequency and intensity of extreme weather events, thereby developing effective nitrogen management strategies to maintain high crop productivity. This finding supports our results from glasshouse experiments.

Expt. 5* – Field chamber experiment *(PhD thesis)

Impacts of elevated CO_2 and temperature on soil nitrification and ammonia-oxidizing communities in cotton farming.

Background and aims

In this study, cotton was used as a model crop to investigate the responses of soil nitrification and ammonia-oxidizing communities to climate change including elevated CO_2 and temperature. Australian cotton crops have been indicated to be vulnerable to climate change (Glover et al., 2008). Increasing atmospheric CO_2 concentration generally increases cotton photosynthesis and biomass, potentially resulting in enhanced crop productivity. Cotton photosynthetic rate increased 30-34% when plants exposed to the CO_2 concentration of 500-900 ppm (Idso et al., 1994). Cotton biomass and yield increased approximately 37% and 43%, respectively when grown at 550 ppm CO_2 (Mauney *et al*., 1994). However, these benefits may be offset due to increasing air temperatures (Streck, 2005). Cotton is known to be dependent on nitrogen fertilizers to sustain high crop productivity. Thus, understanding of how soil nitrification responds to climate change will give more insights into the effects of projected climate change on cotton crop productivity, thereby potentially developing the effective nitrogen management strategies to sustain high crop yields.

In order to achieve these above objectives, the field-based environmentally-controlled chambers were used to simulate elevated CO_2 and temperature at Australian Cotton Research Institute (ACRI) in Narrabri. We hypothesized that elevated temperature and CO_2 will alter nitrification rate due to changes in ammonia-oxidizer abundance and composition; but the effects are related to cotton growth.

Materials and methods

Field site

This field experiment was conducted at the Australian Cotton Research Institute (ACRI) at Narrabri (30.31^{o}S, 149.78^{o}E) in north-west New South Wales, Australia (Fig. 35). This region, a semiarid ecosystem, has hot summers with maximum and minimum daily temperatures of 35^{o}C and 18^{o}C, respectively. Annual rainfall is about 644 mm, of which one-

third falls in summer months. The soil is cracking grey clay soil (vertosols) and alkaline with pH ranged from 7.5 to 8.0, designated as Ug 5.25 in Northcote classification (Northcote et al., 1975). In this experiment, cotton was sown in late summer (February, 2015) and the field experiment ended in late autumn. Mean maximum and minimum daily temperatures during this period were 30^{o}C and 16^{o}C, respectively (Bureau of Meteorology, NSW).

Fig. 35 The location of field chamber experiment at ACRI in Narrabri, NSW.

Cotton cultivar

CSIRO cotton cultivar Sicot BRF71 was planted on ridges spaced one meter apart and separated by a furrow. Water was applied as furrow-flood irrigation (Constable & Hearn, 1981).

Experimental design

Field plots were exposed to different CO_2 and temperature treatments, including ambient CO_2 and ambient temperature (C_aT_a); ambient CO_2 and elevated temperature (C_aT_e); and elevated CO_2 and elevated temperature (C_eT_e). Two replicates were used for each treatment. C_aT_a plots were used as field controls and did not have chambers. The C_aT_e and C_eT_e treatments were maintained in field-based environmentally-controlled chambers. The growth chambers (4 x 4 m^2) were installed in the field and were operational 20 days after planting (Fig. 36).

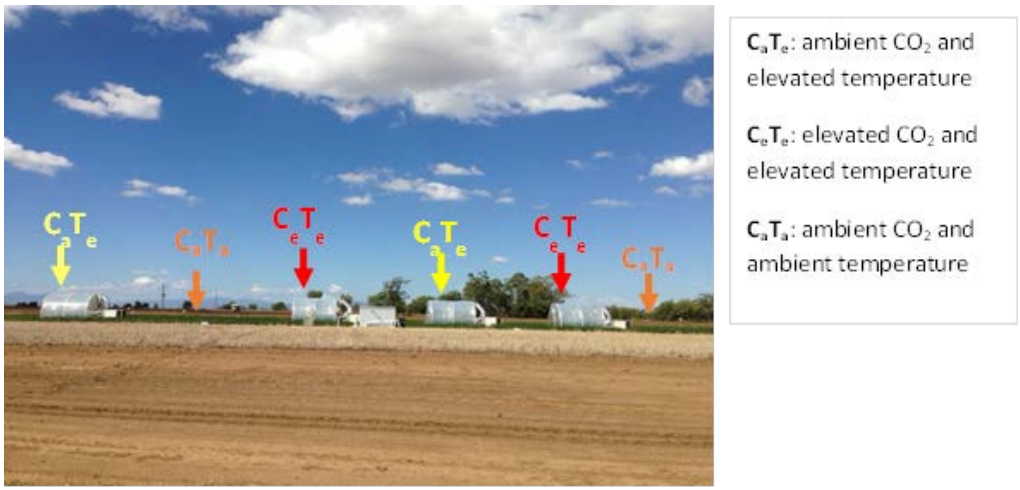


Fig. 36 Photo of field chamber experiment at ACRI in Narrabri

An air conditioner unit was used to maintain air temperature inside the chambers between 2-4^{o}C higher than ambient air temperature. CO_2 gas was released into the chambers to maintain the CO_2 concentration at ambient (400 ppm) and elevated (550 ppm) CO_2. Plots were furrow irrigated four times during the growing season. Drip irrigation was also applied inside the chambers to maintain similar watering regimes in the outside plots and inside the chambers.

Soil sampling

Soil samples were collected four times during the growing season, based on the development stage of cotton plants. The first sampling campaign was conducted before the chamber installation, corresponding to 15 days after planting (DAP). The other three sample collections were conducted 4 days after chamber installation (27 DAP) early squaring stage (41 DAP) and early flowering stage (70 DAP). Five samples were collected each time for each treatment at a soil depth of 10 cm. Soil samples were transferred to the soil laboratory at Hawkesbury Institute for the Environment (HIE), Western Sydney University, NSW for analyses. At HIE, soil samples were passed through a 4 mm sieve to remove plant residue, then kept at 4^{o}C until analysis. Subsamples of soil were kept at -20^{o}C prior to molecular manipulations.

Soil physicochemical analyses, potential nitrification rate and microbial community analyses

Please see the materials and methods in the field waterlogging experiment for detailed description of these analyses.

Results

Soil physicochemical properties

Soil pH ranged from 7.2 to 7.8 across all treatments (Fig. 37). An upward trend of soil pH was observed during the growing season. Soil pH increased approximately 8.4% after 70 DAP corresponding to the early flowering stage. One-way ANOVA and followed Tukey's HSD test showed no significant effect of treatments on soil pH at each developmental stage. Repeated measure ANOVA showed that crop growth stages significantly affect soil pH (P= 0.002) (Table 26). Warming and the interaction of warming and time had no significant effect on soil pH (P= 0.31 and P=0.08, respectively. Elevated CO_2 and its interaction with time also did not significantly affect soil pH (P=0.262 and P=0.09, respectively) (Table 26).

Soil moisture varied in the range of 19% -26% across all treatments and crop development stage (Fig. 37). One-way ANOVA and Tukey's HSD indicated that there were significant treatment effects of warming on soil moisture at seedling establishment and early flowering (P= 0.01 and P=0.007, respectively). Similarly, there was a significant difference of soil moisture between C_aT_e and C_eT_e chamber plots at the early flowering stage (70DAP) (Fig. 37). Repeated measure ANOVA indicated significant effects of crop development stage and warming on soil moisture (P< 0.0005 and P=0.001, respectively). In contrast, no significant effect was observed for elevated CO_2, and the interaction of warming and time, elevated CO_2 and time (Table 26).

Soil inorganic nitrogen decreased during the growing season. In particular, soil NH_4^+ concentration dropped from 12 mg/kg soil dwt to approximately 6 mg/kg soil dwt at early flowering stage (70 DAP). No significant treatment effect was observed at every development stage (Fig. 37). Repeated measure ANOVA indicated a significant effect of crop development stages on soil NH_4^+ concentration (P=0.004). However, no significant effect was observed for warming, elevated CO_2 and their interaction with crop development stages (Table 26).

A decrease in soil NO_3^- was also observed along the time course of experiment for all treatments (Fig. 37). Soil NO_3^- of ambient and elevated temperature plots decreased 37% and 44%, respectively whereas that of combined elevated CO_2 and temperature plots reduced 53% after 70 DAP corresponding to the early flowering stage. One-way ANOVA showed that there was no significant difference of soil NO_3^- between C_aT_a and C_aT_e treatments (P>0.05) at all development stage. In contrast, soil NO_3^- level of C_eT_e was significantly

different from that of C_aT_e and C_eT_e ($P< 0.001$) after 70 DAP corresponding to the early flowering stage whereas no significant difference was observed among all treatments at the other development stages (Fig. 37). Repeated measure ANOVA showed that crop development stage, elevated CO_2 and its interaction with crop development stage on soil NO_3^- concentration ($P< 0.0005$ and $P=0.035$). In contrast, no significant effect of warming and its interaction with time was observed (Table 26).

Soil total N decreased along the time course of experiment (Fig. 37). The amount of soil total N decreased approximately 20% after 70 DAP. One-way ANOVA indicated no significant difference of soil total N among all treatments at every development stage (Fig. 37). Only crop development stages have significant effect of soil total N ($P=0.001$) whereas no significant effect of warming, elevated CO_2 and their interaction with time was observed (Table 26).

Soil total C also decreased during the experiment. Soil total C varied from 10.9 g/kg soil dwt to 12.9 g/kg soil dwt. Under C_aT_a and C_aT_e treatments, soil total C decreased approximately 12% and 14%, respectively after 70 DAP whereas only 5.4 % of total C was observed under C_eT_e treatment. One-way ANOVA showed that there were significant differences in soil total C between C_eT_e and C_aT_e at 70DAP that is corresponding to the early flowering stage (Fig. 37). Repeated measure ANOVA indicated significant effects of time, and elevated CO_2 x time on total C ($P< 0.0005$ and $P= 0.002$, respectively) (Table 26).

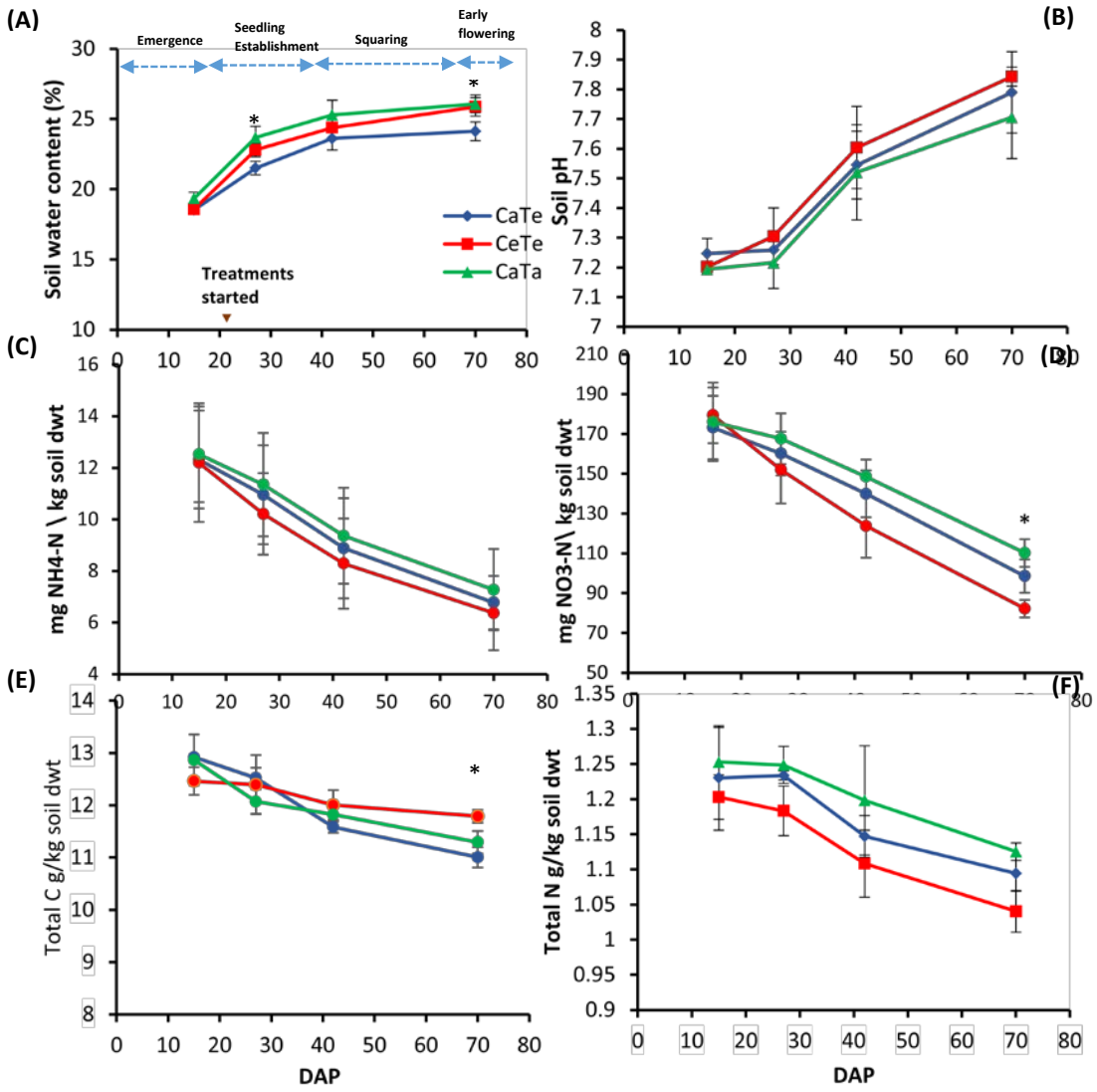


Fig. 37 Changes in soil physicochemical properties under climate treatments during the experiment. (**A**) Soil moisture, (**B**) soil pH, (**C**) soil NH_4^+, (**D**) soil NO_3^-, (**E**) soil total N, and (**F**) soil total C. The asterisk indicates significant difference between different treatments ($P<0.05$).

Table 26 Repeated measures ANOVA for the effects of climate change factors and cotton development stage on soil physicochemical properties. T_e= elevated temperature, C_e= elevated CO_2. Bold values indicate a significant difference at $P<0.05$.

Factor	Soil moisture	pH	NH_4^+	NO_3^-	Total C	Total N
T_e	**0.001**	0.31	0.52	0.12	0.693	0.45
C_e	0.724	0.262	0.34	**<0.0005**	0.398	0.32
Time (T)	**<0.0005**	**0.002**	**0.004**	**<0.0005**	**<0.0005**	**0.001**
T_e x T	0.105	0.08	0.07	0.41	0.203	0.074
C_e x T	0.496	0.09	0.21	**0.035**	**0.002**	0.08

Potential nitrification rate

Potential nitrification rate (PNR) varied from 0.81 to 0.95 mg N/kg soil dwt/hr across all treatments. PNR increased approximately 9.8% for C_aT_a and C_aT_e plots after 70 DAP corresponding to the early flowering stage. Under C_eT_e treatment, PNR increased approximately 13% at the early flowering stage (70 DAP). One-way ANOVA showed that there was no significant differences in PNR between C_aT_a and C_aT_e plots at every development stage. In contrast, PNR of samples collected from C_eT_e plots were significantly different with that of C_aT_e at the early flowering stage (70 DAP) whereas no significant difference was observed at the other development stages (Fig. 38). Repeated measure ANOVA indicated significant effects of crop development stage, elevated CO_2 and its interaction with crop development stage on PNR ($P< 0.0005$, $P= 0.005$ and $P= 0.002$, respectively). In contrast, warming and its interaction with time did not significantly affect PNR ($P= 0.063$ and $P= 0.061$, respectively).

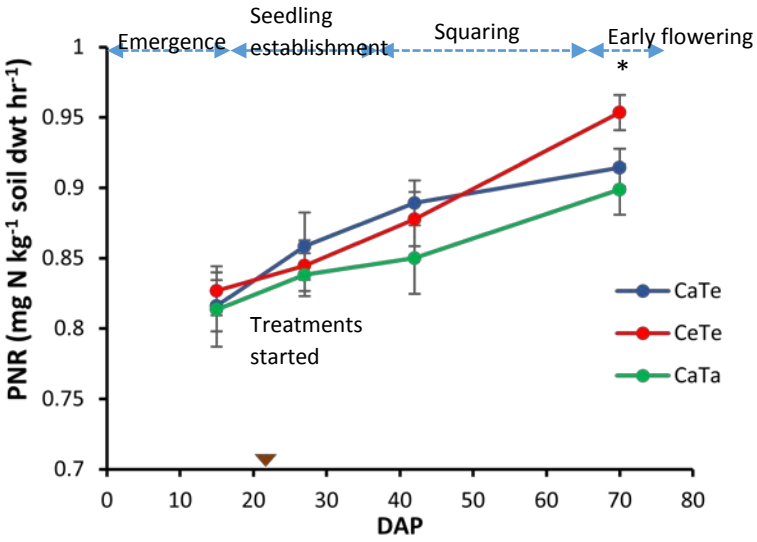


Fig. 38 Changes in potential nitrification rate (PNR) under climate treatments during the experiment. The asterisk indicates significant difference between different treatments ($P<0.05$).

Ammonia-oxidizer communities

The abundance of AOB and AOA amoA genes

The abundance of AOB *amoA* genes varied from $6.06x10^6$ to $7.98 x10^6$ copies/ g soil dwt whereas that of AOA *amoA* genes was approximately 10-fold higher, ranging from $7.06 x10^7$ to $8.43x10^7$ across all treatments. There were upward trends for both AOB and AOA *amoA*

gene copy number. In particular, the abundance of AOB *amoA* genes increased 13.6% and 22% for samples collected from C_aT_a and C_aT_e treatments, respectively after 70 DAP, however, no significant difference was observed at every development stage (Fig. 39). Under C_eT_e treatment, the abundance of AOB *amoA* genes increased 29% after 70 DAP and there was significant treatment effect on AOB *amoA* gene copy number at the early flowering stage (70DAP) (Fig. 39). Repeated measure ANOVA showed significant effect of time on AOB abundance ($P<0.0005$). Warming and warming x time had no significant effect on AOB abundance ($P= 0.054$ and $P= 0.087$, respectively) whereas elevated CO_2 and its interaction with time had significant effects on AOB abundance ($P=0.004$ and $P= 0.008$, respectively) (Table 27).

The abundance of AOA amoA genes also increased during the experiment. It increased approximately 9.86% for ambient condition after 70 DAP. Under C_aT_e and C_eT_e treatments, AOA abundance increased approximately 15% and 19% after 70 DAP, respectively. At the early flowering stage (70DAP), there were significant treatment effects on the abundance of AOA community between C_aT_e and C_eT_e; C_aT_a and C_aT_e ($P= 0.009$ and $P=0.002$, respectively) (Fig. 39). Repeated measure ANOVA indicated significant effect of crop development stage on AOA abundance ($P<0.0005$). Warming and warming x time had significant effects on AOA abundance ($P= 0.023$ and $P = 0.012$, respectively). Elevated CO_2 and elevated CO_2 x time also had significant effects on AOA abundance ($P= 0.003$ and $P= 0.002$) (Table 27).

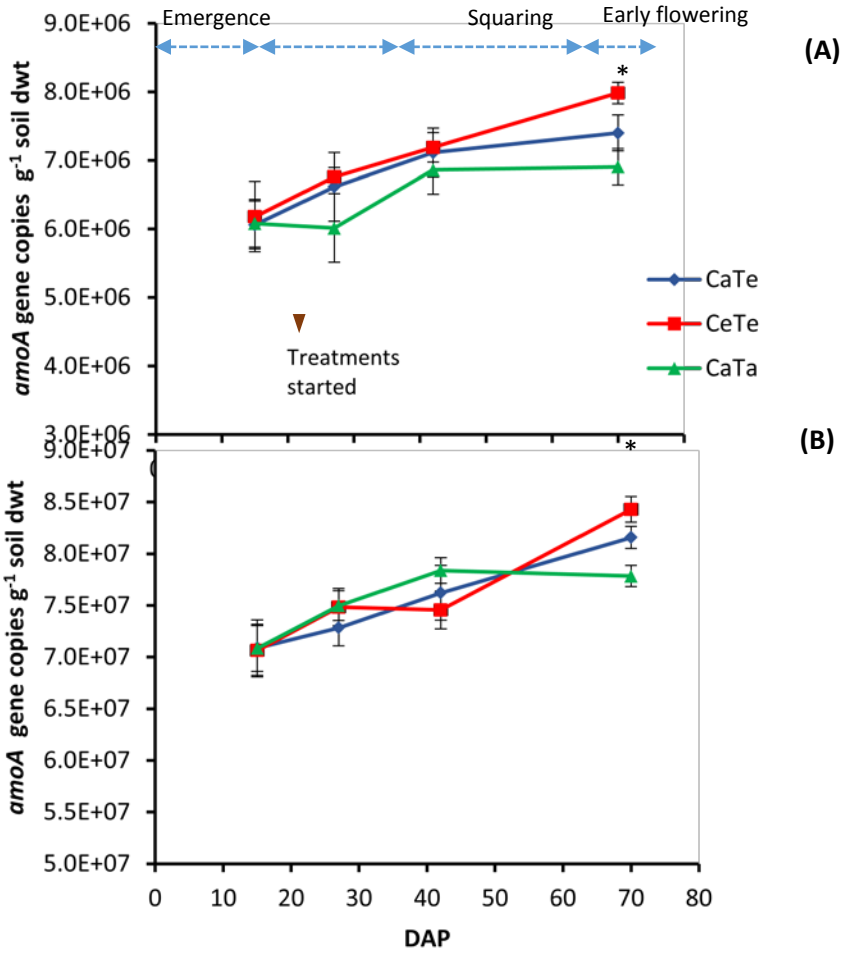


Fig. 39 Changes in the abundance of AOB and AOA *amoA* genes under climate treatments during the experiment (**A**) AOB amoA genes, (**B**) AOA *amoA* genes. The asterisk indicates significant difference between different treatments (P<0.05).

Table 27 Repeated measures ANOVA for the effects of climate change factors and cotton development stage on potential nitrification rate (PNR), AOB and AOA abundance. T_e= elevated temperature; C_e= elevated CO_2. Bold values indicate a significant effect at P< 0.05.

Factor	PNR	AOB abundance	AOA abundance
T_e	0.063	0.054	0.023
C_e	**0.005**	**0.004**	**0.003**
Time (T)	**<0.0005**	**<0.0005**	**<0.0005**
T_e x T	0.061	0.087	**0.012**
C_e x T	**0.002**	0.008	**0.002**

The AOB and AOA community compositions

The analysis of terminal restriction fragment length polymorphism (TRFLP) indicated four and eight different TRFs for AOB and AOA *amoA* genes, respectively. The AOB community has three dominant TRFs including TRF-55, 149 and 251 (Fig. 40). Relative abundance of TRFs showed that TRF-74, 243 and 251 were dominant in the AOA community (Fig. 40). Under C_aT_a and C_aT_e treatments, the AOB communities marginally changed during the duration of the experiment. In contrast, under C_eT_e treatment, the relative abundance of TRF-149 and 251 significantly increased ($P<0.001$) while that of TRF-55 significantly decreased ($P=0.003$) at the early flowering stage (70 DAP). In terms of AOA community composition, no big change was observed at seedling establishment (27DAP) and early flowering stage (70DAP) across all treatments. However, the relative abundance of TRF-54 and 91 significantly increased ($P<0.001$ and $P=0.009$, respectively) whereas that of TRF-74 significantly decreased in response to C_aT_e and C_eT_e treatments at the early flowering stage (70DAP) ($P= 0.01$).

Non metric multidimensional scaling (NMDS) analysis indicated clear separation of AOB communities under C_eT_e treatment and AOA communities under both C_aT_e and C_eT_e treatments at the early flowering stage (70DAP) (Fig. 41). PerMANOVA tests showed significant treatment effect of warming and combined elevated CO_2 and warming on AOA community ($P=0.001$) whereas only the latter had significant effect on AOB community ($P=0.001$).

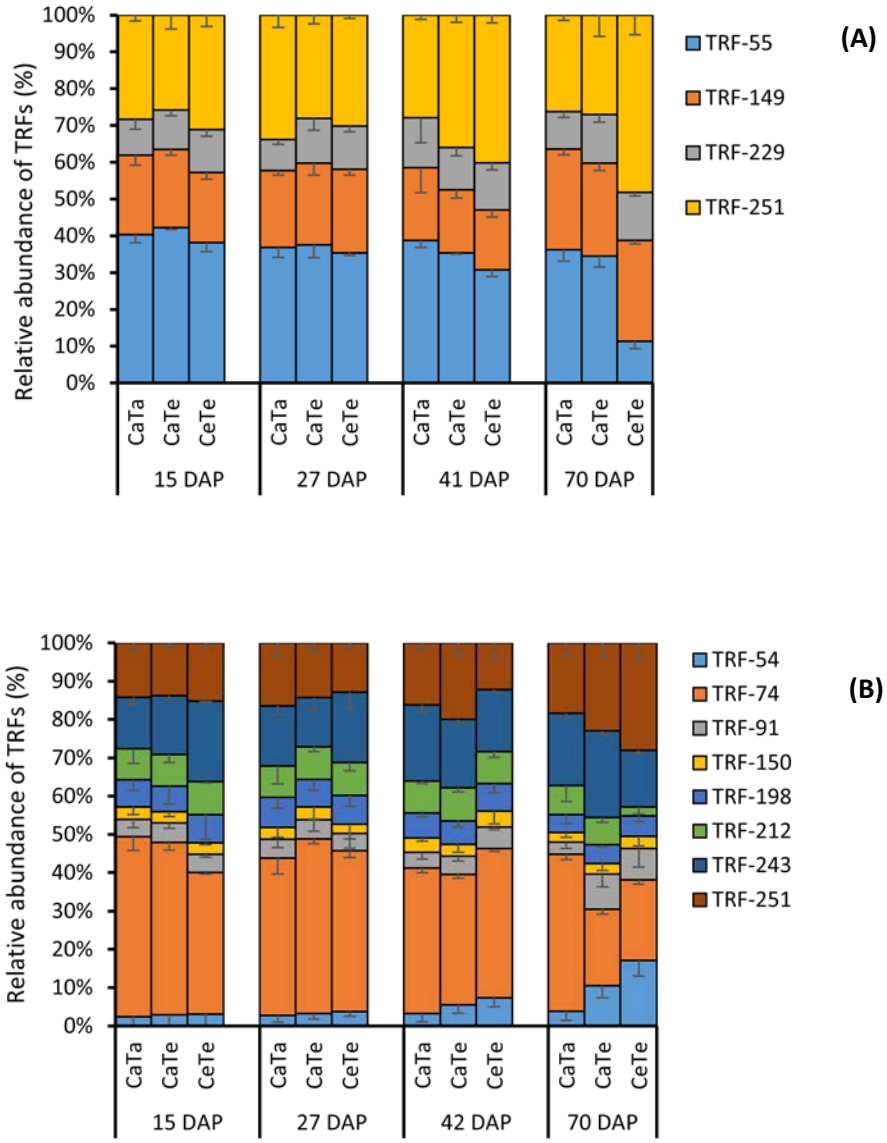


Fig. 40 Terminal restriction fragment length polymorphism (TRFLP) fingerprints of *amoA* gene fragments under climate treatments. (**A**) TRFLP fingerprint of AOB *amoA* gene, (**B**) TRFLP fingerprint of AOA *amoA* gene.

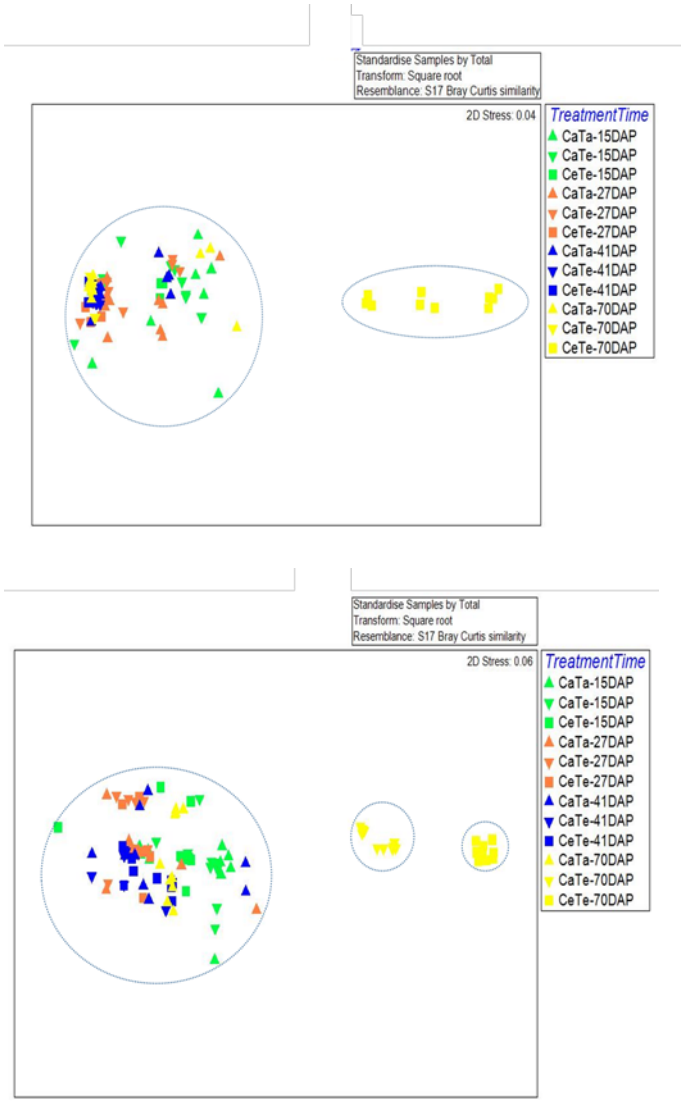


Fig. 41 Non-metric multidimensional scaling (NMDS) ordinations derived from the Bray-Curtis dissimilarity matrices showing differences in (**A**) AOB and (**B**) AOA community compositions under climate treatments at different development stages including emergence (15DAP), seedling establishment (27DAP), early squaring stage (41DAP) and early flowering stage (70DAP). C_aT_a= ambient CO_2 and temperature, C_aT_e= ambient CO_2 and elevated temperature, and C_eT_e= elevated CO_2 and elevated temperature.

Correlation analysis

The relationship of AOB, AOA abundance, potential nitrification rate (PNR) and soil physicochemical properties were examined by Spearman's rank correlation analysis. There were positive correlations between PNR and the abundance of AOB and AOA, which were statistically significant (r_S= 0.81, P<0.01 and r_S=0.507, P<0.01, respectively). AOB abundance and PNR were significantly correlated with soil pH. Also, both the abundance of AOB/AOA and PNR were significantly correlated with total C (Table 28). Using RELATE function in Primer v6, the relationship between AOB/AOA compositions and PNR was

evaluated. Our results indicated that both AOB and AOA structure had significant correlation with PNR (P=0.001) (Fig. 42).

Table 28 Correlation coefficients of soil physicochemical properties, potential nitrification rate (PNR) and the abundance of AOB and AOA communities. Significant difference at P< 0.05 (*).

	Soil moisture	pH	NH_4^+	NO_3^-	Total C	Total N	PNR
AOA	0.179	0.359	-0.326	-0.353	-0.543*	-0.323	0.507*
AOB	0.266	0.590*	-0.266	-0.267	-0.536*	-0.421	0.81*
PNR	0.321	0.685*	-0.467	-0.127	-0.531*	-0.265	-

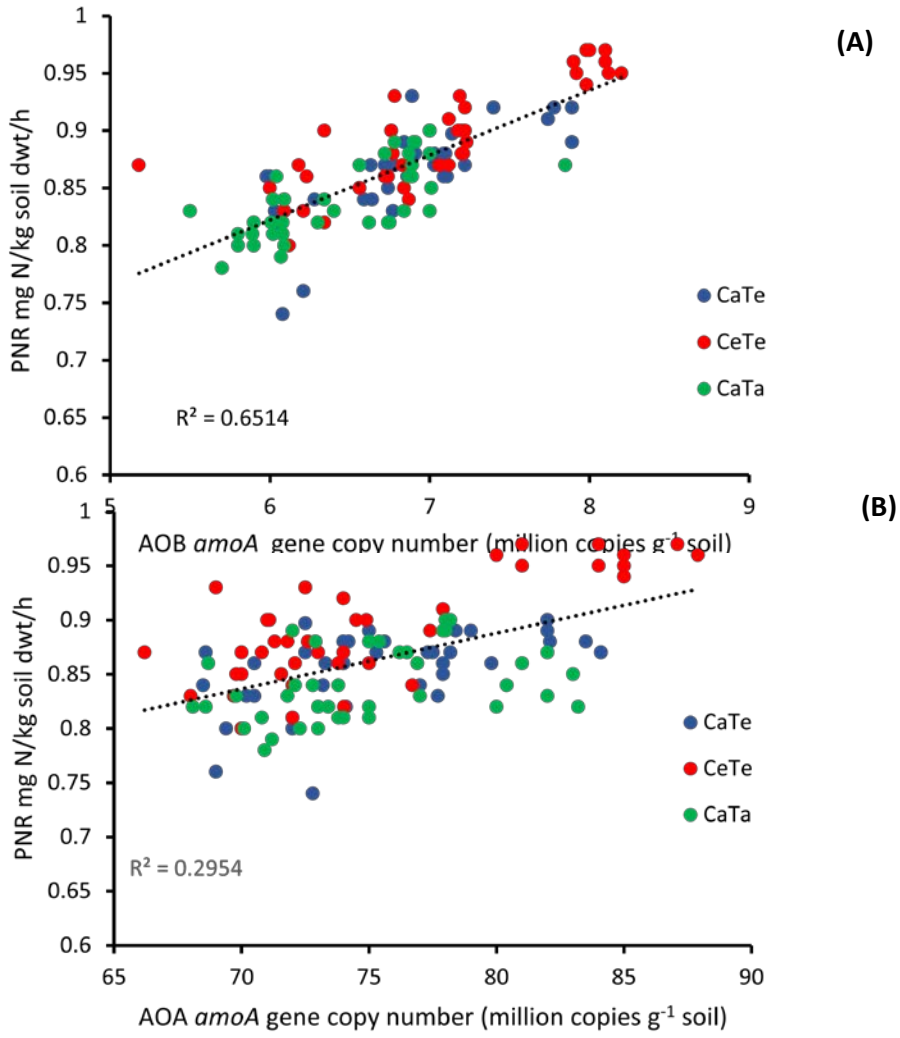


Fig. 42 The relationship between PNR and the abundance of (**A**) AOB and (**B**) AOA

Discussion / conclusions

Our results indicated that the 2-4^{o}C warming had no significant effects on soil properties, PNR and AOB community. Elevated temperature had significant effects on AOA abundance and composition at the early flowering stage of cotton. In contrast, elevated CO_2 significantly affected PNR and AOB/AOA abundance and compositions. These effects were only seen at the early flowering stage. Elevated CO_2 modified soil properties which may interact with altered ammonia-oxidizer communities, resulting in changes in nitrification rate. These changes could potentially lead to alterations in soil nitrogen availability which are related to crop productivity.

6. Please describe any:-

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);
b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and
c) required changes to the Intellectual Property register.

Not applicable.

Conclusion

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

Conclusions are found at the end of each experimental result section.
For overall conclusions, please see Part 4 – Final Report Executive Summary.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.
(b) for the future presentation and dissemination of the project outcomes.
(c) for future research.

<u>Extension activities</u>

Incorporating soil health in management practises to sustainably increase productivity and Profitability, 12-13 October 2015, Australia Cotton Research Institute, Narrabri, Australia

Harnessing rhizosphere-soil-microbial interactions for increasing farm productivity and resilience of farming systems, 10-11 October 2015, Sydney, Australia

Cotton Climate Change Workshop, 9 -10 September 2014, Hawkesbury Institute for the Environment, Western Sydney University, Richmond, NSW

Soil Biology Masterclass 2014 Hawkesbury Institute for the Environment, Western Sydney University (participants ~20 growers and consultants over 2 days).

Soil Biology Masterclass 2013, Hawkesbury Institute for the Environment, Western Sydney University (participants ~20 growers and consultants over 2 days).

*Future Soil Biology Masterclass (annual event) will incorporate the findings from this project as key components of the program.

Conference presentation

Singh BK. Managing soil health. Plenary presentation delivered at 2nd Australian Cotton Research Conference, 8-10 September 2015, Toowoomba, Australia.

Osanai Y, Tissue DT, Bange MP, Anderson IC, Braunack MV, Singh BK. Climate change impact on crop productivity: legacy effect through plant-soil feedback. Oral presentation delivered at 2nd Australian Cotton Research Conference, 8-10 September 2015, Toowoomba, Australia.

Osanai Y, Tissue DT, Bange MP, Anderson IC, Braunack MV, Singh BK. Interactive effects of extreme weather events, elevated CO_2 and temperature on cotton productivity and soil nutrient status.Poster presentation delivered at 2nd Australian Cotton Research Conference, 8-10 September 2015, Toowoomba, Australia.

Nguyen L, Anderson IC, Tissue DT, Bange MP, Braunack MV, Osanai Y, Singh BK. Impact of waterlogging on nitrification and ammonia-oxidizing community in cotton farming. Poster presentation delivered at 2nd Australian Cotton Research Conference, 8-10 September 2015, Toowoomba, Australia.

Osanai Y, Tissue DT, Bange MP, Anderson IC, Braunack MV, Singh BK.The impacts of extreme weather events on crop productivity and soil fertility under future climate. Poster presentation delivered at the 20thWorld Congress of Soil Science: Soils Embrace Life and Universe, 8-13 June 2014,Jeju, South Korea.

Osanai Y, Tissue DT, Bange MP, Anderson IC, Braunack MV, Singh BK.The impact of climate change and extreme weather events on cotton productivity. Invited oral presentation delivered at the Cotton Climate Change Workshop, 8 September 2014, University of Western Sydney, Australia.

Osanai Y, Tissue DT, Bange MP, Anderson IC, Braunack MV, Singh BK. Cotton industry adaptation to extreme weather and climate change. Poster presentation delivered at Crop Science Workshop, 21-22 May 2014, Melbourne, Australia.

Osanai Y, Tissue DT, Bange MP, Anderson IC, Braunack MV, Singh BK. Cotton industry adaptation to extreme weather and climate change. Poster presentation delivered at 1st Australian Cotton Research Conference, 8-11 September 2013, Narrabri, Australia.

*Presenter is underlined.

9. A. List the publications arising from the research project and/or a publication plan.

(NB: Where possible, please provide a copy of any publication/s)

Book

Bange MP, Baker JT, Bauer PJ, Broughton KJ, Constable GA, Luo Q, Oosterhuis DM, Osanai Y, Payton P, Tissue DT, Reddy KR and Singh BK (2016) Climate change and cotton production in modern farming systems, CABI Publishing, UK.

Journal article

Osanai Y, Tissue DT, Bange MP, Anderson IC, Braunack MV, Singh BK (under review) Plant-soil interactions and nutrient availability determine the impact of elevated CO_2 and temperature on cotton productivity. *Plant and Soil*. (see **Appendix 2**)

Osanai Y, Tissue DT, Bange MP, Braunack MV, Anderson IC, Singh BK (ready for submission) Interactive effects of elevated CO_2, warmer temperature and extreme weather events on soil nutrients and crop productivity indicate increased variability of crop production under future climate regimes. (see **Appendix 3**)

Osanai Y, Zhang CJ, Tissue DT, Bange MP, Braunack MV, Anderson IC, Singh BK (in prep.) Climate change impact on crop productivity: legacy effect through plant-soil feedback.

Osanai Y, Zhang CJ, Delgado-Baquerizo M, Tissue DT, Bange MP, Braunack MV, Anderson IC, Singh BK (in prep.) Extreme weather legacy on cropping system under the current and future CO_2 and temperature regimes.

Osanai Y*, Zhang CJ*, Tissue DT, Bange MP, Braunack MV, Anderson IC, Singh BK (in prep.) The impacts of elevated CO_2 and temperature on soil bacterial community in irrigated cotton system.

Osanai Y*, Zhang CJ*, Tissue DT, Bange MP, Braunack MV, Anderson IC, Singh BK (in prep.) The impacts of elevated CO_2, temperature and extreme weather events on soil bacterial community in irrigated cotton system.

Additionally,

A PhD thesis will be written by Ms Linh Nguyen (to be submitted in the next couple of months, and the copy will be available upon request). Two journal articles will be written from the work conducted as a part of this PhD thesis in due course.

Industry magazines

"CSIRO and UWS: Partners In Our Cotton Industry Future" in the Australian Cottongrower magazine (2013).

"Preparing Australian cotton for a future climate" in the Australian Cottongrower magazine (Dec-Jan 2014).

"Focus on the importance of soil health" in Spotlight (Autumn 2016).

"Getting back to your roots" in Spotlight (Autumn 2016).

"Workshop for improving soil health" in The Australian Cottongrower (Feb-March 2016).

"Harnessing rhizosphere-soil-microbial interactions" in The Australian Cottongrower (Feb-March 2016).

B. Have you developed any online resources and what is the website address?

We have a webpage describing the project overview and goals within Hawkesbury Institute for the Environment website, and we will continue to maintain this webpage.

http://www.uws.edu.au/hie/research/research_projects/cotton_adapting_to_climate

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

Adapting to extreme weather events under current and future climate conditions will be necessary to maintain industry profitability and sustainability. To develop strategies to adapt and assist recovery from these extreme climate events, a robust adaptation knowledge framework must be developed within the context of climate change scenarios. The objectives of this project was to examine the impact of extreme events (flooding and drought) under current and future climate (elevated CO_2 and temperature) on soil fertility and function; and how these changes in soil processes affect cotton productivity through better understanding of soil-plant interaction and environmental sustainability.

Climate change impacts

- T_E was the dominant factor in cotton productivity, accelerating the rate of plant development and vegetative growth while elevated CO_2 (C_E) had a strong impact on leaf physiology.
- Vegetative growth was dominated by the interactive effects of elevated temperature (T_E) and C_E on phenology, physiology and soil nutrients, and crop responses were similar in the two soils.
- However, during reproductive growth, the effects of T_E and C_E were limited by soil N availability, inducing changes in resource allocation between vegetative and reproductive growth.
- Such positive responses observed in the first season disappeared in the second season, as cotton productivity was influenced by legacies of C_E and T_E on soil and microbial properties.
- In particular, the legacy of C_E effect through crop residue on soil and microbial properties strongly reduced soil N availability, altering resource allocation more towards belowground and less towards seed cotton yield.
- C_E and T_E also altered the abundance and composition of soil bacterial community, which showed strong correlations with soil chemical properties and soil processes, suggesting that shifts in soil microbial community could impact crop productivity through changes in nutrient cycling and availability.

Implications for Growers

- More N fertiliser will be required to prevent N limitation at C_E for crop production under future climate.
- The responses of soil nutrients and microbial community should be an integral part of climate adaptation strategies.
- The response of non-harvestable biomass to these environmental changes should also be considered and implemented as a part of residue management strategies.

Extreme weather impacts

- The magnitude of flooding and drought impact on cotton productivity was greater at future CO_2 and temperature regimes, suggesting that inter-annual variability in yield is likely to increase under more extreme climates.
- We also found that flooding and drought had contrasting consequences for soil N availability, with drought-induced loss of biological activity resulting in a large amount of residual N in the soil.

- Flooding and drought events occurred in the previous season can affect the soil and microbial properties and that those changes can indirectly influence cotton productivity of the subsequent season.
- In particular, changes in the abundance of nitrifier communities were strongly linked to soil processes that provide plant available N.

Implications for Growers

- Differential fertiliser management strategies are needed to minimise the legacy impact of extreme weather events. Our results suggest that more N fertiliser will be needed to ensure the productivity of subsequent crop following a flooding event, while the opposite is needed following a prolonged drought event.
- Soil microbial communities play an important role in minimising impact of climate change and extreme weather events on cotton productivity. Thus, the management practice which promotes soil health and microbes should be adopted for improved and sustainable cotton production.

Appendix 1: Supplementary figures

Appendix 2: Manuscript 1A (submitted)

Appendix 3: Manuscript 1B (ready for submission)

Supplementary information

TABLES

Table S1 Pearson's correlation between seed cotton yield against measured variables and that of partial correlation controlling for the effect of CO_2 and temperature.

Variables	Pearson's correlation		Partial correlation	
	r	*P*	*r*	*P*
Early growth rate	0.86	**<0.0001**	0.21	0.16
Boll size	0.84	**<0.0001**	0.54	**<0.0001**
Leaf N% (harvest)	-0.69	**<0.0001**	-0.35	**0.02**
Leaf C:N (harvest)	0.58	**<0.0001**	0.25	*0.09*
Leaf C:N (flowering)	-0.56	**<0.0001**	0.03	0.83
Boll number	0.55	**<0.0001**	0.41	**0.004**
Nitrate (late flowering)	0.51	**0.0002**	-0.08	0.59
Height (flowering)	0.50	**0.0003**	-0.06	0.71
Stomatal conductance	0.50	**0.0003**	-0.07	0.62
Leaf C% (harvest)	-0.48	**0.001**	-0.23	0.12
Node (flowering)	-0.44	**0.002**	-0.004	0.98
Leaf N% (flowering)	-0.33	**0.02**	-0.06	0.70
Leaf C% (flowering)	-0.33	**0.02**	-0.07	0.66
Phosphate (early flowering)	0.27	*0.07*	0.09	0.56
Nitrate (early flowering)	0.26	*0.07*	-0.22	0.14
Phosphate (harvest)	0.24	0.28	0.21	0.15
Photosynthetic rate	0.16	0.08	0.05	0.72
iWUE	-0.15	0.30	0.17	0.26
Nitrate (harvest)	0.13	0.39	-0.11	0.48
Phosphate (late flowering)	-0.08	0.60	0.17	0.25

Table S2 Results of analysis of variance (ANOVA) showing the effect of sampling (pre- and post-extreme weather treatment), soil, CO_2 and temperature on nitrate concentrations of well-watered control soils and flooded/drought soils planted with cotton under the four climate change treatments. Flooding effect was examined by comparing changes in nitrate concentrations at pre-flooding treatment (at early flowering) and post-flooding treatment (at 7 days after the end of flooding period) for well-watered and flooded soils. Drought effect was examined by comparing changes in nitrate concentrations at pre-drought treatment (at early flowering) and post-drought treatment (at harvest) for well-watered and drought soils. Values are probability with significant results (P<0.05) shown in bold, marginally significant results (P<0.1) in italic and non-significant results (P>0.1) as n.s.

	Flooding effect		Drought effect	
	Well-watered	Flooded	Well-watered	Drought
Sampling (pre vs post)				
Sampling	**0.001**	**<0.0001**	**<0.0001**	n.s.
Sampling x Soil	n.s.	*0.09*	**<0.0001**	n.s.
Sampling x CO_2	n.s.	n.s.	n.s.	n.s.
Sampling x Temp	n.s.	**<0.0001**	**0.001**	n.s.
Sampling x Soil x CO_2	n.s.	n.s.	n.s.	n.s.
Sampling x Soil x Temp	n.s.	n.s.	n.s.	*0.08*
Sampling x CO_2 x Temp	n.s.	n.s.	*0.06*	**0.01**
Sampling x Soil x CO_2 x Temp	n.s.	n.s.	n.s.	n.s.
Others				
Soil	**<0.0001**	*0.09*	**<0.0001**	**0.001**
CO_2	n.s.	n.s.	n.s.	n.s.
Temp	**<0.0001**	**<0.0001**	**0.0004**	**<0.0001**
Soil x CO_2	n.s.	n.s.	n.s.	*0.08*
Soil x Temp	n.s.	n.s.	n.s.	n.s.
CO_2 x Temp	n.s.	n.s.	n.s.	**0.03**
Soil x CO_2 x Temp	n.s.	n.s.	n.s.	n.s.

Supplementary information

FIGURES

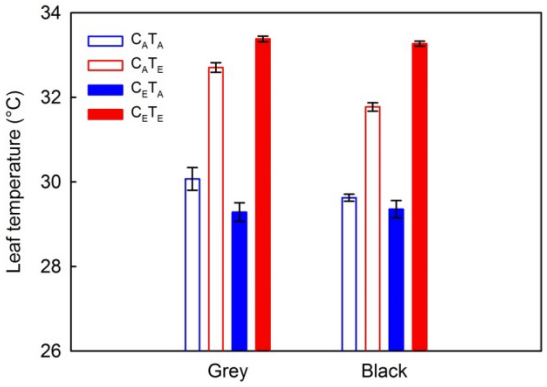


Fig. S1 Leaf temperature of cotton plants grown on grey vertosol and black vertosol under the four climate change treatments measured at early flowering (n=6). Measurements were taken over 3 days at early flowering stage of cotton growth.

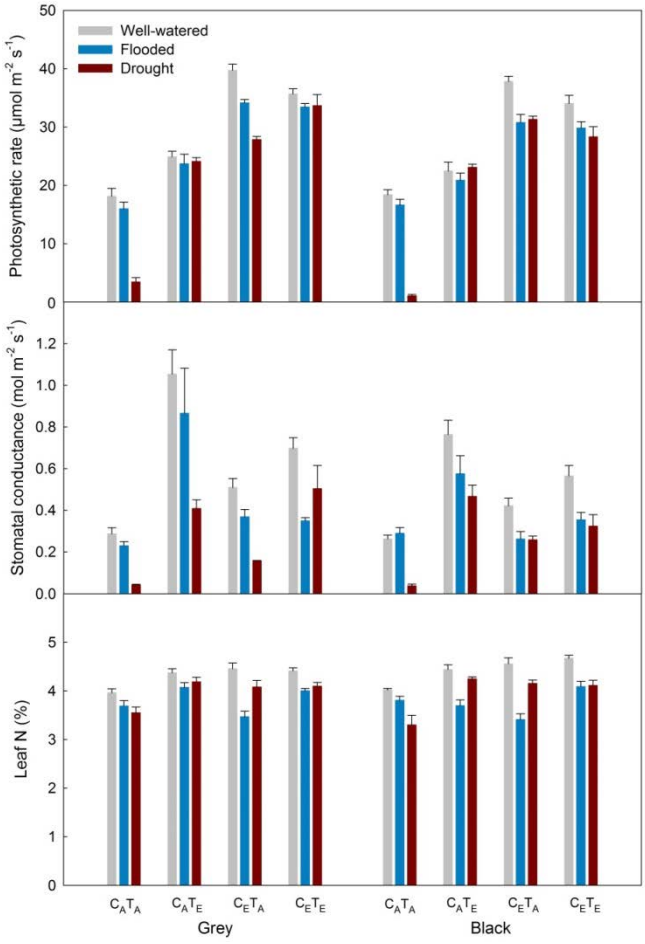


Fig. S2 Photosynthetic rate, stomatal conductance and leaf N concentrations of well-watered, flooded and drought cotton plants grown on grey vertosol and black vertosol under the four climate treatments measured at the end of flooding and early drought period.

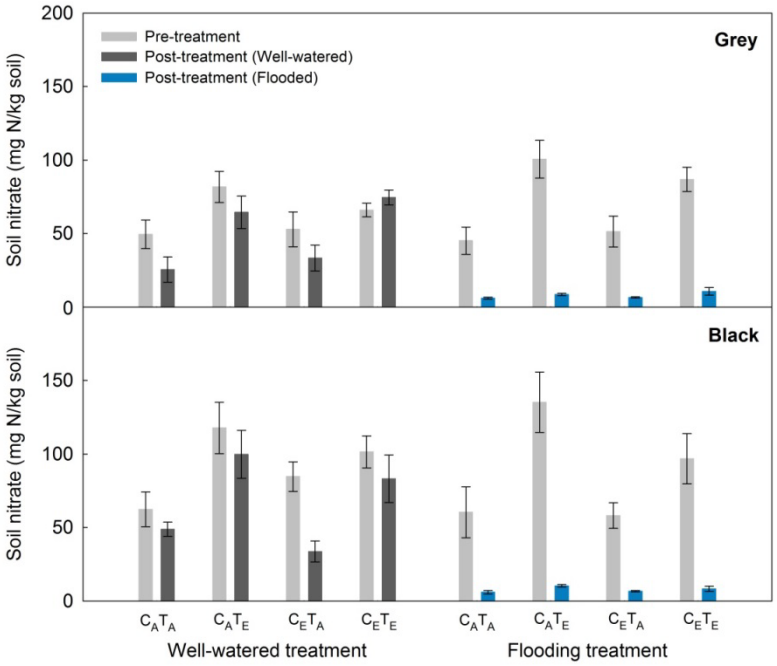


Fig. S3 Soil nitrate concentrations of grey vertosol and black vertosol planted with cotton under climate treatments at pre-water treatment (at early flowering) and post-water treatment (at 7 days after the end of flooding period) for well-watered and flooded soils.

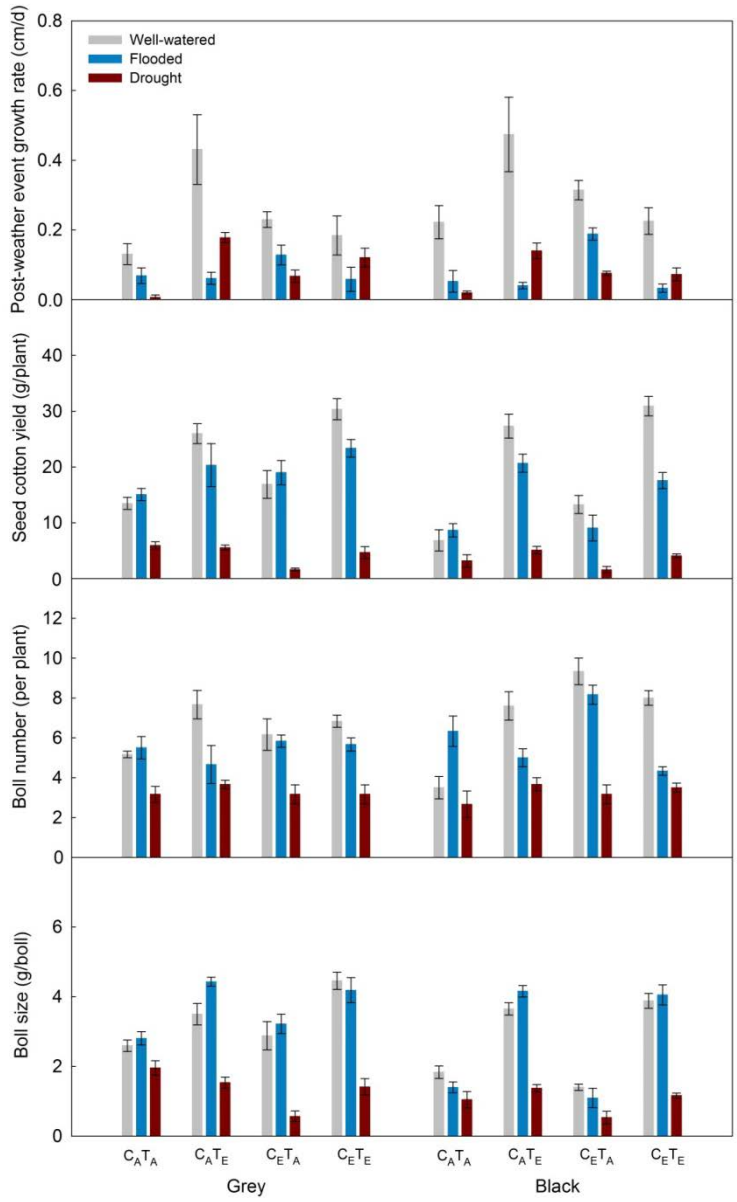


Fig. S4 Post-flooding/drought vegetative growth rate, seed cotton yield, boll number and boll size of well-watered and flooded/drought cotton plants grown on grey vertosol and black vertosol under the four climate treatments.

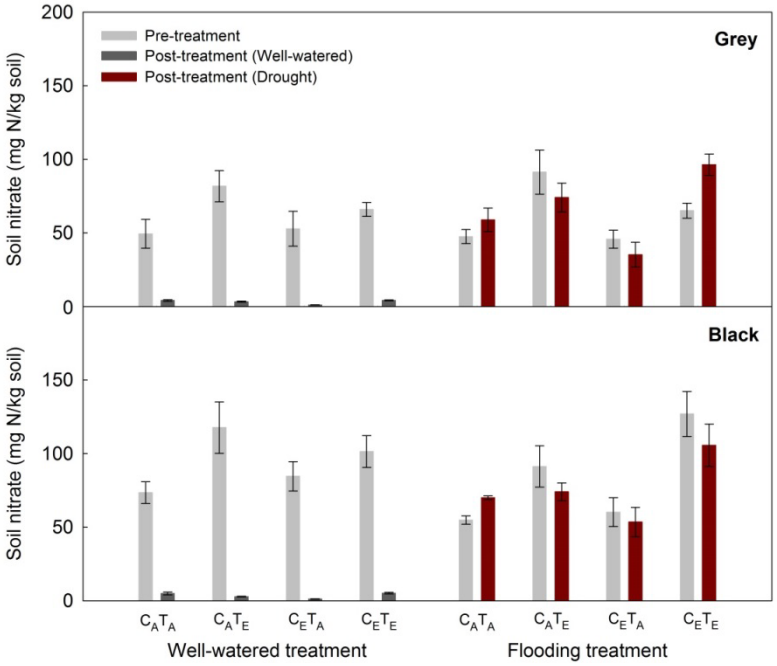


Fig. S5 Soil nitrate concentrations of grey vertosol and black vertosol planted with cotton under climate treatments at pre-water treatment (at early flowering) and post-water treatment (at harvest) for well-watered and drought soils.

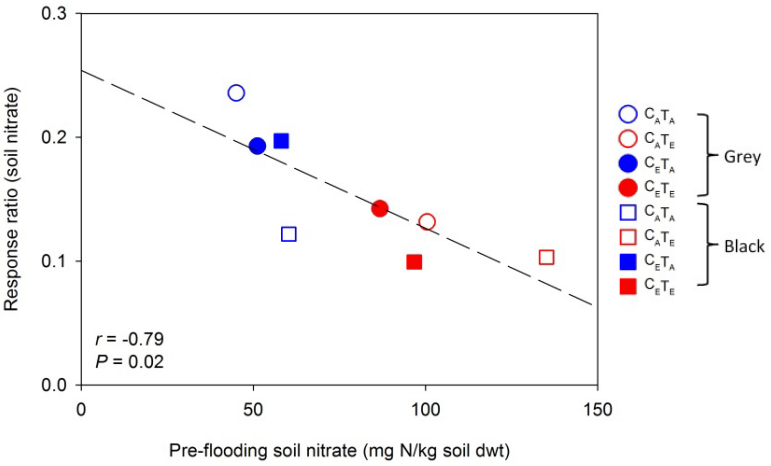


Fig. S6 The relationship between response ratio of soil nitrate to the flooding treatment and soil nitrate concentrations at pre-flooding treatment.

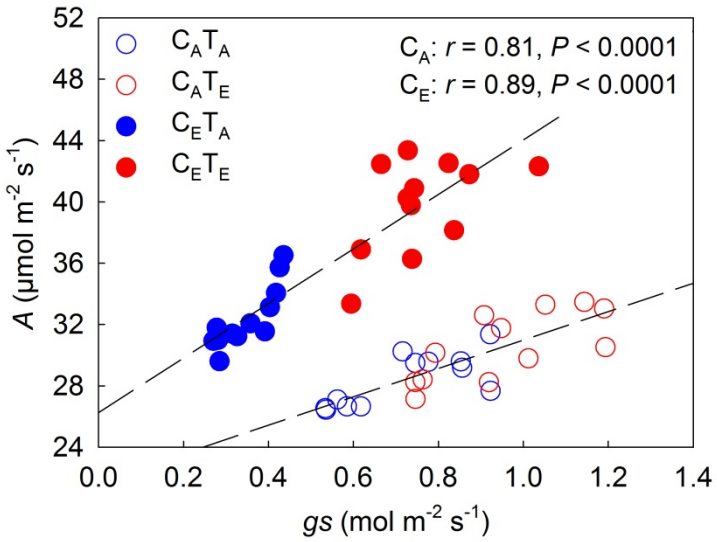


Fig. S7 The relationship between photosynthetic rate (*A*) and stomatal conductance (*gs*) at each CO_2 level of cotton plants grown at the four climate change treatments. Measurements were taken over 3 consecutive days at early flowering stage.

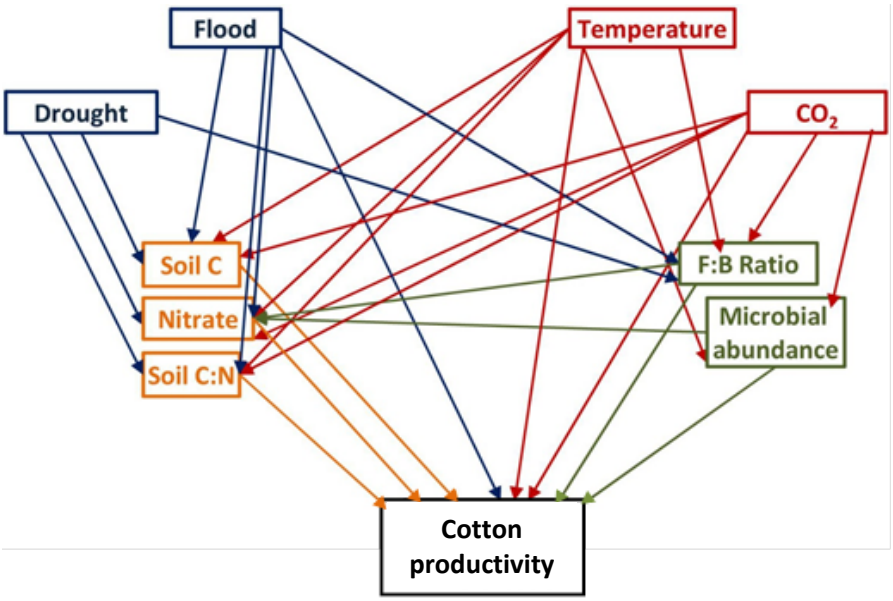


Fig. S7 A prior model

Plant-soil interactions and nutrient availability determine the impact of elevated CO_2 and temperature on cotton productivity

Number of text pages: 35

Number of tables: 4

Number of figures: 5

Running head: Plant-soil interactions under climate change

Yui Osanai[1*], David T. Tissue[1], Michael P. Bange[2], Ian C. Anderson[1], Michael V. Braunack[2], Brajesh K. Singh[1, 3]

[1]Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, New South Wales 2751, Australia

[2]CSIRO Agriculture, Australian Cotton Research Institute, Locked Bag 59, Narrabri, New South Wales 2390, Australia

[3] Global Centre for Land Based Innovation, Western Sydney University, Locked Bag 1797, Penrith, New South Wales 2751, Australia

*Corresponding author: tel: +612 4570 1911, fax: +612 4570 1103, e-mail: y.osanai@westernsydney.edu.au

Keywords: climate change, cotton (*Gossypium hirsutum* L.), elevated CO_2, elevated temperature, plant-soil interaction, soil nutrient

Abstract

Background and aims: Elevated CO_2 (C_E) and temperature (T_E) can not only affect crop physiology and growth but also soil nutrient availability, which could in turn influence crop response to C_E and T_E. However, such indirect impacts of C_E and T_E on crop productivity are often unexplored, potentially underestimating the impact of C_E and T_E at a system level.

Methods: To explore the possible role of soil nutrient availability in mediating crop response to C_E and T_E, we examined the effects of C_E and T_E on cotton productivity and soil nutrient availability in two soils.

Results: Early vegetative growth response was dominated by the interaction between C_E and T_E; T_E accelerated vegetative growth while C_E enhanced photosynthesis and vegetative growth at ambient temperature. When soil nitrogen availability became low during the reproductive phase, altered soil nitrogen availability at C_E and T_E and differences in soil characteristics influenced resource allocation and yield response, resulting in a doubling of cotton yield at T_E but only a marginal increase at C_E.

Conclusions: Changes in soil nutrient availability induced by C_E and T_E during crop development can enhance or limit yield responses to C_E and T_E. Thus, soil responses should also be considered when developing adaptation strategy for climate change.

Introduction

The response of primary production to single climate change factors such as elevated [CO_2] (C_E) and elevated temperature (T_E) has been studied for many crop species (e.g. Cure and Acock 1986; Jablonski et al. 2002; Kimball 1983; Lobell and Field 2007). Crops generally respond positively to C_E with higher photosynthetic rates and biomass production, and lower stomatal conductance, thereby increasing water use efficiency (Kimball et al. 2002). Meta-analysis by Rustad *et al.* (2001) showed that T_E generally increased plant productivity through increased biomass production and lengthening of the growing season, particularly in regions where temperature currently limits growth. However, T_E has also been shown to reduce yields of major crop species (Asseng et al. 2015; Challinor et al. 2014; Lobell and Field 2007), with responses dependent on the magnitude of T_E and crop species (Schlenker and Roberts 2009; Tubiello et al. 2002). Importantly, the interactive effect of C_E and T_E on crop productivity may not be additive (Reddy et al. 1998; Sun et al. 2012; Yoon et al. 2009), which may limit the predictive capacity of current models based on single climate factor experiments.

The impact of C_E and T_E on plant productivity depends on other limiting resources, such as water and nutrient availability (Hovenden et al. 2014; Reich et al. 2006; Reich et al. 2014; Rustad et al. 2001), which may become limiting over longer periods of time (Luo *et al.* 2004). Stimulation of plant photosynthesis and productivity by C_E is often limited in nutrient-poor soils (Oechel et al. 1994; Reich et al. 2006; Reich et al. 2014; Tissue et al. 1993), as soil nutrients limit the capacity of plants to utilise increased carbon (C) availability. Such reductions in plant responsiveness to C_E have also been observed in well-fertilised cropping systems (Bloom et al. 2014; Franzaring et al. 2011; Li et al. 2003; Rogers et al. 1996)

59 suggesting that the initial stimulation in C_E may not be maintained over the long-term in any
60 system.

61 Soil nutrient availability may be affected by C_E and T_E, either directly or indirectly through
62 plant-mediated changes in soil processes that regulate nutrient cycling (Bardgett et al. 2008;
63 Reich and Hobbie 2013), particularly in non-cropping systems (Dieleman et al. 2012; Drake
64 et al. 2011; Hovenden et al. 2008; Phillips et al. 2011; Sherry et al. 2008; Zhou et al. 2012).
65 T_E often increases soil N mineralisation and availability (Bai et al. 2013) while the effect of
66 C_E on soil N varies in direction and magnitude amongst ecosystems (de Graaff et al. 2006;
67 Langley et al. 2009). Investigating the impact of changes in soil N availability in highly
68 productive cropping systems may be complicated, in part due to the addition of fertiliser and
69 concomitant changes in crop development and nutrient uptake. Thus, the importance of C_E
70 and T_E on crop productivity, through their effect on soil nutrient availability, is relatively
71 unknown.

72 Given the importance of plant-soil interactions in regulating soil nutrient cycling and
73 availability, differences in the physical and chemical properties of soils may modulate plant
74 responses to climate drivers due to their ability to hold and release resources for plant uptake
75 (Barton et al. 2005; Tolk et al. 1999). Soils may also contribute substantially to the regulation
76 of microbial community composition and their functions (Lauber et al. 2008; Wakelin et al.
77 2008), which mediates key soil processes that directly impact nutrient availability. While the
78 role of soil in mediating plant responses to C_E and T_E may have less importance in cropping
79 systems, where resource availability is often optimised, experimental evidence for this is
80 limited. Thus, it is unknown whether the response of a crop species to C_E and T_E is similar
81 across different soils. In a climate modelling study, Ludwig and Asseng (2006) found
82 significant differences in simulated yield response of wheat to climate change drivers (C_E, T_E

and precipitation) between acid sandy loam, duplex and clay soils, indicating that differences in soil properties may influence crop responses to climate change drivers. Given the importance of crop management in ensuring high agricultural productivity in future climate regimes (Challinor et al. 2014; Howden et al. 2007), greater understanding of the interaction between C_E, T_E and soil are required to develop crop management strategies to maintain crop productivity in future climates.

The aim of this study was to examine the main and interactive effects of soil, C_E and T_E on crop physiology, growth and soil nutrients. We chose cotton as the model system, since cotton is grown world-wide in high temperature regions, which may be particularly susceptible to rising temperature, yet is fast-growing, often responsive to C_E and strongly dependent on temperature and N supply for high productivity (Kimball and Mauney 1993; Mauney et al. 1978; Reddy et al. 1997). Using a naturally lit glasshouse, we grew cotton in a full factorial design: ambient (C_A) and elevated (C_E) [CO_2], ambient (T_A) and elevated (T_E) temperature, and two soils (grey and black vertosols) from seed until cotton lint formation. We hypothesised that (1) C_E will increase photosynthetic rate and increase the rate of vegetative growth and reproductive output; (2) T_E will accelerate the rate of vegetative growth and increase reproductive output; (3) C_E and T_E will interactively increase photosynthetic rate, the rate of vegetative growth and reproductive output; (4) C_E and T_E will increase soil nutrient availability thereby further contributing to the positive effect of C_E and T_E on crop productivity; and (5) crop response to C_E and T_E will differ between soils due to differences in physical and chemical properties.

Materials and methods

Soil, plant material and experimental conditions

A glasshouse experiment was set up in April 2013 using two soils (grey vertosol and black vertosol, Isbell 1996) collected from cotton growing regions in New South Wales, Australia. Grey vertosol (USDA Soil Taxonomy: Typic Haplustert) was collected at the Australian Cotton Research Institute in Narrabri (30°10'S, 149°40'E) and black vertosol (Ustic Pellustert) was collected from a farm in Spring Ridge (31°21'S, 150°12'E). Top-soil (0 – 20 cm) and sub-soil (20 – 40 cm) were collected separately at each of the two field sites and transported to the glasshouse facility at Western Sydney University in Richmond, NSW, Australia (the physical and chemical characteristics of these soils are listed in Table S1). The top-soil and sub-soil were placed into large pots (24 L, 26 x 26 x 40 cm deep). The pots were watered to field-capacity and allowed to drain for two weeks prior to planting cotton seeds.

Four naturally sun-lit glasshouse compartments were used to simulate four climate change treatments: (ambient [CO_2], ambient temperature; C_AT_A), (ambient [CO_2], elevated temperature; C_AT_E), (elevated [CO_2], ambient temperature; C_ET_A), and (elevated [CO_2], elevated temperature; C_ET_E). C_A was targeted at 400 ppm (averaged 421±1.3 ppm) and C_E was targeted at 640 ppm (646±7.3 ppm) [CO_2]. The target temperatures (day/night) for T_A were 28/16 °C (averaged 28.8±0.06/16.8±0.06 °C) and for T_E were 32/20 °C (averaged 33.2±0.06/20.0±0.01 °C) throughout the experiment. We simulated diurnal changes in temperature within each compartment by ramping up temperatures during the day and ramping temperatures down in the night; this occurred five times over each 24h period. Humidity was not controlled, and allowed to vary in each glasshouse compartment, as expected in the field. Subsequently, vapour pressure deficit (VPD) differed between the temperature treatments, with the mean of 1.06±0.003 kPa (ranged 0.61 – 2.72 kPa) for T_A and 1.25±0.003 kPa (ranged 0.61 – 3.15 kPa) for T_E. VPD did not vary between CO_2 treatments. Pots were rotated within and between glasshouse compartments on a monthly basis to avoid pseudoreplication and minimise potential effects on plant performance associated with

environmental conditions in each glasshouse compartment (see Ghannoum *et al.* (2010) for details on glasshouse environmental control).

Four cotton seeds (*Gossypium hirsutum* L. Cv, 71BRF [Bollgard II® Roundup Ready Flex®], CSIRO Australia; Stiller, 2008) were sown into each pot filled with grey or black vertosol and were thinned to one plant per pot at 1 to 2 leaf stage. Thus, the experiment consisted of a total of 48 plants (2 x CO_2 treatments, 2 x temperature treatments, 2 x soils, 6 x replicates). Pots were maintained under the [CO_2] and temperature treatments until plants were harvested (at 142 days after planting for T_E and 196 days after planting for T_A).

Pots were fertilised with Multigro® fertiliser (8 g, 10.1% N, 3.5% P, 5.5% K, 16.3% S, 7.8% Ca, Incitec Pivot Ltd, Melbourne) and 500 mL of Aquasol® (1.6 g/L, 23.0% N, 40% P, 18.0% K, 0.05% Zn, 0.06% Cu, 0.0013% Mo, 0.15% Mn, 0.06% Fe, 0.011% B, Hortico, Vic) to achieve a N fertiliser amount of 190 kg N ha^{-1}, which is commonly applied to irrigated cotton in the field (Braunack 2013). Fertiliser was applied once before cotton seeds were sown. Soil volumetric water content (VWC) was monitored using TDR (time domain reflectometer) probes. All pots were watered to maintain optimal VWC (40-60%) throughout the experiment.

Plant growth measurements

Plant measurements (height and number of nodes) were taken periodically to examine treatment effects on vegetative growth. The number of days to attain phenological stages (first square [flower bud], first flower and first open boll) were recorded for each plant to examine treatment effects on the rate of plant development. Vegetative growth rate was calculated by the plant height at the early flowering stage divided by the number of days to achieve it. At harvest, the number of open bolls, green bolls, flowers, squares and fruiting

154 sites were recorded to examine treatment effects on reproductive growth. As this study was a
155 part of two-season long experiment, we were unable to determine vegetative biomass
156 production for this experiment. Seed cotton was harvested from all bolls and weighed after
157 being oven-dried at 70 °C for a week.

158 *Leaf physiology and nutrient measurements*

159 Net photosynthesis and stomatal conductance were measured over 3 days at the early
160 flowering stage on fully expanded leaves using a portable open gas exchange system (LI-
161 6400XT, LI-COR, Lincoln, NE, USA). The measurements were taken at midday (between
162 1100 h and 1400 h) at saturating light (photosynthetic photon flux density of 1800 μmol m^{-2}
163 s^{-1}), using mid-day growth temperature (28 °C or 32 °C) and [CO_2] (400 ppm or 640 ppm) for
164 each climate treatment combination; three measurements from the same leaf were recorded
165 and averaged. The measurements for each plant over 3 days were then averaged to account
166 for daily fluctuations in photosynthetic response to the treatments. A leaf sample was taken
167 from each plant at flowering (from recently fully expanded leaves) and at harvest (from
168 canopy) for the determination of total C and N concentrations. Leaf samples were oven-dried
169 at 70 °C and ground to powder prior to analysis using an elemental analyser (CE1110 CHN-S,
170 Carlo Erba, Milan, Italy).

171 *Soil sampling and nutrient analyses*

172 Soil samples were taken at plant growth stages of early flowering, late flowering and harvest
173 by taking two cores from the top 10 cm of the pot (2.5 cm in diameter). The samples were
174 homogenised by passing through a 4 mm-sieve which also removed large roots, litter and
175 gravel. Sub-samples were taken for determination of soil pH_{water} and soil gravimetric water
176 content by oven-drying at 105°C for 24 h. Plant available N was assessed by measuring

ammonium (NH_4^+) and nitrate (NO_3^-) concentrations of soil samples extracted with 2*M* KCl and plant available phosphorus (P) was determined by bicarbonate-extractable P using the Colwell method (Rayment and Lyons 2010). The extracts were analysed for NH_4^+, NO_3^- and phosphate concentration using an AQ2 discrete analyser (SEAL Analytical, Wisconsin, USA).

Statistical analyses

All data were analysed by three-way analysis of variance (ANOVA) in R statistical software (ver. 3.2.2, R Core Team 2014) to test the main and interactive effects of soil, C_E and T_E. We treated the six pots for each soil in each climate change treatment as a true replicate by rotating pots within and between the glasshouse compartments. All data were further analysed by two-way ANOVA for each soil to compare means using the Tukey's *post hoc* comparison. Plant height and node data were analysed by repeated measures ANOVA to test the temporal effect of the main and interactive effects of soil, C_E and T_E. All data were checked for normality and heteroscedasticity, and log-transformed where necessary. Relationships between the seed cotton yield and measured variables were examined by Spearman's correlation (*r*) and further by partial correlation analyses while controlling for the effect of C_E and T_E using 'ggm' package in R 3.2.2 (Marchetti et al. 2015).

Results

Plant development and vegetative growth

Plant development (number of days to attain phenological stages) and vegetative growth (plant height and number of nodes) were significantly influenced by C_E and T_E (Table 1, 2), with a strong interaction between C_E and T_E on plant development and plant height in both soils (CO_2 x temperature interaction, $P<0.0001$ and $P=0.0002$, respectively). For plants

grown at C_AT_E, the rate of early plant development was increased by more than 50% relative to C_AT_A, with plants reaching first square on average 51 and 61 days earlier in grey vertosol and black vertosol, respectively; this difference was maintained until harvest (Table 1, Fig. 1). Plants grown in C_ET_A also had increased rates of early development compared to C_AT_A; 24% and 28% faster in grey vertosol and black vertosol, respectively. Plants grown in C_ET_E showed a similar response to that of C_AT_E, thus the effect of C_E was only evident at T_A and not at T_E in both soils. Plant development and vegetative growth (plant height and number of nodes) differed significantly between the two soils, with faster growth observed for plants grown on grey vertosol than black vertosol (Table 1, 2). Soil interacted with T_E to the first phenological event (i.e. the development of first square), with a greater difference between the two soils at T_A (temperature x soil interaction, P=0.03).

The effect of C_E and T_E on the rate of vegetative growth measured as change in plant height reflected the differences in the rate of phenological development; however, the strength of their effects changed with time (repeated ANOVA, time x CO_2 x temperature interaction, P<0.0001), with the greatest differences between the treatments observed around 100 days after planting (DAP) in both soils (Fig. 1a, b). T_E accelerated the rate of vegetative growth at C_A and C_E, with plants reaching their maximum height at 109 DAP in both soils (Fig. 1a, b). Plants grown in C_ET_A reached their maximum height at 142 DAP compared to 161 DAP for plants grown in C_AT_A. C_E and T_E, either singly or in combination, increased the final height of the plants by 22% on average for both soils (CO_2 x temperature interaction, P=0.0002, Table 1). The rate of vegetative growth also reflected the difference in the rate of development between the two soils and was faster in the grey than black vertosol soils throughout the experiment (repeated ANOVA, P=0.01).

The effect of C_E and T_E on node development interacted with time in both soils (repeated ANOVA, time x CO_2 x temperature interaction, P<0.0001), with the greatest differences

between the treatments being observed around 100 DAP (Fig. 1c, d). T_E increased the rate of node development at C_A and C_E, in both soils and C_E also increased the rate of node development, but only at T_A. Although C_E and T_E increased the rate of node development, both reduced the total number of nodes produced per plant (P=0.001, P=0.0002 respectively, Table 1). The final node number was higher in black vertosol compared to grey vertosol (P=0.001); however, the effect of C_E and T_E on final node number did not differ between the two soils. Plants grown in the grey and black vertosol also differed in the rate of node development, although the nature of this difference changed with time (repeated ANOVA, time x soil interaction, P=0.0004). Node development was faster for plants grown on grey vertosol compared to those in black vertosol at early stages of growth; however, this difference disappeared by the flowering stage and eventually reversed by harvest (Fig. 1c, d).

Leaf physiology

There was a strong positive effect of C_E on net photosynthetic rate in both soils (P<0.0001, Table 2, Fig. 2a); however, the magnitude of this effect depended on the temperature treatment (CO_2 x temperature interaction, P<0.0001), as the positive effect of C_E was significantly greater at T_A compared to T_E, in both soils. There were no significant differences in net photosynthetic rate between plants grown on grey vertosol and black vertosol (Table 2). Stomatal conductance was significantly increased by T_E (P<0.0001, Fig. 2b), particularly at C_A (CO_2 x temperature interaction, P<0.0001), which more than doubled stomatal conductance in plants grown at C_AT_E when compared to plants in C_AT_A in both soils. There was also a significant difference in stomatal conductance between the two soils (Table 2), where plants grown on grey vertosol had a higher stomatal conductance than plants grown on black vertosol. The instantaneous water use efficiency (iWUE) calculated from the net photosynthetic rate over transpiration rate was strongly increased by C_E in both soils

(P<0.0001) particularly at T_A (CO_2 x temperature interaction, P<0.0001), with the effect of C_E and T_E being antagonistic (Fig. 2c). C_ET_A increased the iWUE by 90% and 79% in the grey and black vertosol, respectively, compared to C_AT_A, while C_ET_E increased iWUE by 33% and 24 % in the grey and black vertosol, respectively, compared to C_AT_A. There were no differences in iWUE between the two soils (Table 2).

Cotton yield and yield components

There were significant positive effects of T_E and C_E on seed cotton yield (P<0.0001, P=0.001 respectively, Table 2, Fig. 3a), which did not interact with each other nor with soils. The highest yield was observed at C_ET_E with more than a 2-fold increase compared to C_AT_A, followed by C_AT_E and C_ET_A (Fig. 3a). There was only a marginal difference in seed cotton yield between the two soils (P=0.07) where plants grown on grey vertosol produced more seed cotton yield than that of black vertosol. The total number of bolls produced per plant was significantly influenced by the interaction between CO_2, temperature and soil (CO_2 x temperature x soil interaction, P=0.03, Table 2), giving no consistent pattern in the effect of climate change treatments (Fig. 3b). In both soils, however, plants grown at C_AT_A produced the least number of bolls. The average size of each boll differed between CO_2 and temperature treatments; however, each treatment also interacted with soil (CO_2 x soil interaction, P<0.05, temperature x soil interaction, P=0.01), largely driven by the lack of C_E effect and greater effect of T_A on boll size in black vertosol compared to grey vertosol (Fig. 3c).

Leaf nutrients

Leaf total C and N concentrations and C:N ratios differed substantially between developmental stages (Table 2, 3). At flowering, C_E significantly increased C concentrations,

but only at T_A (CO_2 x temperature interaction, P=0.001) in both soils. C_E and T_E alone or in combination increased leaf N concentration in both soils (CO_2 x temperature interaction, P=0.004) by 16% compared with C_AT_A. This increase was largely reflected in leaf C:N ratios with plants grown at C_AT_E, C_ET_A and C_ET_E having lower C:N ratios than plants grown at C_AT_A (CO_2 x temperature interaction, P=0.02). There were no significant differences in leaf C and N concentrations and C:N ratios between the plants grown on the two soils, although T_E decreased leaf C concentrations only in black vertosol (temperature x soil interaction, P<0.05). At harvest, leaf C contents were higher than during flowering but generally showed a similar pattern to that of flowering, although there was a significant CO_2 x temperature x soil interaction (P<0.0001). Leaf N concentration on the other hand showed a substantial decrease at harvest (by 43% on average). C_E and T_E alone or in combination reduced leaf N concentration at harvest in both soils (CO_2 x temperature interaction, P<0.0001). Leaf C:N ratios reflected this change in the effect of C_E and T_E, with plants grown at C_AT_E, C_ET_A and C_ET_E having higher C:N ratios that that of C_AT_A (CO_2 x temperature interaction, P<0.0001). There were significant differences in leaf N concentrations and C:N ratios between the two soils (P=0.001 and P=0.003, respectively).

Soil nutrients

Soil nitrate concentrations declined gradually as plants matured (Table 4). The rate of soil nitrate depletion was greater at T_E in both soils (P=0.0001). Soil nitrate concentrations were 47% and 128% higher under T_E than T_A for grey and black vertosol at early and late flowering, respectively. By harvest, little nitrate remained in the soil, and nitrate concentrations were particularly low under C_ET_A for both soils (CO_2 x temperature interaction, P<0.0001). Soil ammonium concentrations were less than 5 mg N kg soil^{-1} for both soils and did not change throughout the experiment (data not shown). Soil phosphate

concentrations differed significantly between the soils (P<0.0001) and fluctuated throughout the experiment, with no consistent effect from C_E or T_E. Soil inorganic N and P concentrations differed significantly between the two soils; however, responses to climate change treatments were generally similar between soils.

Relationships between yields, vegetative growth, plant physiology and leaf and soil nutrient status

Correlation analyses were performed to examine the relationship between the seed cotton yield and yield components, vegetative growth, leaf physiology and leaf and soil nutrient status (Table S2). Seed cotton yield was correlated with boll size (r=0.84, P<0.0001, Fig. 4a) and also correlated with vegetative growth rate (calculated as the height at first flowering divided by the number of days to reach the stage of first flowering), with T_E driving this relationship (r=0.86, P<0.0001, Fig. 4b). There were also significant relationships between seed cotton yield and soil nitrate concentrations at late flowering (r=0.51, P=0.0002, Fig. 4c) and leaf N concentrations at harvest, (r=–0.69, P<0.0001, Fig. 4d), with T_E also driving these relationships. We further examined these relationships using partial correlation analysis while controlling for the effect of climate change treatment and found that significant relationships were maintained for boll size and leaf N concentrations at harvest (Table S2). However, this was not the case for vegetative growth rate and soil nitrate concentrations at late flowering, suggesting that climate change treatments were the underlying drivers of these relationships (Table S2).

Discussion

Our study found that there were complex interactions between C_E, T_E and soil that influenced vegetative and reproductive growth differently as soil N availability and resource allocation

changed throughout crop development (Fig. 5). T_E was the dominant factor in cotton productivity, accelerating the rate of plant development and vegetative growth while C_E had a strong impact on leaf physiology. T_E, and C_E to a lesser extent, increased seed cotton yield; however, there was an interaction between C_E and T_E on phenology, vegetative growth, leaf physiology and nutrient status that influenced the crop response during the development. T_E impacted soil N availability, with greater soil N at T_E than T_A at flowering contributing to a greater seed cotton yield at T_E than T_A. Changes in soil N availability and leaf N status during crop development and their relationships with seed cotton yield indicate that the differences in soil N availability became an important determinant of reproductive growth and resource allocation within the plant (Fig. 5b). Plant responses to C_E and T_E were generally similar between the two soils during vegetative growth; however, differences in soil properties exerted a stronger impact on the response of yield components to C_E and T_E, suggesting that differences in their ability to continuously provide N impacted reproductive response to C_E and T_E. This study therefore provides some insights into the complex interactions between C_E and T_E on crop productivity through plant-soil interactions and highlights the importance of incorporating soil responses in understanding the mechanisms by which C_E and T_E impact crop productivity.

Factors affecting vegetative growth – interactive effects of T_E and C_E

The rate of vegetative growth and development was strongly affected by both T_E and C_E; although the positive effect of C_E on vegetative growth was only evident at T_A. Temperature is the primary environmental factor that controls phenology and growth of many crop species, with a moderate increase in temperature increasing the rate of plant development and growth in cotton (Bradow and Bauer 2010; Reddy et al. 1992). Reddy *et al.* (1992) found that an increase of maximum temperature from 25 °C to 30 °C doubled plant height and increased

node number by 30% during the early vegetative growth in cotton. The effect of T_E on early vegetative growth was stronger in our study, with 3-4 times faster growth than Reddy *et al.* (1992). This is most likely due to the greater diurnal temperature difference used in our study (12 °C) compared to their study (8 °C), accentuating the temperature effect, as lower night temperatures have also been shown to reduce vegetative growth in cotton (Warner and Burke 1993).

The effect of C_E on the rate of vegetative growth was relatively small and depended on temperature, with no apparent increase in vegetative growth in C_E observed at T_E. The positive effect of C_E on early vegetative growth at T_A is comparable to the findings of Mauney *et al.* (1994) who found that C_E (350 ppm to 550 ppm) increased total biomass of cotton by 50% by the early flowering stage. The interactive effect of C_E and T_E was observed in the study by Reddy *et al.* (1995) who observed that C_E (350 ppm to 700 ppm) had no effect on cotton growth at low temperature (20 °C/12 °C), while C_E significantly increased vegetative biomass with higher temperatures (the maximum ranged from 25 °C to 35 °C) under continuous nutrient supply. Their findings contrast with our results; however, the lack of C_E effect on vegetative growth at T_E we observed is most likely due to the shift in carbohydrate allocation from vegetative to reproductive growth, as indicated by the accelerated rate of plant development at C_ET_E. By examining the effect of C_E and T_E on the entire plant development process culminating in seed cotton production, our study was able to capture the effect of C_E at T_E on all cotton growth phases, which otherwise may have been underestimated by examining early vegetative growth only.

Leaf physiology was responsive to C_E and T_E. C_E had a strong positive effect on photosynthetic rate while T_E increased stomatal conductance; these results are in line with the findings of previous studies in cotton (Mauney et al. 1978; Radin et al. 1987; Zhao et al. 2004). The combined effect of C_E and T_E on leaf physiology was not simply additive, but

highly interactive. The limited response to C_E at T_E may indicate that down-regulation of photosynthesis may have occurred at C_ET_E in response to nutrient-limitation (Tissue and Oechel 1987) or sink-limitation (Woodrow 1994), although it is not likely that either of these limitations occurred during the early flowering stage to significantly affect subsequent growth. Another explanation for the reduction in photosynthetic rate is the increased photorespiration, as a result of increased leaf temperature following reduced stomatal conductance at C_E (Drake et al. 1997). We found that stomatal conductance was smaller and leaf temperature was higher at C_ET_E than C_ET_A (Fig. S1). Thus, this may explain the limited C_E effect on photosynthetic rate at T_E than T_A and may have contributed to the limited growth response to C_E at T_E. The increase in stomatal conductance at C_E, particularly at C_ET_A, is somewhat unexpected, as C_E generally decrease stomatal conductance (Kimball et al. 2002). Stomatal conductance is highly sensitive to environmental variations (Damour et al. 2010), thus by opting to standardise the developmental stage of the plants, the measurement dates differed between the treatments. Thus small daily variations in the glasshouse environment (e.g. vapour pressure deficit, light, and air movement) may have contributed to the increase in stomatal conductance at C_ET_A.

Factors affecting reproductive growth – plant-soil interactions at C_E and T_E

Reproductive growth was positively correlated with vegetative growth, and we found a strong positive yield response to T_E, while C_E also increased yield although to a lesser extent. Previous studies on the impact of C_E and T_E on cotton yield found that cotton yield responded to both C_E and T_E, with 60% increase in yield when [CO_2] was increased from 350 ppm to 650 ppm (Kimball and Mauney 1993). The cotton yield response to higher growth temperature is dependent on the relationship to the optimal temperature (Reddy et al. 1995; Wanjura et al. 1992; Yoon et al. 2009). Reddy *et al.* (1995) found that raising the maximum

391 temperature from 25 to 30 °C increased reproductive output (boll and square dry weight)
392 three-fold, while raising [CO_2] from 350 ppm to 700 ppm had less effect. Yoon *et al.* (2009)
393 on the other hand observed a 50% reduction in yield across all CO_2 treatments when
394 temperature was increased to a supra-optimal temperature of 35 °C. Here, we found that
395 raising the growth temperature from 28 to 32 °C resulted in a strong positive growth response,
396 clearly indicating that 32 °C was at or below the optimal temperature for cotton growth
397 (Conaty et al. 2014).

398 Reproductive response to C_E and T_E differed from that of vegetative response in that the
399 response was strongly influenced by soil N availability through its effect on the ability of
400 plants to sustain boll growth (Fig. 5b). Boll growth is influenced by changes in source/sink
401 relationships which control assimilate partitioning and determine the amount of carbohydrate
402 supply to developing bolls (Baker and Baker 2010). Imbalance in the source/sink relationship
403 has been demonstrated to cause reduced growth, flowering and boll retention (Guinn 1985).
404 Rogers *et al.* (1996) investigated sink strength in cotton under C_E and found that soil N
405 supply had a marked effect on sink strength and therefore the responsiveness to C_E treatment.
406 We found that leaf N did not differ between treatments at flowering and that the
407 concentrations were comparable to those reported for well-fertilised cotton plants (Milroy et
408 al. 2001; Rogers et al. 1996). This suggests that soil N supply was not limiting at early
409 flowering stage, thus a strong positive plant response to C_E could be maintained. However,
410 the relationship between soil nitrate at the late flowering stage and seed cotton yield suggests
411 that during reproductive growth, soil N availability became limiting and reduced the effect of
412 C_E and T_E on reproductive growth.

413 Our study provides evidence that T_E can influence crop productivity indirectly through their
414 impact on soil nutrient availability, as greater soil N availability at T_E is likely to have added
415 to the positive effect of T_E on reproductive growth. Such indirect effect through soil N

availability is often difficult to detect due to concomitant increase in plant growth and thus is rarely explored in highly productive fertilised systems. Evidence from unfertilised/natural systems shows that T_E often increases N mineralisation, nitrification and soil inorganic N availability (Bai et al. 2013), particularly where T_E does not lead to soil moisture limitation. While the number of studies is limited, T_E also increased N mineralisation and N availability in fertilised cropping systems (Patil et al. 2010; Zhang et al. 2013), suggesting that soil responses to T_E can contribute to the overall effect of T_E on crop productivity. Furthermore, plant available N such as ammonium and nitrate can be immobilised by the soil microbial community or lost through denitrification, ammonia volatilisation or nitrate leaching. While studies have found that T_E increases denitrification and nitrous oxide fluxes (Bai et al. 2013; Patil et al. 2010), accelerated crop development at T_E meant that T_E also reduced the time in which soil N becomes unavailable for plant uptake, thereby further contributing to the positive effect of T_E on crop productivity.

The impacts of C_E on soil N are often found to be plant-mediated, such as reduced plant N demand following decreased leaf N concentrations (Ainsworth and Long 2005) and altered N availability through increased C allocation to belowground /root biomass, rhizodeposition or root exudates (Pendall et al. 2004; Zak et al. 1993) that could increase or decrease soil N availability by promoting microbial mineralisation and immobilisation of N (de Graaff et al. 2006). However, Wood *et al.* (1994) found no effect of C_E on N mineralisation, despite an increase in belowground C input over the three years of cotton production at C_E. We also found no evidence that C_E increased soil N availability nor reduced leaf N concentrations. Thus, unlike T_E, the lack of a positive effect of C_E on soil N availability suggests that limited soil N availability during the reproductive phase may have limited the potential effect of C_E on reproductive growth.

Indeterminate crop species such as cotton can maintain active vegetative growth even during the reproductive phase depending on environmental conditions and resource availability. Excessive N and water supply have been linked to excessive vegetative growth (rank growth), delaying the attainment of a full fruit load and maturity (Halevy et al. 1987; Mullins and Burmester 2010). We found differences in resource allocation (i.e. carbohydrates and N) at T_E and C_E, suggesting that indirect effects of T_E and C_E on reproductive growth may occur via their impact on soil N availability. The lack of vegetative growth of plants at C_ET_E during the reproductive phase suggests that soil N may have limited the yield potential of these plants. Similar observations were made by Halevy *et al.* (1987) who found that vegetative growth ceased in low N treatments after the end of flowering and that translocation of N occurred from older leaves to the developing bolls. Furthermore, they found that in severely N-limited plants, boll development was completely stopped. This may explain the negative relationship we found between leaf N concentrations at harvest and seed cotton yield, and unexpectedly high leaf N found in plants at C_AT_A suggesting that these plants may have ceased their reproductive growth due to the low soil N availability.

The role of soil N availability in determining the effect of C_E and T_E is also evident from the importance of soil properties at influencing the reproductive responses to C_E and T_E. Soils with different physical and chemical properties differ in their ability to store and supply water and nutrients to plants. Thus, the effect of soil may be minimal when water and nutrients are readily available but exerts a strong influence on plant growth when water or nutrients become limiting. Ping *et al.* (2004) examined the relationship between cotton yield, quality and soil properties from an irrigated cotton field in Texas that comprised three soil types (variations within sandy loam and sandy clay loam) over three years. They found that the relationship differed significantly between years, reflecting differences in rainfall and fertiliser N management. For instance, they found that soil texture was more important to

cotton yield in a drier year than a wet year. Such interactions between soil properties and resource availability are in agreement with our findings, and further suggest that differences in soil properties can modulate crop responses to C_E and T_E through their interaction with resource availability.

Implications for future cotton productivity

The positive yield response to C_E and T_E we found suggests that cotton productivity may be improved under future climatic conditions. C_E is likely to benefit productivity, provided that soil N and water availability are adequately managed during crop development (Mauney et al. 1978). However, a recent meta-analysis by Feng *et al.* (2015) has indicated that C_E may reduce plant N acquisition regardless of soil N availability, and that different mechanisms and processes such as reduced N mineralisation (Luo et al. 2004), increased microbial N immobilisation (de Graaff et al. 2006), reduced nitrate assimilation (Bloom et al. 2014), reduced demand for Rubisco (Long et al. 2004) and reduced transpiration-driven mass flow of soil N (McDonald et al. 2002; McGrath and Lobell 2013) act in parallel to influence plant N acquisition. Thus, identifying relative importance of these mechanisms in controlling N acquisition in cotton is necessary to develop an adequate N management strategy to maintain enhanced cotton productivity at C_E.

The positive effect of T_E is restricted to moderate temperature increases and will be reversed if temperatures increase beyond the optimal growth temperature (Reddy et al. 1995; Yoon et al. 2009). A strong response to T_E has also been observed in other crop species (Jumrani and Bhatia 2014; Lobell and Field 2007) as temperature is the main climatic factor that influences the growth of most crop species. Fluctuations in temperature during the growing season are likely to affect crop growth, with both maximum and minimum temperatures outside the optimal growth temperature. Thus, one needs to be cautious when extrapolating the results

489 from this temperature controlled glasshouse study into the highly variable environment of the
490 field. Nonetheless, measuring crop response in a controlled environment is necessary to
491 unravel complex interactions between multiple climatic factors that affect crop productivity.
492 Thus, this study provides some insight into mechanisms that likely regulate plant
493 performance in the field.

494 The extent to which C_E and T_E may affect yield response is an important aspect in assessing
495 the sustainability of agricultural productivity. It has been suggested that the effect of C_E may
496 be short-lived, due to resource limitations required to support enhanced plant growth (Luo et
497 al. 2004). Thus, if resource availability is adequately managed, the positive impact of C_E may
498 be sustained (Liberloo et al. 2007). This requires an understanding of plant-soil interactions
499 and whether climate-induced changes have feedback effects on subsequent crop production
500 through changes in soil fertility and function. Changes in belowground C input may induce
501 changes in soil microbial community structure and function, which may impact nutrient
502 cycling and availability (de Graaff et al. 2006; Singh et al. 2010). Such changes in soil
503 processes are likely to be influenced by differences in soil physical, chemical and biological
504 properties. Thus, the understanding of long-term effects of climate change drivers on crop
505 productivity needs to integrate both above- and below-ground responses as well as feedbacks
506 between these responses on crop productivity.

507 Our study showed that T_E was the dominant factor in cotton productivity, accelerating the
508 rate of plant development and vegetative growth while C_E had a strong impact on leaf
509 physiology. Vegetative growth was dominated by the interactive effects of T_E and C_E on
510 phenology, physiology and soil nutrients, and crop responses were similar in the two soils.
511 However, during reproductive growth, the effects of T_E and C_E were limited by soil N
512 availability, inducing changes in resource allocation between vegetative and reproductive
513 growth. The reduction in soil N availability during reproductive growth also caused

differences in soil properties to exert stronger influence over the crop response to C_E and T_E. Together, these results highlight the complex interactions between C_E, T_E and soil on crop productivity that are highly dynamic in their impacts depending on soil resource availability and resource allocation within the plants during crop development. This study demonstrated the importance of examining both plant and soil responses in understanding the mechanisms by which C_E, T_E and soil impact crop productivity, and provides evidence that differences in soil properties can modulate crop responses to C_E and T_E by their ability to continuously provide resources as per the crop's demand. Such knowledge is crucial in developing effective adaptation strategy for crop production under future climate, and future research should explicitly consider plant-soil interactions in assessing the impact of climate change on crop production.

Acknowledgements

We acknowledge the financial assistance of the Cotton Research and Development Corporation in order to undertake this project. We thank M. Hovenden and P. Trivedi for their helpful comments on the manuscript and J. Grinyer, R. Smith and M. Thiessen for their assistance with glasshouse and laboratory work. We also thank Ben Clift 'Tathra' Spring Ridge for access to the soil for the study. We have no conflict of interest to declare.

References

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. New Phytologist 165: 351-371.

Asseng S, Ewert F, Martre P, Rotter RP, Lobell DB, Cammarano D, Kimball BA, Ottman MJ, Wall GW, White JW, Reynolds MP, Alderman PD, Prasad PVV, Aggarwal PK, Anothai J, Basso B, Biernath C, Challinor AJ, De Sanctis G, Doltra J, Fereres E, Garcia-Vile M, Gayler S, Hoogenboom G, Hunt LA, Izaurralde RC, Jabloun M, Jones CD, Kersebaum KC, Koehler AK, Muller C, Kumar SN, Nendel C, O'Leary G, Olesen JE, Palosuo T, Priesack E, Rezaei EE, Ruane AC, Semenov MA, Shcherbak I, Stockle C, Stratonovitch P, Streck T, Supit I, Tao F, Thorburn PJ, Waha K, Wang E, Wallach D, Wolf I, Zhao Z, Zhu Y (2015) Rising temperatures reduce global wheat production. Nature Climate Change 5: 143-147.

Bai E, Li SL, Xu WH, Li W, Dai WW, Jiang P (2013) A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. New Phytologist 199: 441-451.

Baker DN, Baker JT (2010) Cotton source/sink relationships. Physiology of Cotton: 80-96.

Bardgett RD, Freeman C, Ostle NJ (2008) Microbial contributions to climate change through carbon cycle feedbacks. Isme J 2: 805-814.

Barton L, Schipper LA, Barkle GF, McLeod M, Speir TW, Taylor MD, McGill AC, van Schaik AP, Fitzgerald NB, Pandey SP (2005) Land application of domestic effluent

552 onto four soil types: Plant uptake and nutrient leaching. Journal of Environmental
553 Quality 34: 635-643.

554 Bloom AJ, Burger M, Kimball BA, Pinter PJ (2014) Nitrate assimilation is inhibited by
555 elevated CO_2 in field-grown wheat. Nature Climate Change 4: 477-480.

556 Bradow JM, Bauer PJ (2010) Germination and seedling development. In: JM Stewart, DM
557 Oosterhuis, JJ Heitholt, JR Mauney (eds) Physiology of Cotton. Springer, New York.

558 Braunack MV (2013) Cotton farming systems in Australia: factors contributing to changed
559 yield and fibre quality. Crop Pasture Sci 64: 834-844.

560 Challinor AJ, Watson J, Lobell DB, Howden SM, Smith DR, Chhetri N (2014) A meta-
561 analysis of crop yield under climate change and adaptation. Nature Climate Change 4:
562 287-291.

563 Conaty WC, Mahan JR, Neilsen JE, Constable GA (2014) Vapour pressure deficit aids the
564 interpretation of cotton canopy temperature response to water deficit. Funct Plant Biol
565 41: 535-546.

566 Cure JD, Acock B (1986) Crop responses to carbon dioxide doubling - a literature survey.
567 Agr Forest Meteorol 38: 127-145.

568 Damour G, Simonneau T, Cochard H, Urban L (2010) An overview of models of stomatal
569 conductance at the leaf level. Plant Cell Environ 33: 1419-1438.

570 de Graaff MA, van Groenigen KJ, Six J, Hungate B, van Kessel C (2006) Interactions
571 between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis.
572 Glob Change Biol 12: 2077-2091.

573 Dieleman WIJ, Vicca S, Dijkstra FA, Hagedorn F, Hovenden MJ, Larsen KS, Morgan JA,
574 Volder A, Beier C, Dukes JS, King J, Leuzinger S, Linder S, Luo YQ, Oren R, de
575 Angelis P, Tingey D, Hoosbeek MR, Janssens IA (2012) Simple additive effects are
576 rare: a quantitative review of plant biomass and soil process responses to combined
577 manipulations of CO_2 and temperature. Glob Change Biol 18: 2681-2693.

578 Drake BG, GonzalezMeler MA, Long SP (1997) More efficient plants: a consequence of
579 rising atmospheric CO_2? Annual Review of Plant Physiology and Plant Molecular
580 Biology 48: 609-639.

581 Drake JE, Gallet-Budynek A, Hofmockel KS, Bernhardt ES, Billings SA, Jackson RB,
582 Johnsen KS, Lichter J, McCarthy HR, McCormack ML, Moore DJP, Oren R,
583 Palmroth S, Phillips RP, Pippen JS, Pritchard SG, Treseder KK, Schlesinger WH,
584 DeLucia EH, Finzi AC (2011) Increases in the flux of carbon belowground stimulate
585 nitrogen uptake and sustain the long-term enhancement of forest productivity under
586 elevated CO_2. Ecol Lett 14: 349-357.

587 Feng ZZ, Rutting T, Pleijel H, Wallin G, Reich PB, Kammann CI, Newton PCD, Kobayashi
588 K, Luo YJ, Uddling J (2015) Constraints to nitrogen acquisition of terrestrial plants
589 under elevated CO_2. Glob Change Biol 21: 3152-3168.

590 Franzaring J, Weller S, Schmid I, Fangmeier A (2011) Growth, senescence and water use
591 efficiency of spring oilseed rape (*Brassica napus* L. cv. Mozart) grown in a factorial
592 combination of nitrogen supply and elevated CO_2. Environ Exp Bot 72: 284-296.

593 Ghannoum O, Phillips NG, Conroy JP, Smith RA, Attard RD, Woodfield R, Logan BA,
594 Lewis JD, Tissue DT (2010) Exposure to preindustrial, current and future atmospheric

595 CO_2 and temperature differentially affects growth and photosynthesis in *Eucalyptus*.
596 Glob Change Biol 16: 303-319.

597 Guinn G (1985) Fruiting of cotton .III. Nutritional stress and cutout. Crop Sci 25: 981-985.

598 Halevy J, Marani A, Markovitz T (1987) Growth and NPK uptake of high-yielding cotton
599 grown at different nitrogen levels in a permanent-plot experiment. Plant Soil 103: 39-
600 44.

601 Hovenden MJ, Newton PCD, Carran RA, Theobald P, Wills KE, Schoor JKV, Williams AL,
602 Osanai Y (2008) Warming prevents the elevated CO2-induced reduction in available
603 soil nitrogen in a temperate, perennial grassland. Glob Change Biol 14: 1018-1024.

604 Hovenden MJ, Newton PCD, Wills KE (2014) Seasonal not annual rainfall determines
605 grassland biomass response to carbon dioxide. Nature 511: 583.

606 Howden SM, Soussana JF, Tubiello FN, Chhetri N, Dunlop M, Meinke H (2007) Adapting
607 agriculture to climate change. Proc Natl Acad Sci U S A 104: 19691-19696.

608 Isbell RF (1996) The Australian Soil Classification. CSIRO Publishing, Collingwood, Vic,
609 Australia.

610 Jablonski LM, Wang XZ, Curtis PS (2002) Plant reproduction under elevated CO_2
611 conditions: a meta-analysis of reports on 79 crop and wild species. New Phytologist
612 156: 9-26.

613 Jumrani K, Bhatia VS (2014) Impact of elevated temperatures on growth and yield of
614 chickpea (*Cicer arietinum* L.). Field Crop Res 164: 90-97.

615 Kimball BA (1983) Carbon dioxide and agricultural yield - an assemblage and analysis of
616 430 prior observations. Agron J 75: 779-788.

617 Kimball BA, Kobayashi K, Bindi M (2002) Responses of agricultural crops to free-air CO_2
618 enrichment. Adv Agron 77: 293-368.

619 Kimball BA, Mauney JR (1993) Response of cotton to varying CO_2, irrigation, and nitrogen:
620 yield and growth. Agron J 85: 706-712.

621 Langley JA, McKinley DC, Wolf AA, Hungate BA, Drake BG, Megonigal JP (2009) Priming
622 depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to
623 elevated CO_2. Soil Biol Biochem 41: 54-60.

624 Lauber CL, Strickland MS, Bradford MA, Fierer N (2008) The influence of soil properties on
625 the structure of bacterial and fungal communities across land-use types. Soil Biol
626 Biochem 40: 2407-2415.

627 Li FS, Kang SZ, Zhang JH (2003) CO_2 enrichment on biomass accumulation and nitrogen
628 nutrition of spring wheat under different soil nitrogen and water status. J Plant Nutr
629 26: 769-788.

630 Liberloo M, Tulva I, Raim O, Kull O, Ceulemans R (2007) Photosynthetic stimulation under
631 long-term CO_2 enrichment and fertilization is sustained across a closed *Populus*
632 canopy profile (EUROFACE). New Phytologist 173: 537-549.

633 Lobell DB, Field CB (2007) Global scale climate - crop yield relationships and the impacts of
634 recent warming. Environ Res Lett 2.

635 Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants
636 face the future. Annu Rev Plant Biol 55: 591-628.

637 Ludwig F, Asseng S (2006) Climate change impacts on wheat production in a Mediterranean
638 environment in Western Australia. Agr Syst 90: 159-179.

639 Luo Y, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, McMurtrie RE, Oren R,
640 Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB (2004) Progressive nitrogen
641 limitation of ecosystem responses to rising atmospheric carbon dioxide. Bioscience
642 54: 731-739.

643 Marchetti GM, Drton M, Sadeghi K (2015) ggm: Functions for graphical Markov models. R
644 package version 2.3. http://CRAN.R-project.org/package=ggm.

645 Mauney JR, Fry KE, Guinn G (1978) Relationship of photosynthetic rate to growth and
646 fruiting of cotton, soybean, sorghum, and sunflower. Crop Sci 18: 259-263.

647 Mauney JR, Kimball BA, Pinter PJ, Lamorte RL, Lewin KF, Nagy J, Hendrey GR (1994)
648 Growth and yield of cotton in response to a free-air carbon-dioxide enrichment
649 (FACE) environment. Agr Forest Meteorol 70: 49-67.

650 McDonald EP, Erickson JE, Kruger EL (2002) Can decreased transpiration limit plant
651 nitrogen acquisition in elevated CO_2? Funct Plant Biol 29: 1115-1120.

652 McGrath JM, Lobell DB (2013) Reduction of transpiration and altered nutrient allocation
653 contribute to nutrient decline of crops grown in elevated CO_2 concentrations. Plant
654 Cell Environ 36: 697-705.

655 Milroy SP, Bange MP, Sadras VO (2001) Profiles of leaf nitrogen and light in reproductive
656 canopies of cotton (*Gossypium hirsutum*). Ann Bot 87: 325-333.

657 Mullins GL, Burmester CH (2010) Relation of growth and development to mineral nutrition.
658 In: JM Stewart, DM Oosterhuis, JJ Heitholt, JR Mauney (eds) Physiology of Cotton.
659 Springer, New York.

660 Oechel WC, Cowles S, Grulke N, Hastings SJ, Lawrence B, Prudhomme T, Riechers G,
661 Strain B, Tissue D, Vourlitis G (1994) Transient nature of CO_2 fertilization in Arctic
662 tundra. Nature 371: 500-503.

663 Patil RH, Laegdsmand M, Olesen JE, Porter JR (2010) Effect of soil warming and rainfall
664 patterns on soil N cycling in Northern Europe. Agric Ecosyst Environ 139: 195-205.

665 Pendall E, Mosier AR, Morgan JA (2004) Rhizodeposition stimulated by elevated CO_2 in a
666 semiarid grassland. New Phytologist 162: 447-458.

667 Phillips RP, Finzi AC, Bernhardt ES (2011) Enhanced root exudation induces microbial
668 feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecol Lett 14:
669 187-194.

670 Ping JL, Green CJ, Bronson KF, Zartman RE, Dobermann A (2004) Identification of
671 relationships between cotton yield, quality, and soil properties. Agron J 96: 1588-
672 1597.

673 R Core Team (2014) R: A language and environment for statistical computing. R Foundation
674 for Statistical Computing, Vienna, Austria.

675 Radin JW, Kimball BA, Hendrix DL, Mauney JR (1987) Photosynthesis of cotton plants
676 exposed to elevated levels of carbon dioxide in the field. Photosynth Res 12: 191-203.

677 Rayment GE, Lyons DJ (2010) Soil Chemical Methods - Australasia. CSIRO Publishing,
678 Melbourne, Australia.

679 Reddy KR, Hodges HF, McKinion JM (1997) A comparison of scenarios for the effect of
680 global climate change on cotton growth and yield. Aust J Plant Physiol 24: 707-713.

681 Reddy KR, Reddy VR, Hodges HF (1992) Temperature effects on early season cotton growth
682 and development. Agron J 84: 229-237.

683 Reddy KR, Robana RR, Hodges HF, Liu XJ, McKinion JM (1998) Interactions of CO_2
684 enrichment and temperature on cotton growth and leaf characteristics. Environ Exp
685 Bot 39: 117-129.

686 Reddy VR, Reddy KR, Hodges HF (1995) Carbon dioxide enrichment and temperature
687 effects on cotton canopy photosynthesis, transpiration, and water-use efficiency. Field
688 Crop Res 41: 13-23.

689 Reich PB, Hobbie SE (2013) Decade-long soil nitrogen constraint on the CO_2 fertilization of
690 plant biomass. Nature Climate Change 3: 278-282.

691 Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, Knops JMH, Naeem S,
692 Trost J (2006) Nitrogen limitation constrains sustainability of ecosystem response to
693 CO_2. Nature 440: 922-925.

694 Reich PB, Hobbie SE, Lee TD (2014) Plant growth enhancement by elevated CO_2 eliminated
695 by joint water and nitrogen limitation. Nat Geosci 7: 920-924.

696 Rogers GS, Milham PJ, Thibaud MC, Conroy JP (1996) Interactions between rising CO_2
697 concentration and nitrogen supply in cotton .1. Growth and leaf nitrogen
698 concentration. Aust J Plant Physiol 23: 119-125.

699 Rustad LE, Campbell JL, Marion GM, Norby RJ, Mitchell MJ, Hartley AE, Cornelissen JHC,
700 Gurevitch J (2001) A meta-analysis of the response of soil respiration, net nitrogen
701 mineralization, and aboveground plant growth to experimental ecosystem warming.
702 Oecologia 126: 543-562.

703 Schlenker W, Roberts MJ (2009) Nonlinear temperature effects indicate severe damages to
704 US crop yields under climate change. Proc Natl Acad Sci U S A 106: 15594-15598.

705 Sherry RA, Weng ES, Arnone JA, Johnson DW, Schimel DS, Verburg PS, Wallace LL, Luo
706 YQ (2008) Lagged effects of experimental warming and doubled precipitation on
707 annual and seasonal aboveground biomass production in a tallgrass prairie. Glob
708 Change Biol 14: 2923-2936.

709 Singh BK, Bardgett RD, Smith P, Reay DS (2010) Microorganisms and climate change:
710 terrestrial feedbacks and mitigation options. Nat Rev Microbiol 8: 779-790.

711 Stiller WN (2008) Sicot 71BRF. Plant Varieties Journal 21: 194-197.

712 Sun P, Mantri N, Lou HQ, Hu Y, Sun D, Zhu YQ, Dong TT, Lu HF (2012) Effects of
713 elevated CO_2 and temperature on yield and fruit quality of strawberry (*Fragaria* x
714 *ananassa* Duch.) at two levels of nitrogen application. PLoS One 7.

715 Tissue DT, Oechel WC (1987) Response of *Eriophorum vaginatum* to elevated CO_2 and
716 temperature in the Alaskan tussock tundra. Ecology 68: 401-410.

717 Tissue DT, Thomas RB, Strain BR (1993) Long-term effects of elevated CO_2 and nutrients
718 on photosynthesis and rubisco in loblolly pine seedlings. Plant Cell Environ 16: 859-
719 865.

720 Tolk JA, Howell TA, Evett SR (1999) Effect of mulch, irrigation, and soil type on water use
721 and yield of maize. Soil Till Res 50: 137-147.

722 Tubiello FN, Rosenzweig C, Goldberg RA, Jagtap S, Jones JW (2002) Effects of climate
723 change on US crop production: simulation results using two different GCM scenarios.
724 Part I: Wheat, potato, maize, and citrus. Clim Res 20: 259-270.

725 Wakelin SA, Macdonald LM, Rogers SL, Gregg AL, Bolger TP, Baldock JA (2008) Habitat
726 selective factors influencing the structural composition and functional capacity of
727 microbial communities in agricultural soils. Soil Biol Biochem 40: 803-813.

728 Wanjura DF, Upchurch DR, Mahan JR (1992) Automated irrigation based on threshold
729 canopy temperature. T Asae 35: 153-159.

730 Warner DA, Burke JJ (1993) Cool night temperatures alter leaf starch and photosystem II
731 chlorophyll fluorescence in cotton. Agron J 85: 836-840.

732 Wood CW, Torbert HA, Rogers HH, Runion GB, Prior SA (1994) Free-air CO_2 enrichment
733 effects on soil carbon and nitrogen. Agr Forest Meteorol 70: 103-116.

734 Woodrow IE (1994) Optimal acclimation of the C_3 photosynthetic system under enhanced
735 CO_2. Photosynth Res 39: 401-412.

736 Yoon ST, Hoogenboom G, Flitcroft I, Bannayan M (2009) Growth and development of
737 cotton (*Gossypium hirsutum* L.) in response to CO_2 enrichment under two different
738 temperature regimes. Environ Exp Bot 67: 178-187.

739 Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel R, Randlett DL (1993) Elevated
740 atmospheric CO_2 and feedback between carbon and nitrogen cycles. Plant Soil 151:
741 105-117.

742 Zhang NY, Guo R, Song PA, Guo JX, Gao YZ (2013) Effects of warming and nitrogen
743 deposition on the coupling mechanism between soil nitrogen and phosphorus in
744 Songnen Meadow Steppe, northeastern China. Soil Biol Biochem 65: 96-104.

745 Zhao DL, Reddy KR, Kakani VG, Mohammed AR, Read JJ, Gao W (2004) Leaf and canopy
746 photosynthetic characteristics of cotton (*Gossypium hirsutum*) under elevated CO_2
747 concentration and UV-B radiation. J Plant Physiol 161: 581-590.

748 Zhou JZ, Xue K, Xie JP, Deng Y, Wu LY, Cheng XH, Fei SF, Deng SP, He ZL, Van
749 Nostrand JD, Luo YQ (2012) Microbial mediation of carbon-cycle feedbacks to
750 climate warming. Nature Climate Change 2: 106-110.

751

752

753 **Table 1** The effects of CO_2 and temperature treatments on the rate of crop development (the
754 number of days to each developmental stage with standard errors in parenthesis) and the
755 effect size relative to the ambient CO_2 and ambient temperature treatment given as a
756 percentage change for each soil. Treatments are C_AT_A (ambient CO_2, ambient temperature),
757 C_AT_E (ambient CO_2, elevated temperature), C_ET_A (elevated CO_2, ambient temperature), C_ET_E
758 (elevated CO_2, elevated temperature). Different letters within columns indicate significant
759 differences between the treatments within each soil (P<0.05).

Soil	Treatment	Number of days			Percentage change		
		1st square	1st flower	1st open boll	1st square	1st flower	1st open boll
Grey	C_AT_A	97.2 (3.3)^a	131.2 (2.3)^a	186.5 (2.6)^a	-	-	-
	C_AT_E	45.8 (1.0)^c	76.0 (1.4)^c	136.5 (1.4)^b	-52.9	-42.1	-26.8
	C_ET_A	73.5 (2.0)^b	109.2 (2.6)^b	191.3 (1.9)^a	-24.4	-16.8	2.6
	C_ET_E	44.8 (0.8)^c	72.5 (1.4)^c	129.3 (1.6)^b	-53.9	-44.7	-30.7
Black	C_AT_A	109.8 (3.6)^a	139.3 (1.3)^a	193.7 (0.4)^a	-	-	-
	C_AT_E	48.7 (0.8)^c	77.3 (1.0)^c	138.5 (2.0)^b	-55.6	-44.5	-28.5
	C_ET_A	79.2 (1.7)^b	115.3 (1.3)^b	195.7 (0.4)^a	-27.9	-17.2	1.0
	C_ET_E	47.0 (0.9)^c	77.0 (1.0)^c	134.5 (2.8)^b	-57.2	-44.7	-30.6

760 **Table 2** Results of analysis of variance (ANOVA) showing the effect of CO_2, temperature, soil and their interactions on phenology, vegetative
761 growth, yield components, physiology, leaf nutrients and soil nutrients. Values are probability with significant results (P<0.05) shown in bold
762 and marginally significant results (P<0.1) in italic.

			CO_2	Temp	Soil	CO_2 x Temp	CO_2 x Soil	Temp x Soil	CO_2 x Temp x Soil
Phenology	1st square		**<0.0001**	**<0.0001**	**0.0002**	**<0.0001**	0.19	**0.03**	0.28
	1st flower		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.80	*0.07*	0.27
	1st open boll		0.41	**<0.0001**	**0.001**	**0.001**	0.95	0.41	0.26
Vegetative growth	Final height		0.32	**<0.0001**	0.35	**0.0002**	0.84	0.37	0.66
	Final node number		**0.001**	**0.0002**	**0.001**	0.36	0.82	0.50	0.12
Yield components	Seed cotton		**0.001**	**<0.0001**	*0.07*	0.88	0.41	0.13	0.88
	Boll size		*0.08*	**<0.0001**	**0.0002**	*0.06*	**0.05**	**0.01**	0.99
	Boll number		**0.0003**	**0.001**	0.12	**<0.0001**	**0.001**	0.81	**0.03**
Physiology	Photosynthetic rate		**<0.0001**	0.001	0.33	**<0.0001**	0.90	0.98	0.24
	Stomatal conductance		**0.03**	**<0.0001**	**0.05**	**<0.0001**	0.68	0.54	0.29
	iWUE		**<0.0001**	**<0.0001**	0.18	**<0.0001**	0.33	*0.08*	*0.09*
Leaf nutrients	C	Flowering	**<0.0001**	**<0.0001**	0.30	**0.001**	0.20	**0.05**	*0.06*
		Harvest	**<0.0001**	**<0.0001**	0.22	**<0.0001**	0.57	**0.01**	**0.02**
	N	Flowering	**<0.0001**	**0.001**	*0.06*	**0.004**	0.34	0.49	0.53
		Harvest	**<0.0001**	**<0.0001**	**0.001**	**<0.0001**	0.96	**0.02**	0.58
	C:N	Flowering	**<0.0001**	**<0.0001**	0.10	**0.02**	0.63	0.22	0.96
		Harvest	**<0.0001**	**<0.0001**	**0.003**	**<0.0001**	0.47	0.22	0.60
Soil nutrients	NO_3^-	Early flowering	0.85	**0.001**	**0.001**	*0.08*	0.57	0.41	0.55
		Late flowering	0.65	**<0.0001**	**0.03**	0.98	0.10	0.50	0.90
		Harvest	**0.01**	**0.01**	0.19	**<0.0001**	0.63	0.74	0.11
	PO_4^-	Early flowering	0.78	**0.004**	**<0.0001**	0.10	0.63	0.49	0.42
		Late flowering	**0.003**	0.12	**<0.0001**	0.30	0.73	0.77	0.29
		Harvest	0.38	0.41	**<0.0001**	**<0.0001**	0.81	**0.003**	0.47

763 **Table 3** The effects of CO_2 and temperature treatments on leaf C, N and C:N ratios of cotton
764 plants grown on grey vertosol and black vertosol under the four climate change treatments.
765 Leaf samples were collected at flowering and at harvest. Values are means with standard
766 errors in parenthesis. Different letters within columns indicate significant differences
767 (P<0.05) between the treatment within each soil.

		C (%)		N (%)		C:N	
Soil	Treatment	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest
Grey	C_AT_A	39.92 (0.28)^b	41.75 (0.32)^b	3.96 (0.08)^b	3.41 (0.08)^a	10.09 (0.16)^a	12.26 (0.28)^b
	C_AT_E	40.35 (0.19)^b	42.30 (0.26)^b	4.37 (0.09)^a	2.48 (0.10)^b	9.25 (0.22)^b	17.19 (0.72)^a
	C_ET_A	41.39 (0.13)^a	44.16 (0.21)^a	4.46 (0.11)^a	2.43 (0.07)^b	9.31 (0.22)^b	18.23 (0.59)^a
	C_ET_E	39.94 (0.18)^b	41.69 (0.21)^b	4.41 (0.06)^a	2.43 (0.12)^b	9.07 (0.16)^b	17.42 (1.12)^a
Black	C_AT_A	40.54 (0.06)^b	42.43 (0.40)^b	4.02 (0.03)^b	4.07 (0.20)^a	10.09 (0.08)^a	10.55 (0.47)^b
	C_AT_E	39.65 (0.25)^b	40.91 (0.27)^c	4.44 (0.10)^a	2.54 (0.12)^b	8.95 (0.17)^b	16.24 (0.68)^a
	C_ET_A	41.81 (0.43)^a	44.08 (0.18)^a	4.56 (0.12)^a	2.97 (0.19)^b	9.20 (0.21)^b	15.16 (1.04)^a
	C_ET_E	40.31 (0.17)^b	41.51 (0.29)^{bc}	4.67 (0.06)^a	2.59 (0.15)^b	8.64 (0.15)^b	16.25 (0.82)^a

768 **Table 4** The effects of CO_2 and temperature treatments on soil nitrate and phosphate
769 concentrations at early flowering, late flowering and harvest for each soil. Values are means
770 and standard errors in parenthesis. Different letters within columns indicate significant
771 differences (P<0.05) between the treatment within each soil (shown only when CO_2 x
772 temperature interactions were significant).

		Soil nitrate (mg N/kg soil dwt)			Soil phosphate (mg P/kg soil dwt)		
Soil	Treatment	Early flower	Late flower	Harvest	Early flower	Late flower	Harvest
Grey	C_AT_A	49.4 (9.7)	25.4 (8.6)	4.1 (0.6)^{a}	139.5 (13.8)	130.9 (15.7)	133.3 (10.5)^{ab}
	C_AT_E	81.7 (10.6)	64.4 (11.0)	3.3 (0.4)^{a}	137.9 (9.9)	97.7 (9.3)	145.1 (8.8)^{a}
	C_ET_A	52.9 (11.9)	33.3 (8.8)	1.1 (0.1)^{b}	121.8 (2.9)	89.9 (14.7)	151.9 (6.8)^{a}
	C_ET_E	65.9 (4.7)	74.5 (5.0)	4.1 (0.3)^{a}	171.8 (21.1)	91.9 (3.8)	113.8 (3.3)^{b}
Black	C_AT_A	62.3 (11.8)	48.7 (4.8)	5.0 (0.9)^{ab}	56.5 (9.1)	56.6 (16.0)	50.2 (6.7)^{b}
	C_AT_E	117.7 (17.5)	99.7 (16.3)	2.9 (0.3)^{bc}	86.2 (19.8)	46.0 (13.1)	105.8 (12.4)^{a}
	C_ET_A	84.6 (10.0)	33.7 (7.1)	1.3 (0.2)^{c}	45.3 (10.4)	27.5 (8.6)	79.6 (5.8)^{ab}
	C_ET_E	101.4 (10.9)	83.1 (16.2)	5.2 (0.6)^{a}	93.1 (20.0)	16.5 (4.1)	69.0 (5.4)^{b}

773

774

775 **Figure captions**

776 **Fig. 1** The effects of CO_2 and temperature treatments on plant height (a, b) and number of
777 nodes (c, d) of cotton plants grown on grey vertosol and black vertosol under the four climate
778 change treatments (n=6). Plants were harvested at 142 and 196 days after planting (DAP) for
779 T_E and T_A respectively.

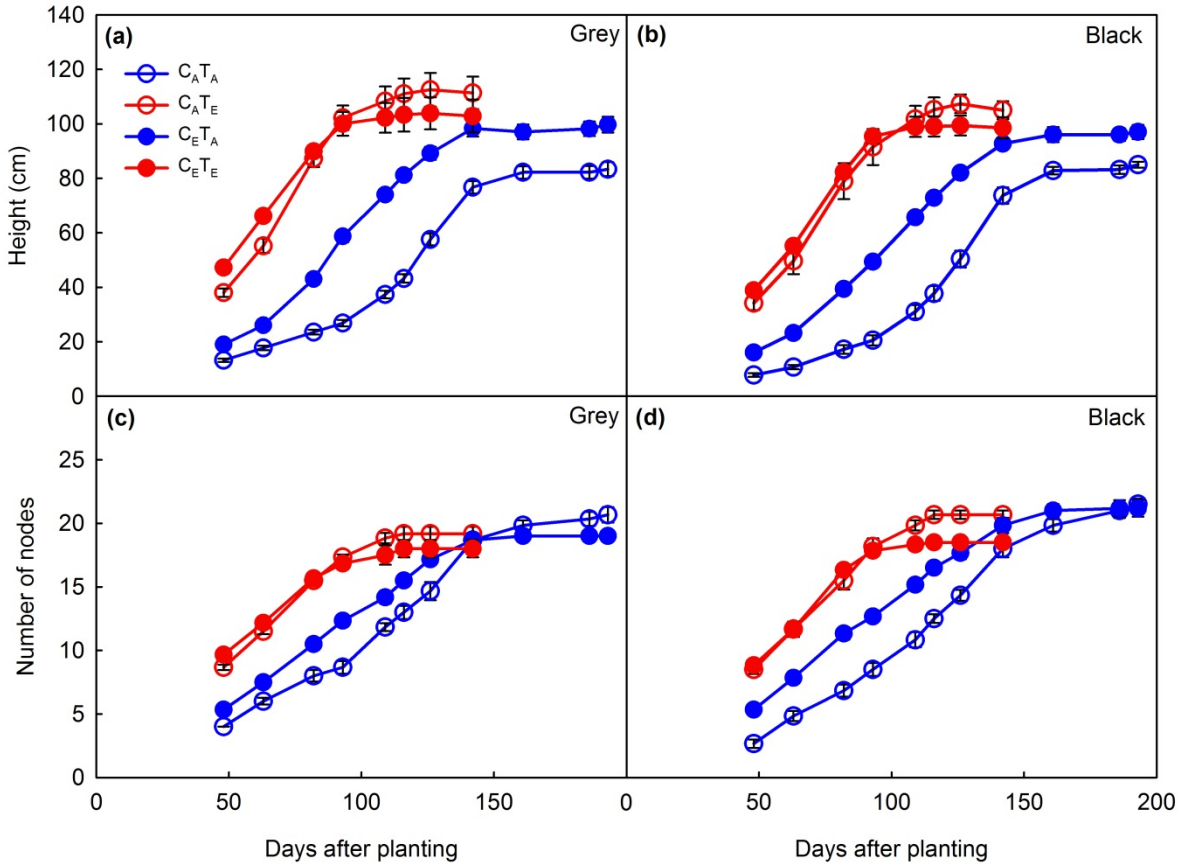
780 **Fig. 2** The effects of CO_2 and temperature treatments on leaf photosynthetic rate (a), stomatal
781 conductance (b) and instantaneous water use efficiency (c) of cotton plants grown on grey
782 vertosol and black vertosol under the four climate change treatments (n=6). Measurements
783 were taken over 3 days at early flowering stage of cotton growth. Different letters indicate
784 significant differences (P<0.05) between the treatment within each soil (shown only when
785 CO_2 x temperature interactions were significant).

786 **Fig. 3** The effects of CO_2 and temperature treatments on cotton yields (a), the number of bolls
787 per plant (b) and boll size (c) of cotton plants grown on grey vertosol and black vertosol
788 under the four climate change treatments (n=6). Plants were harvested at 142 and 196 days
789 after planting for T_E and T_A respectively. Different letters indicate significant differences
790 (P<0.05) between the treatment within each soil (shown only when CO_2 x temperature
791 interactions were significant).

792 **Fig. 4** Relationship between seed cotton yield and boll size (a), vegetative growth rate (b),
793 soil nitrate concentrations at late flowering (c) and leaf N concentrations at harvest (d) of
794 cotton plants grown on grey vertosol and black vertosol under the four climate change
795 treatments.

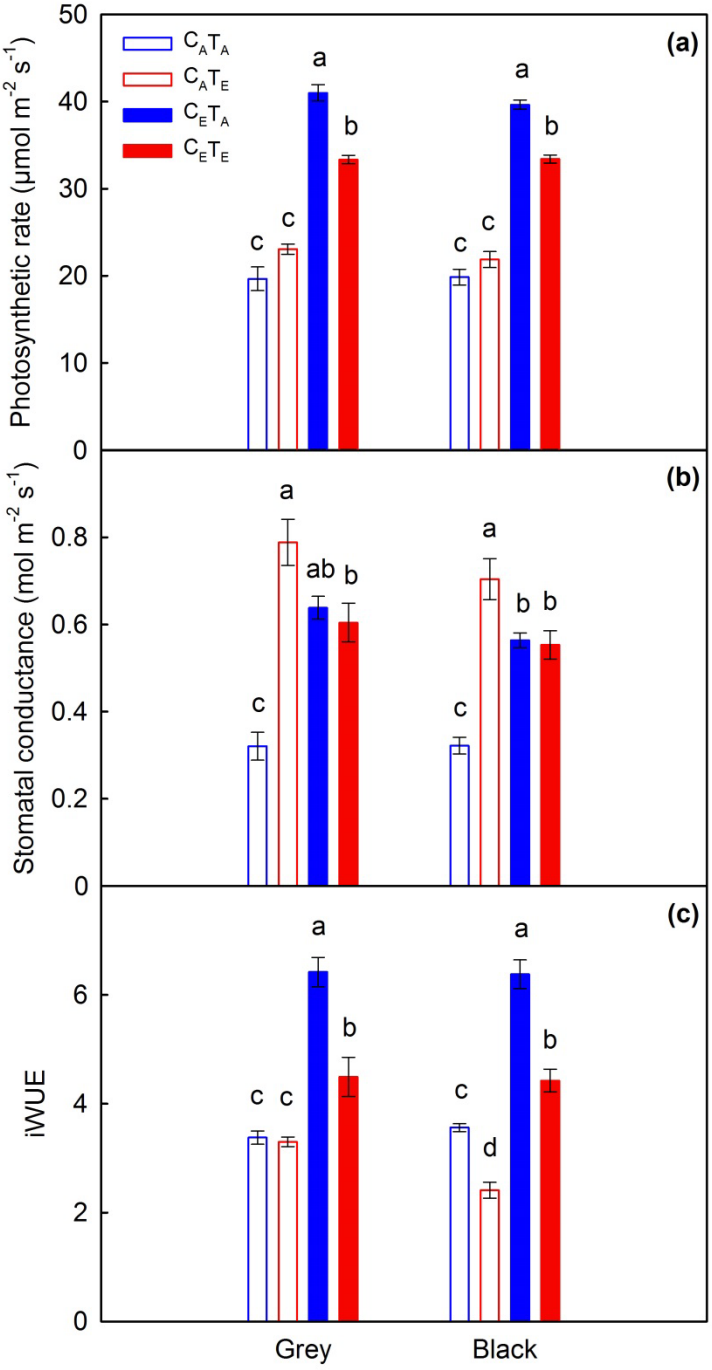
796 **Fig. 5** Conceptual diagrams summarising the main and interactive effects of C_E, T_E and soil
797 on cotton productivity. (a) Early vegetative growth is strongly influenced by T_E which

accelerates the growth and development of cotton plants. C_E also increases the rate of growth via its strong positive effect on photosynthetic rate which accompanies increased stomatal conductance in the absence of water limitation. The combined C_E and T_E (i.e. C_ET_E) increases vegetative growth and photosynthetic rate and induces early transition to reproductive growth. T_E increases the amount of N remaining at flowering, while C_E only increases it marginally. Differences in soil properties affect stomatal conductance and vegetative growth, but have little effect on crop responses to C_E and T_E. (b) Response to C_E and T_E during reproductive phase is strongly influenced by the remaining soil N availability (indicated by dotted arrows) and resource allocation within the plant. Differences in soil properties start to exert a stronger influence over reproductive growth via their effect on soil N availability. The effect of C_E on reproductive growth is limited by soil N availability and resource allocation to vegetative growth, while the greater soil N availability at T_E allows plants to allocate more resources to reproductive growth while maintaining some vegetative growth. Despite the greater soil N availability at T_E, plants at C_ET_E allocate all resources to reproductive growth, suggesting that vegetative growth is limited by soil N availability. However, combined with the greater photosynthetic capability at C_E, plants at C_ET_E produce the greatest reproductive output. Thickness of arrows indicates the strength of the relationship/effect.


(a)
Grey
(b)
Black
(c)
Grey
(d)
Black
C_AT_A
C_AT_E
C_ET_A
C_ET_E
Height (cm)
Number of nodes
Days after planting
Days after planting

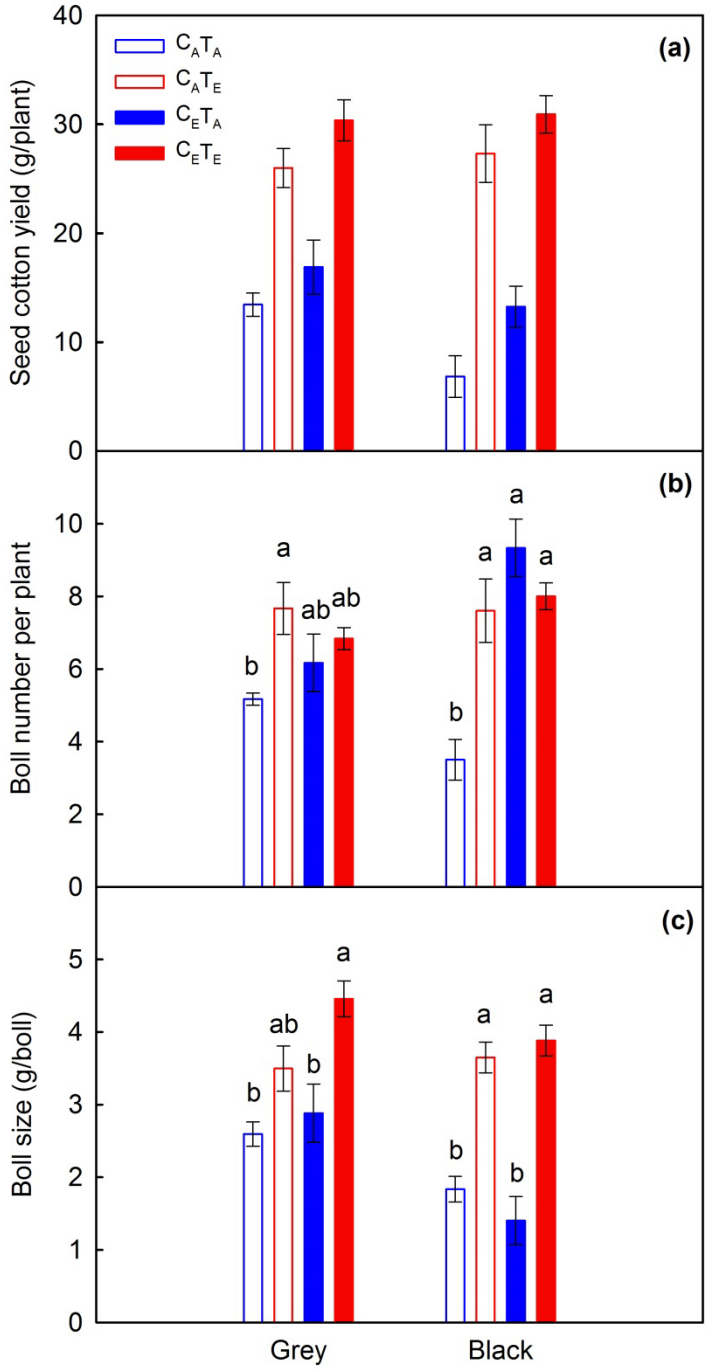
815

816 **Fig. 1**


(a)
(b)
(c)
C_AT_A
C_AT_E
C_ET_A
C_ET_E
Photosynthetic rate (μmol m^{-2} s^{-1})
Stomatal conductance (mol m^{-2} s^{-1})
iWUE
Grey
Black

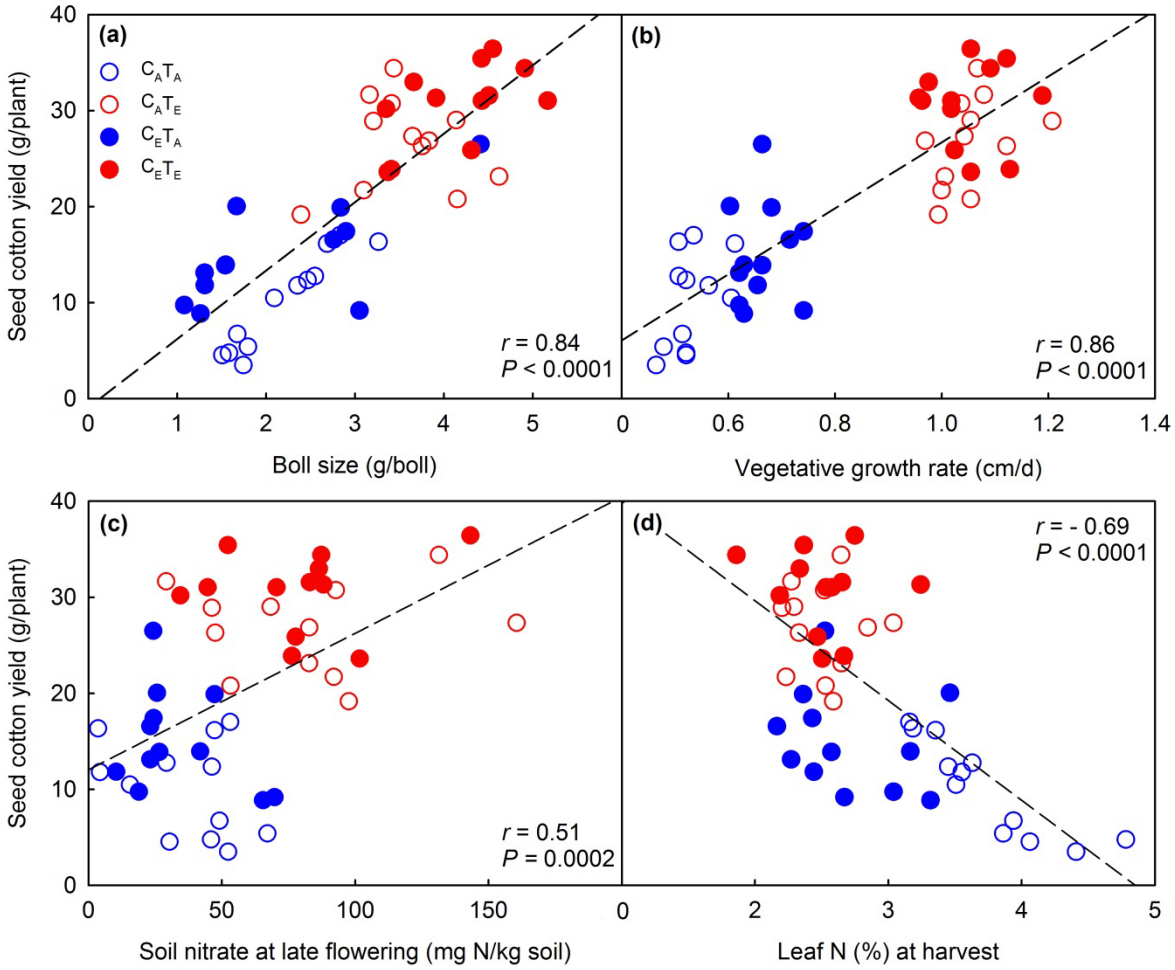
817

818 **Fig. 2**


(a)
(b)
(c)
C_AT_A
C_AT_E
C_ET_A
C_ET_E
Seed cotton yield (g/plant)
Boll number per plant
Boll size (g/boll)
Grey
Black

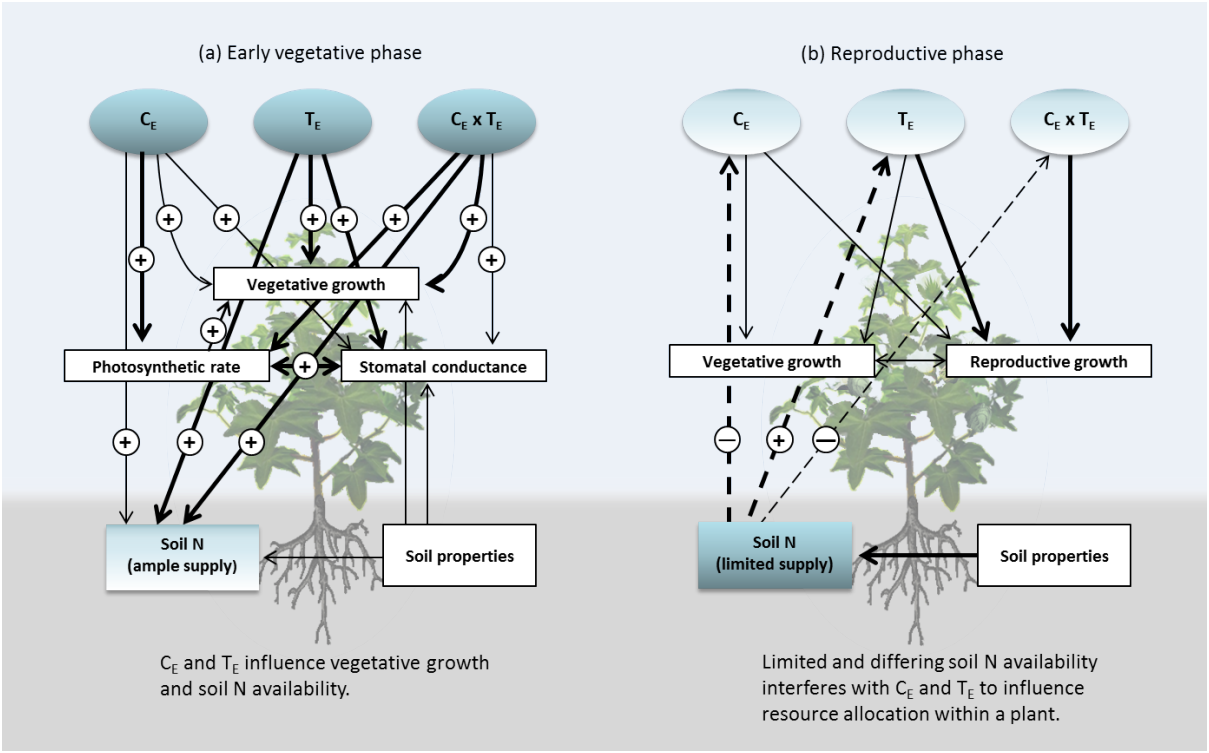
819

820 **Fig. 3**


(a)
C_AT_A
C_AT_E
C_ET_A
C_ET_E
r = 0.84
P < 0.0001
Seed cotton yield (g/plant)
Boll size (g/boll)
(b)
r = 0.86
P < 0.0001
Vegetative growth rate (cm/d)
(c)
r = 0.51
P = 0.0002
Soil nitrate at late flowering (mg N/kg soil)
(d)
r = - 0.69
P < 0.0001
Leaf N (%) at harvest

821

822 **Fig. 4**


(a) Early vegetative phase
C_E
T_E
C_E x T_E
Vegetative growth
Photosynthetic rate
Stomatal conductance
Soil N
(ample supply)
Soil properties
C_E and T_E influence vegetative growth
and soil N availability.
(b) Reproductive phase
C_E
T_E
C_E x T_E
Vegetative growth
Reproductive growth
Soil N
(limited supply)
Soil properties
Limited and differing soil N availability
interferes with C_E and T_E to influence
resource allocation within a plant.

823

824 **Fig. 5**

Interactive effects of elevated CO_2, warmer temperature and extreme weather events on soil nutrients and crop productivity indicate increased variability of crop production under future climate regimes.

Running head: Extreme weather events and climate change affects crop productivity

Yui Osanai[1], David T. Tissue[1], Michael P. Bange[2], Michael V. Braunack[2], Ian C. Anderson[1], Brajesh K. Singh[1, 3*]

[1]Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, New South Wales 2751, Australia

[2]CSIRO Agriculture, Australian Cotton Research Institute, Locked Bag 59, Narrabri, New South Wales 2390, Australia

[3] Global Centre for Land Based Innovation, Western Sydney University, Locked Bag 1797, Penrith, New South Wales 2751, Australia

*Corresponding author: tel: +612 4570 1911, fax: +612 4570 1103, e-mail: y.osanai@westernsydney.edu.au, b.singh@westernsydney.edu.au

Keywords: flooding, drought, elevated CO_2, elevated temperature, cotton (*Gossypium hirsutum* L.), soil nitrogen

Type of Paper: Original article

Abstract (200 words limit)

Aims: Projected increases in global atmospheric concentration of CO_2 (C_E), temperature (T_E) and extreme weather events are expected to affect agricultural production. However, our knowledge on interactions between them is limited, thus potentially underestimating the impact of future climates on agriculture.

Methods: Using a large glasshouse experiment, we examined whether flooding and drought affected cotton productivity and soil fertility when grown in future CO_2 and temperature regimes, and whether these responses differed between different soils.

Results: In well-watered conditions, T_E significantly increased yield. Flooding induced immediate physiological responses in cotton, and reduced soil nitrogen, particularly at T_E. Flooding reduced vegetative growth (by 68~ 91%) and caused a significant yield loss at T_E. Drought had detrimental impacts on physiology, growth and yield, and resulted in a large amount of residual nitrogen in the soil, particularly at T_E. While interactions between C_E, T_E and soil were also evident in some responses to flooding/drought, T_E dominantly interacted with flooding/drought impacts on those responses.

Conclusions: Despite its positive effect, the greater yield reduction upon flooding/drought at T_E suggests that inter-annual variability in yield is likely to increase under more extreme climates. Adaptation strategies should focus on both plant and soil responses to minimise yield loss under future climates.

Introduction

Rising atmospheric carbon dioxide (CO_2) and temperature, and associated increased frequency of extreme climate events (drought and flood), are predicted to occur in the coming decades (IPCC 2014), and in combination may have catastrophic impacts on agricultural production. While elevated CO_2 (C_E) has the potential to increase crop productivity (Tubiello et al. 2007), the effect of elevated temperature (T_E) depends on the temperature optimum of crop species and therefore have either negative or positive consequences (Hatfield et al. 2011; Challinor et al. 2014). The interactive effects of C_E and T_E are not necessarily additive (Dieleman et al. 2012), making prediction of their impacts more challenging. Furthermore, water and nutrient availability strongly determines the magnitude of C_E and T_E impacts on crop productivity (Rogers et al. 1996; Li et al. 2003; Bloom et al. 2014) such that projected increase in the frequency and intensity of flooding and drought events may significantly impact crop productivity under future CO_2 and temperature regimes (Porter and Semenov 2005; Tubiello et al. 2007). Surprisingly, few studies have investigated the impact of these extreme weather events on crop productivity under future climatic conditions (Robredo et al. 2007; Robredo et al. 2011; Shimono et al. 2012), and rarely have impacts of extreme climate been studied in conjunction with the main and interactive effects of C_E and T_E on crop productivity.

Drought and flooding may have a direct impact on crop physiology and growth by generating dry soil and waterlogged conditions. Crops under these conditions often exhibit immediate physiological responses such as reduced stomatal conductance, photosynthetic rate, water and nutrient uptake, which can reduce the yield of many crop species (Bange et al. 2004; Farooq et al. 2009; Milroy and Bange 2013). The magnitude of these extreme events on yield depends on the timing (Cannell and Belford 1980; Wright et al. 1991; Bange et al. 2004;

63 Shao et al. 2013) and duration of the event (Hodgson 1982; Wright et al. 1991; Glaz and
64 Lingle 2012; Yaduvanshi et al. 2012), as well as management strategies to assist recovery
65 (Hodgson 1982; Sugimoto et al. 1989; Swarup and Sharma 1993; Huang et al. 1994; Singh et
66 al. 2002). In cotton, Bange et al (2004) found that a waterlogging event at an early
67 developmental stage, but not at a later developmental stage, caused a significant reduction in
68 yield due to reduced fruit production. Not only its direct impact on crop growth but also
69 changes in soil nutrient status may further contribute to the loss of crop productivity
70 following a waterlogging event. Indeed, nutrient addition following the flooding
71 (waterlogging) event may alleviate the adverse impact on crop yield (Hodgson 1982; Swarup
72 and Sharma 1993; Singh et al. 2002), because flooding impairs nutrient uptake by roots and
73 reduces soil nitrogen (N) availability through increased leaching, run-off and denitrification
74 (Cameron and Haynes 1986). Thus, the impact of flooding on soil nutrients is likely to hinder
75 crop recovery, adding to the adverse impact of flooding on crop productivity. The impact of
76 moderate drought on crop productivity may be reduced by adding soil nutrients (Saneoka et
77 al. 2004; Gimeno et al. 2014), although, it is unclear whether adding soil nutrients may play a
78 significant role in reducing crop loss under severe water limitation.

79 The effect of extreme weather events on crop productivity in future CO_2 and temperature
80 regimes remains relatively unknown. In our previous study on cotton in well-watered
81 conditions, C_E and T_E increased seed cotton yield (Osanai et al submitted). C_E increased
82 photosynthetic rate by 109% in T_A and by 46% in T_E, contributing to higher growth rates and
83 greater seed cotton yield. T_E strongly accelerated the rate of vegetative growth, which
84 resulted in greater light capturing capacity to support higher cotton yield. T_E significantly
85 increased stomatal conductance and reduced instantaneous water use efficiency, reflecting a
86 greater evapotranspiration demand at T_E. Such changes in crop physiology at C_E and T_E may
87 affect cotton response to flooding and drought events. For instance, T_E is likely to accelerate

88 the onset and/or magnitude of drought stress on crops through increased evapotranspiration
89 and soil drying, while C_E may reduce the impact of drought and flooding on crop growth by
90 allowing crops to maintain photosynthesis and carbohydrate supply to support cellular
91 functions under reduced stomatal conductance. Understanding the potential interactions
92 between extreme weather events (drought and flooding) and climate change drivers (C_E and
93 T_E) on crop physiology may provide valuable insights into the mechanisms affecting crop
94 productivity under future climatic conditions. Such knowledge will be essential for predicting
95 the vulnerability of crop production in future climates.

96 Differences in soil nutrient availability may affect the impact of extreme weather events,
97 particularly flooding, on crop productivity, as a function of the effect of C_E and T_E on soil N
98 cycling and availability (de Graaff et al. 2006; Hovenden et al. 2008; Bai et al. 2013). Greater
99 soil N availability may buffer crops from flooding-induced loss of soil N, and enhanced
100 mineralisation may also assist crop recovery by increasing plant available N in the soil. The
101 effect of C_E and T_E on crop productivity may differ in different soils, particularly when
102 applied nutrients become limiting (Osanai et al submitted), reflecting differences in their
103 inherent capacity to supply nutrients and water. Differences in water infiltration rates,
104 drainage and water holding capacity of soil may determine the severity and duration of soil
105 waterlogging conditions and the magnitude of N loss during flooding events (Aulakh et al.
106 1991; Sogbedji et al. 2000), as well as the rate of recovery which can determine final yield
107 (Hodgson 1982; Malik et al. 2001). Similarly, differences in water-holding capacity of soil
108 are likely to influence the rate of soil drying and the onset of drought stress in crops. Crop
109 production occurs across a range of soils that differ in physical, chemical and microbial
110 properties that can influence nutrient and water availability, yet the possible role of soils in
111 mediating crop response to these extreme weather events are rarely explored. This lack of
112 understanding could limit our ability to predict yield loss associated with extreme weather

events, and hamper development of adequate management strategies to minimise the impact on productivity under projected future climate regimes.

Here, we set up a large glasshouse experiment to investigate the impact of flooding and severe drought events on cotton productivity in two different soils, under current (ambient CO_2 and ambient temperature; C_AT_A) and future CO_2 and temperature regimes (C_AT_E, C_ET_A and C_ET_E) in a factorial design to examine how flooding and drought may interact with the main and interactive effects of C_E and T_E. We proposed the following hypotheses:

1) The flooding event will saturate the soil and have an immediate impact on crop growth and physiology. C_E will ameliorate the impact of flooding on crop growth by maintaining photosynthetic rate at reduced stomatal conductance, while T_E will exacerbate the impact by reducing evaporative cooling at reduced stomatal conductance. Flooding effects on yield will depend on the impact on soil N status and recovery under each climatic condition.
2) Impacts of drought will be greater under T_E due to increased evapotranspiration and reduced access to soil water, leading to a greater reduction in yield. C_E will reduce the impact of the drought on yield by maintaining photosynthesis for longer periods of time under limiting water conditions. Soil N availability will play little role in mediating crop response to severe drought.
3) Given the importance of soil properties in mediating water and nutrient movements, the impact of extreme weather events on cotton productivity will differ between soils due to differences in soil characteristics.

Materials and methods

Soil and plant materials and climate conditions in the glasshouse

A large-scale glasshouse experiment was set up in 2013 using two soils (grey and black vertosols) collected from two adjacent cotton growing regions in New South Wales, Australia. The majority of irrigated cotton production in Australia occurs on heavy clay soils (Cattle and Field 2013). The grey vertosol (USDA Soil Taxonomy: Typic Haplustert) was collected at the Australia Cotton Research Institute in Narrabri (30°10'S, 149°40'E) and black vertosol (Ustic Pellustert) was collected from a farm in Spring Ridge (31°21'S, 150°12'E). Top-soil (0 – 20 cm) and sub-soil (20 – 40 cm) were collected separately at each of the two field sites, and were re-assembled into large pots (26 x 26 x 40 cm deep). These soils differed in physical and chemical properties (Table S1). The pots were watered to field-capacity and allowed to drain for two weeks prior to planting cotton seeds.

Cotton seeds (*Gossypium hirsutum* L. Cv, 71BRF [Bollgard II® Roundup Ready Flex®], CSIRO Australia, Stiller 2008) were sown into pots filled with grey or black vertosol, and maintained under [CO_2] and temperature treatments for *ca.* six months. Pots were fertilised with Multigro® fertiliser (8 g, 10.1% N, 3.5% P, 5.5% K, 16.3% S, 7.8% Ca, Incitec Pivot Ltd, Melbourne) and 500 mL of Aquasol® (1.6 g/L, 23.0% N, 40% P, 18.0% K, 0.05% Zn, 0.06% Cu, 0.0013% Mo, 0.15% Mn, 0.06% Fe, 0.011% B, Hortico, Vic) to achieve a N fertiliser rate of 180 kg N ha^{-1}, which is commonly applied to irrigated cotton in the field (Braunack 2013) in addition to the pre-existing soil inorganic N. The pre-existing inorganic N for grey and black vertosols were 74 kg N ha^{-1} and 100 kg N ha^{-1} respectively. Fertiliser was applied once before cotton seeds were sown.

Four adjacent naturally lit glasshouse compartments (3.0 x 5.0 x 3.5 m, w x l x h each) were used to simulate current and future CO_2 and temperature regimes in a factorial design, consisting of one current (ambient [CO_2] and ambient temperature; C_AT_A) and three future CO_2 and temperature regimes (ambient [CO_2] and elevated temperature; C_AT_E, elevated

160 [CO_2] and ambient temperature; C_ET_A, and elevated [CO_2] and elevated temperature; C_ET_E).
161 C_A was maintained at 400 ppm and C_E was maintained at 640 ppm [CO_2]. The temperatures
162 for T_A were 28/16 °C (day/night) and for T_E were 32/20 °C (day/night), with diurnal changes
163 in temperature within each compartment by ramping up temperatures during the day and
164 ramping temperatures down in the night. Humidity was not controlled, and allowed to vary in
165 each glasshouse compartment, as expected in the field. Subsequently, vapour pressure deficit
166 (VPD) differed between the temperature treatments, with the mean of 1.06±0.003 kPa
167 (ranged 0.61 – 2.72 kPa) for T_A and 1.25±0.003 kPa (ranged 0.61 – 3.15 kPa) for T_E. VPD
168 did not vary between the CO_2 treatments. All pots were rotated within and between the
169 glasshouse compartments on a monthly basis to minimise potential effects on plant
170 performance associated with environmental conditions in each glasshouse compartment.
171 Detailed description of glasshouse environmental control is found in Ghannoum et al (2010).

172 *Extreme weather events*

173 Extreme weather events were imposed at the early flowering stage when environmental
174 stresses are most likely to affect the reproductive growth of cotton (Bange et al. 2004;
175 Snowden et al. 2014). The timing of extreme weather events was based on the
176 developmental stage rather than on a particular point in time after sowing. This was done to
177 minimise the confounding effect of differences in plant size and developmental stage, which
178 were generated by the CO_2 and temperature treatments, at any given time period. Prior to the
179 early flowering stage, when 90% of plants in each climate condition produced the first flower,
180 all plants were well-watered to maintain optimal volumetric soil water content (40 – 60%).
181 Plants in each climate condition were then divided into two extreme weather treatments
182 (flooding, drought) and a well-watered control for each soil, with six replicates for each
183 treatment combination (144 plants in total).

184 *Flooding treatment*

185 Flooding conditions were created by maintaining 5-10 cm of standing water above the soil
186 surface for six days to ensure a significant flooding event. Soils can remain saturated for this
187 period of time where a significant rain event follows furrow irrigation. At the end of the
188 flooding period, all pots were left to drain naturally and watered regularly to maintain optimal
189 volumetric water content (40 – 60%) until the end of the experiment. Plant height was
190 measured before, during and after the flooding event to examine the treatment effect on plant
191 growth. Vegetative growth was calculated for post-flooding period by subtracting pre-
192 flooding plant height from the final plant height, and then dividing it by the number of days
193 between the two measurement points.

194 Plants were harvested when 90% of well-watered control plants in each climate condition (i.e.
195 C_AT_A, C_AT_E, C_ET_A and C_ET_E) produced the first open boll. Seed cotton was harvested from
196 both open and closed bolls and weighed, after being oven-dried at 70 °C for a week.

197 Net photosynthesis and stomatal conductance were measured at the early flowering stage
198 before the flooding event on recently fully expanded leaves using a portable open gas
199 exchange system (LI-6400XT, LI-COR, Lincoln, NE, USA). The measurements were taken
200 around midday (between 1100 h and 1400 h) at saturating light (photosynthetic photon flux
201 density of 1800 μmol m^{-2} s^{-1}), using mid-day growth temperature (28 °C or 32 °C) as the air
202 temperature in the cuvette, and CO_2 concentration (400 ppm or 640 ppm) of each climate
203 condition. The impacts of flooding on net photosynthesis and stomatal conductance were
204 measured at the end of the six-day flooding period.

205 A leaf sample was taken from each plant at seven days after the end of flooding period (from
206 recently fully expanded leaves) for the determination of total C and N concentrations. Leaf

samples were oven-dried at 70 °C and ground to powder before being analysed using an elemental analyser (CE1110 CHN-S, Carlo Erba, Milan, Italy).

The impact of flooding on soil N availability was examined by collecting soil samples before the flooding event (at early flowering) and three days after the end of flooding from both well-watered control and flooded soils. Plant available N was assessed by measuring ammonium (NH_4^+) and nitrate (NO_3^-) concentrations using the 2M KCl extraction method (Rayment and Lyons 2010). The extracts were analysed for NH_4^+ and NO_3^- concentration using an AQ2 discrete analyser (SEAL Analytical, Wisconsin, USA). Plant available N predominantly existed as NO_3^- as the concentration of NH_4^+ remained low (less than 5 mg kg $soil^{-1}$) throughout the experiment. Thus, only the response of NO_3^- is presented.

Drought treatment

We simulated an extreme drought event by discontinuing water supply to the plants until the harvest. The treatment simulated an extreme weather event in which a severe water shortage limited the use of irrigation. This was different from episodic water stress that crops may experience during the season. Similar to the flooded plants, the timing of the harvest was determined when 90% of well-watered control plants in each climate change treatment produced the first open boll. Leaf gas exchange was measured before the drought event and six days into the drought treatment, as described above. Plant growth measurements and leaf nutrient analyses were also performed, as described above. The impact of drought on soil N availability was examined by collecting soil samples before the drought treatment (at early flowering) and at harvest from both well-watered control and drought soil. Plant available N was assessed as described above.

Well-watered control

Plants under well-watered conditions were watered regularly to maintain optimal volumetric soil water content (40 – 60 %) throughout the experiment. Plant growth measurements and leaf nutrient analyses were performed, as described above. Leaf gas exchange and soil N availability were measured concomitantly with the measurements taken for flooding and drought treatments.

Statistical analyses

Quantification of extreme weather impacts under climate change treatments

To compare the magnitude of extreme weather events on plant and soil responses between the four climate change treatments, we calculated the response ratio between the well-watered control and flooded/drought plants, under each climate change treatment in each soil, to be used as an effect-size metric (Hedges et al. 1999; Borenstein et al. 2009). We calculated the natural logarithm of the response ratio (InR) as,

$$InR = ln(\bar{X}_t/\bar{X}_c) \quad (1)$$

where $\bar{X}_c$ is the mean of well-watered control and $\bar{X}_t$ is the mean of extreme weather treatment. The variance of the log response ratio ($VlnR$) is computed as,

$$VlnR = S^2_{Pooled}\left(\frac{1}{n_c(\bar{X}c)^2} + \frac{1}{n_t(\bar{X}_t)^2}\right), \quad (2)$$

$$\text{and } S^2_{Pooled} = \sqrt{\left(\frac{(n_c-1)S_C^2 + (n_t-1)S_t^2}{n_c + n_t - 2}\right)} \quad (3)$$

where S^2_{Pooled} is pooled standard deviation, n_c and S_c are the sample size and standard deviation of the well-watered control and n_t and S_t are the sample size and standard deviation of the extreme weather treatments. The 95% confidence interval is calculated from

t-distribution. An effect size is considered significant if the confidence interval does not overlap with 1 in the back-transformed response ratio. Values >1 and <1 indicate positive and negative responses, respectively.

We also tested the statistical significance of the extreme weather treatments and their interactions with CO_2, temperature and soil by using analysis of variance (ANOVA) in R statistical software package (ver. 3.2.2, R Core Team 2014). Firstly, we performed two-way ANOVA to test the effects of flooding/drought and soil on crop and soil responses in the current CO_2 and temperature regime (i.e. C_AT_A). We then used four-way ANOVA to test whether the impacts of flooding/drought were changed in the future CO_2 and temperature regimes by examining the significance of interactions between flooding/drought, CO_2, temperature and soil. The effects of extreme weather treatment on soil nitrate (for flooding) and residual soil nitrate (for drought) were further assessed by comparison between the pre-flood/drought treatment and post-flood/drought treatment data using four-way ANOVA with sampling (pre and post), soil, CO_2 and temperature as factors for each extreme weather treatment. *P* values were obtained for minimal adequate models by deleting non-significant ($P>0.1$) factors from full models (Crawley 2013). All data were checked for normality and heteroscedasticity and log-transformed where necessary.

To examine the relationship between the immediate crop physiological and soil N responses to extreme weather treatments and vegetative growth and yield responses, Pearson's correlation analyses were performed on the response ratios calculated as above.

Results

The impact of flooding at early flowering on leaf physiology in the current and future CO_2 and temperature regimes

In the current CO_2 and temperature regime (C_AT_A), flooding reduced photosynthetic rate in both soils (P=0.03, Table 1), with 12% and 13% reduction in grey and black vertosol, respectively (Fig. 1). Flooding marginally interacted with soil (P=0.08) and reduced stomatal conductance by 21% in grey vertosol, while no difference was observed in black vertosol.

These physiological responses to flooding were altered in future CO_2 and temperature regimes, particularly at C_E (Fig. 1, Table 2). The effect of flooding on photosynthetic rate interacted with CO_2 in both soils (flood x CO_2 interaction, P=0.01) in that the relative reductions in photosynthetic rate between well-watered and flooded plants were greater at C_E (10% and 15% in grey and black vertosol, respectively) compared to C_A (8% and 10% respectively). The impact of flooding on photosynthetic rate was marginally reduced at T_E (flood x temperature interaction, P=0.08), as the relative reductions in photosynthetic rate between well-watered and flooded plants were lower at T_E (5% and 10% in grey and black vertosol, respectively) compared to T_A (13% and 16%, respectively).

The impact of flooding on stomatal conductance interacted with CO_2 (flood x CO_2 interaction, P=0.02). The relative reductions in stomatal conductance between well-watered and flooded plants were greater at C_E (39% and 37% in grey and black vertosol, respectively) compared to C_A (20% and 7%, respectively). Flooding impact was also marginally influenced by temperature (flood x temperature interaction, P=0.07); the relative reduction in stomatal conductance by flooding was greater at T_E (35% and 31% in grey and black vertosol respectively) than at T_A (24% and 14%, respectively).

CO_2 and temperature exhibited significant interactive effects on photosynthetic rate (CO_2 x temperature interaction, P<0.0001, Table 2, Fig. S1) and stomatal conductance (CO_2 x temperature interaction, P<0.0001), and also between soils (soil x CO_2 x temperature interaction, P=0.04); however, CO_2 and temperature did not interact with flooding.

297 *The impact of flooding at early flowering stage on leaf and soil nutrients in current and*
298 *future CO_2 and temperature regimes*

299 In the current CO_2 and temperature regime (C_AT_A), flooding significantly decreased leaf N
300 concentrations of cotton plants in both soils (P=0.01, Fig. 1, Table 1), with 7% and 5%
301 reduction in leaf N in grey and black vertosol, respectively, when compared to with well-
302 watered plants. The strongest negative impact of flooding was observed in its effect on soil
303 nitrate concentrations (P<0.0001), which differed between the two soils (flood x soil
304 interaction, P=0.03, Fig. 1, Table 1). While soil nitrate concentrations showed a small
305 reduction in well-watered plants due to plant uptake, the reduction was far greater for flooded
306 plants (Fig. S2, Table S2). The reduction in soil nitrate concentrations by flooding was
307 greater in black vertosol (88%) than grey vertosol (76%) at C_AT_A.

308 In the future CO_2 and temperature regimes, flooding impact on leaf N strongly interacted
309 with CO_2 (flood x CO_2 interaction, P<0.0001, Table 2, Fig. 1); however, this also interacted
310 with temperature (flood x CO_2 x temperature interaction, P<0.0001) in both soils. This was
311 largely driven by the stronger reduction in leaf N by flooding at C_ET_A (22% and 25%
312 reduction in grey and black vertosol, respectively) compared to C_ET_E (9% and 13%
313 respectively). Flooding impact on soil nitrate concentration was significant in the future CO_2
314 and temperature regimes (P<0.0001, Fig. 1, Fig. S2, Table 2). Flooding impact on soil nitrate
315 concentration was not altered by CO_2; however, it interacted with temperature (flood x
316 temperature interaction, P=0.003, Table 2, Table S2) in both soils. The reduction in soil
317 nitrate concentration by flooding was greater at T_E (86% and 90% in grey and black vertosol,
318 respectively) compared to T_A (79% and 84%, respectively). Leaf N concentrations were also
319 influenced by soil x CO_2 x temperature interaction (P=0.04, Table 2, Fig. S1).

The consequences of flooding on cotton yield and yield components in the current and future CO_2 and temperature regimes

In the current CO_2 and temperature regime (C_AT_A), the rate of vegetative growth following the 6-day flooding event was significantly reduced in both soils (P=0.003, Fig. 2, Table 1), with 48% and 77% reduction in post-flooding vegetative growth rate in grey and black vertosol, respectively. However, flooding had no effect on seed cotton yield in either soil (Fig. 2, Table 1). Flooding impact was evident in boll number and boll size, but only in black vertosol (flood x soil interaction, P=0.04, P=0.08 respectively); flooding increased the number of bolls by 81%, but reduced boll size by 24% in black vertosol. Flooding had no impact on the number and size of bolls in grey vertosol.

Flooding impact on vegetative growth, seed cotton yield and yield components were significantly altered by future CO_2 and temperature regimes (Fig. 2, Table 2). Flooding impact on vegetative growth rate was altered by CO_2 (flood x CO_2 interaction, P=0.01), temperature (flood x temperature interaction, P=0.0005) and their interaction (flood x CO_2 x temperature interaction, P=0.01) in both soils. The impact of flooding on vegetative growth rate was greater at T_E than T_A; however, the effect on CO_2 on flooding impact differed between the temperature treatments, with a greater reduction observed at C_ET_E than C_ET_A in both soils (Fig. 2).

Contrary to its lack of effect on seed cotton yield at the current CO_2 and temperature regime, flooding had a significant impact on seed cotton yield in future CO_2 and temperature regimes (Fig. 2, Table 2). Flooding interacted marginally with CO_2 (flood x CO_2 interaction, P=0.07), but significantly with CO_2 and soil (flood x CO_2 x soil interaction, P=0.03); flooding reduced seed cotton yield by 37% at C_E in black vertosol, while C_E had no impact in grey verosol. Flooding impact was also greater at T_E (flood x temperature interaction, P=0.01) in both soils,

with the relative yield reduction of 23% and 34% by flooding at T_E in grey and black vertosol, respectively. Flooding impact on boll number was altered by CO_2 (flood x CO_2 interaction, P=0.09), temperature (flood x temperature interaction, P<0.0001) and their interaction (flood x CO_2 x temperature interaction, P=0.02). Although the magnitude of its effect differed between the CO_2 treatments, flooding at T_E significantly reduced boll number by 28% in grey vertosol and 40% in black vertosol. Flooding impact on boll size was negligible (Fig. 2, Table 2), with only marginal differences between well-watered and flooded plants observed at C_AT_E in which flooding increased boll size by 27% in grey vertosol and 14% in black vertosol (flood x CO_2 x temperature interaction, P=0.07).

While flooding had a significant impact on vegetative growth and reproductive growth, the positive effect of T_E was evident for both well-watered and flooded plants (Table 2, Fig. S3). Seed cotton yield and boll size were significantly increased by T_E particularly in black vertosol (soil x temperature interaction, P<0.0001).

Relationships between immediate flooding responses and yield responses

Correlation analyses were conducted to examine whether immediate physiological and soil response to flooding correlated with vegetative growth and yield response. Response ratio of post-flooding vegetative growth did not correlate with that of stomatal conductance to flooding (Fig. 3a); however, there was a very strong positive correlation between the impact of post-flooding vegetative growth rate and soil nitrate concentrations (Fig. 3b, r=0.93, P=0.001). Response of seed cotton yield to flooding was positively correlated with stomatal conductance (Fig. 3c, r=0.71, P=0.05). Unlike its effect on vegetative growth, flooding impact on soil nitrate did not correlate with seed cotton yield (Fig. 3d). Changes in seed cotton yield under flooding were positively correlated with boll number (Fig. 3e, r=0.83,

367 *P*=0.01), but not with boll size (Fig. 3f), indicating that reduced boll production induced by
368 flooding at flowering contributed substantially to reduction in yield.

369 *The impact of drought at early flowering on leaf physiology and nutrients under current and*
370 *future CO_2 and temperature regimes*

371 In the current CO_2 and temperature regime (C_AT_A), the detrimental impact of drought on
372 plant physiology and leaf nitrogen was evident by the sixth day of the drought treatment
373 (Table 1, 3). Drought reduced photosynthetic rate (P<0.0001) by 81% and 94% in grey and
374 black vertosol, respectively, when compared to well-watered control plants. Stomatal
375 conductance was strongly reduced (P<0.0001) by 86% in both soils. Drought reduced leaf N
376 (P=0.0002) by 10% and 18% in grey and black soils, respectively. The drought impact did
377 not differ between the two soils at C_AT_A.

378 The impact of drought on leaf physiology and nutrient status was significant in the future
379 CO_2 and temperature regimes (Table 3, 4). Drought impact on photosynthetic rate did not
380 interact with CO_2, but interacted with temperature (drought x temperature interaction,
381 P<0.0001) and with CO_2 and temperature (drought x CO_2 x temperature interaction,
382 P<0.0001), which also differed between the two soils (drought x soil x CO_2 x temperature
383 interaction, P<0.04). While the effects of CO_2 and temperature on photosynthetic response to
384 drought varied in magnitude and between the two soils, both C_E and T_E reduced the negative
385 impact of drought on photosynthetic rate in both soils. Drought impact on stomatal
386 conductance was not altered by CO_2 or temperature; however, it was marginally affected by
387 the interaction between CO_2, temperature and soil (drought x soil x CO_2 x temperature
388 interaction, P<0.09), where both C_E and T_E ameliorated the negative impact of drought.
389 Drought impact on leaf N was not altered by the main effect of CO_2 and temperature but by
390 the interaction between CO_2 and temperature (drought x CO_2 x temperature interaction,

391 *P*=0.04). In both soils, the negative impact of drought on leaf N was reduced at C_AT_E
392 compared to C_AT_A (Table 3).

393 The leaf physiology of both well-watered and drought plants was significantly influenced by
394 CO_2 and temperature treatment. Although there was a significant drought x soil x CO_2 x
395 temperature interaction, the strong positive effect of CO_2 x temperature was evident in
396 photosynthetic rate (*P*<0.0001) and stomatal conductance (*P*<0.0001, Fig. S1).

397 *The consequences of drought on cotton yield, yield components and soil nutrients in the*
398 *current and future CO_2 and temperature regimes*

399 The drought treatment initiated at early flowering led to a significant reduction in vegetative
400 and reproductive growth compared to that of well-watered plants (Fig. 4). In the current CO_2
401 and temperature regime, drought significantly reduced vegetative growth (*P*=0.0001, Table 1)
402 by 95% and 91% in grey and black vertosol, respectively, with its effect marginally
403 differerent between the two soils (drought x soil interaction, *P*=0.08). Drought significantly
404 reduced seed cotton yield (*P*=0.0003) in both soils, with 56% and 54% reduction in seed
405 cotton yield in grey and black vertosol, respectively, compared to well-watered plants. Boll
406 number and size were significantly reduced by drought (*P*=0.01, *P*=0.002 respectively) in
407 both soils. Boll number was reduced by drought by 39% and 25% in grey and black vertosol,
408 respectively, while boll size was reduced by 25% and 43%, respectively. Mortality of cotton
409 plants at the end of the drought treatment led to a large amount of residual nitrate in the soil
410 at harvest (*P*<0.0001, Fig. S4, Table 1) compared to that of well-watered plants. In well-
411 watered plants, soil nitrate concentrations significantly decreased from 49.4 mg N g $soil^{-1}$ at
412 early flowering to 4.1 mg N g $soil^{-1}$ at harvest in grey vertosol and from 62.3 mg N g $soil^{-1}$ to
413 5.1 mg N g $soil^{-1}$ in black vertosol. In drought plants, however, the amount of nitrate in the

414 drought soil at harvest did not differ from the amount of nitrate at the pre-drought
415 concentrations measured at early flowering (Fig. S4, Table S2).

416 In future CO_2 and temperature regimes, drought impact on vegetative growth was not altered
417 by CO_2, but altered by temperature (drought x temperature interaction, $P<0.0001$, Fig. 4,
418 Table 4) and by CO_2 and temperature interaction (drought x CO_2 x temperature interaction,
419 $P=0.005$). Although their effects varied in magnitude, drought impact on vegetative growth
420 was generally ameliorated by T_E and C_E in both soils. Drought impact on seed cotton yield
421 was altered by both CO_2 (drought x CO_2 interaction, $P<0.0001$) and temperature (drought x
422 temperature interaction, $P<0.0001$), with its effect marginally greater in black than grey
423 vertosol (drought x soil x temperature interaction, $P=0.07$). The reduction in seed cotton yield
424 by drought was greater at C_E, with 88% reduction at C_E compared to 67% at C_A in both grey
425 and black vertosol. The reduction in seed cotton yield in drought was greater at T_E, (82% and
426 84% in grey vertosol and black versotol, respectively) compared to T_A (73% and 71%,
427 respectively). Drought impact on boll number was altered by CO_2 (drought x CO_2 interaction,
428 $P=0.002$), temperature (drought x temperature interaction, $P=0.05$) and their interaction
429 (drought x CO_2 x temperature interaction, $P=0.004$) with a marginal difference between the
430 two soil (drought x soil x CO_2 x temperature interaction, $P=0.10$). The negative effect of
431 drought on boll number was greater at T_E (54% reduction on average) than at T_A (44%
432 reduction) in both soils and the largest reduction was observed at C_ET_A in black vertosol
433 (66% reduction), compared to well-watered plants. Drought impact on boll size was altered
434 by CO_2 (drought x CO_2 interaction, $P=0.002$) which differed between the two soils (drought
435 x soil x CO_2 interaction, $P=0.01$), and temperature (drought x temperature interaction,
436 $P<0.0001$). Drought impact on boll size was greater at T_E in both soils, while the impact of
437 drought on boll size at C_ET_A was greater in grey vertosol (80% reduction) than black vertosol
438 (62% reduction). The amount of residual nitrate in the soil was altered by CO_2 (drought x

439 CO_2 interaction, *P*=0.02), and CO_2 and temperature interaction (drought x CO_2 x temperature
440 interaction, *P*=0.001); however, this difference was due to the CO_2 and temperature effect on
441 soil nitrate concentrations at pre-drought (Fig. S4, Table S2) and not at the end of the drought
442 treatment because the eventual mortality of plants due to drought meant that changes in soil
443 nitrate concentrations were minimal during the drought treatment.

444 Due to the strong impact of drought treatment on reproductive growth, the impacts of CO_2
445 and temperature were less pronounced by the end of the experiment (Fig. S3), except for
446 residual soil nitrate which was strongly influenced by the positive effect of temperature on
447 soil nitrate concentrations prior to the drought treatment.

448 *Relationships between immediate drought responses and yield responses*

449 Correlation analyses were conducted to examine whether immediate physiological responses
450 to drought were correlated with vegetative growth and yield responses. The response ratio of
451 seed cotton yield to drought was strongly and positively correlated with boll size (Fig. 5a,
452 *r*=0.91, *P*=0.002) and boll number (Fig. 5b, *r*=0.87, *P*=0.01), reflecting the detrimental
453 impact of drought on boll production, as well as the importance of pre-existing bolls in
454 determining seed cotton yield. In addition, boll development was more strongly affected by
455 drought at C_E compared to C_A relative to well-watered plants, and the two soils differed
456 greatly in their response to drought at T_A.

457 **Discussion**

458 Our results showed that the impact of flooding and drought on cotton productivity differed
459 when plants were grown under future CO_2 and temperature regimes, and both physiological
460 and soil nutrient responses were strongly linked to vegetative and reproductive growth
461 responses to the extreme weather events. At the current CO_2 and temperature regimes,

462 flooding had negative impacts on leaf physiology, vegetative growth, leaf and soil N status
463 but had no effect on seed cotton yield in both soils. Drought had a detrimental impact on leaf
464 physiology, leaf N status, growth and yield and led to a large amount of residual N in the soil.
465 Contrary to expectations, physiological responses to flooding were more pronounced in
466 plants grown at C_E, and C_E did not ameliorate the impact of flooding on cotton yield. T_E did
467 not exacerbate flooding nor drought impact on leaf physiology; however, the impacts of
468 flooding and drought on cotton yield were greater at T_E than T_A. Overall, flooding and
469 drought impacts on cotton yield were greater in the future CO_2 and temperature regimes,
470 largely due to the greater yield potential of cotton in the future CO_2 and temperature regimes
471 than the current regime in the absence of extreme weather events. Therefore, our findings
472 indicate that the instability of crop productivity is likely to increase in the more extreme
473 climates expected under future climate regimes.

474 *Flooding impact under the current and future CO_2 and temperature regimes*

475 The impact of flooding on cotton yield depended on both physiological and soil nutrient
476 responses to the flooding event. Reduction in stomatal conductance is an immediate
477 physiological response commonly observed in plants undergoing waterlogging stress, which
478 is often followed by a reduction in photosynthetic rate and translocation of carbohydrates
479 (Kozlowski 1984). Reduction in stomatal conductance can also impact uptake of water
480 soluble nutrients such as nitrate which enters plant roots along with water by mass flow
481 (Shimono and Bunce 2009; McGrath and Lobell 2013). Such physiological responses may
482 not be temporary, with potential long-term consequences for plant nutrition and growth, as
483 observed previously in cotton (Milroy and Bange 2013). The large reduction in soil nitrate
484 following the flooding event further reduced N nutrition of plants and slowed the rate of
485 recovery. Loss of soil N following flooding can occur through nitrate leaching and

denitrification, and the latter could contribute to substantial loss (30-60%) of applied N in vertosol soils (Rochester and Constable 2000). We found that the magnitude of soil N loss depended on pre-flooding soil nitrate concentrations (Fig. S5) which were higher in T_E than T_A. Thus, the effect of flooding on soil N availability has most likely contributed to the greater cotton yield reduction observed at T_E compared to T_A.

There might be a limit to leaf physiological adjustment during the flooding event that can minimise yield loss due to flooding. We hypothesised that C_E would reduce the impact of flooding because it allows plants to maintain photosynthesis at reduced stomatal conductance. We found that stomatal conductance, but not photosynthesis, was more greatly reduced in response to flooding in C_E compared to C_A; however, C_E did not affect yield response to flooding. Lack of C_E effect on plant productivity in response to waterlogging has been reported in previous studies (Megonigal et al. 2005; Shimono et al. 2012), where even a greater proportion of biomass was reduced in C_E despite higher photosynthesis in C_E (Megonigal et al. 2005). Given that the effect of flooding on yield at C_E was compared to the yield of non-flooded plants at C_E, the discrepancy between the physiological response and yield response may be attributed to the greater photosynthetic potential at C_E under non-flooded conditions. Indeed, photosynthetic rate was still greater at C_E than C_A under flooding, even though a greater reduction in photosynthetic rate under flooding was observed at C_E compared to C_A relative to well-watered plants (Fig. S1). Similarly, the greater impact of flooding on yield at T_E is attributed to enhanced yield at T_E under non-flooded conditions, combined with its greater reduction in soil nitrate upon the flooding event which further magnified the impact of flooding on cotton yield (Fig. S5). Therefore, the relative magnitude of the flooding impact largely depends on the impact of C_E and T_E on the yield potential of plants and soil N availability in the absence of flooding events.

510 *Drought impact on under the current and future CO_2 and temperature regimes*

511 C_E and T_E have been shown to change plant-water relations in many systems (Kimball et al
512 2002), and we hypothesised that the impact of drought events on crop productivity would be
513 exacerbated at T_E and ameliorated at C_E. Contrary to our expectation, we found that drought
514 impact on leaf physiology and vegetative growth was greatest at the current CO_2 and
515 temperature regime (i.e. C_AT_A). Naudts et al (2013) also observed minimal impact of T_E on
516 plant physiological response to drought, while a clear mitigating response was observed when
517 plants were grown at both T_E and C_E. The impact of C_E and T_E on plant productivity has been
518 linked to its effect on soil water availability (Dermody et al. 2007), and a lack of enhanced
519 soil drying has been attributed to the lack of T_E effect on plant productivity (Naudts et al.
520 2013). Thus, other factors (e.g. VPD, air movement, root biomass) that influence the rate of
521 soil drying under these conditions need to be taken into a consideration when examining the
522 effect of T_E and C_E on crop response to drought.

523 The yield response to drought differed substantially from that of physiology and vegetative
524 growth with a greater yield reduction at T_E, primarily reflecting the greater yield potential at
525 T_E in the absence of a severe drought event. However, unlike the effect of flooding in which
526 reduced soil N availability restricted the recovery of flood-affected plants and yield, drought
527 impact on soil N availability played little role in mediating crop responses to drought. In fact,
528 a large amount of residual N was found in the soil as a result of drought-induced mortality of
529 plants. Furthermore, drought has been shown to restrict biological activities in the soil,
530 reducing soil enzymatic activities and nutrient turnover (Sardans and Penuelas 2005; 2010;
531 Hartmann et al. 2013). Our results suggest that minimal changes in soil N occurred during the
532 drought, and that neither C_E nor T_E affected the drought impact on soil N. However, it is
533 possible that altered soil N availability at C_E and T_E may have indirectly contributed to the

drought response through changes in leaf N. It has been suggested that increased plant N nutrition can contribute to drought tolerance by increasing osmotic adjustment capability (Saneoka et al. 2004; Gimeno et al. 2014). We found that leaf N concentrations were lower in C_AT_A compared to future CO_2 and temperature regimes; therefore, the drought response may be partly explained by C_E and T_E impact on crop N status prior to the drought event.

The role of soil characteristics in crop response to flooding and drought

We hypothesised that differences in soil characteristics may affect crop response to flooding and drought due to their effects on soil water and nutrient availability. While the two soils examined occasionally differentially affected crop physiology, growth and yield, relative differences in crop response to flooding and drought between them were small. Soil characteristics that affect water drainage (e.g. soil texture) can be an important determinant of flooding impact on crop productivity, because faster drainage may allow rapid recovery from soil waterlogging, yet it may also generate a rapid loss of soil nitrate which may restrict crop recovery. We found that loss of soil nitrate was greater in black vertosol, which has a higher coarse sand content than grey vertosol (Table S1), and thus this may partly explain the differences observed between the two soils in growth and yield responses to flooding, particularly at C_E. Drought impact on reproductive growth also differed between the two soils, reflecting differences in reproductive growth between the soils under both well-watered and drought conditions. Such an interaction between drought and soil has been previously reported by Rivest et al (2013) who found that 10-day drought reduced grain yield of wheat in a heavy-textured soil, while no reduction was observed in the light-textured soil. Given that both soils were heavy-textured vertosols, our study demonstrates that even small variations in soil characteristics can produce marked differences in crop productivity and its response to extreme weather events.

Implications for agricultural production under projected future climates

A recent review by Thornton et al (2014) highlighted the importance of including climate variability and extreme weather events when evaluating the impact of climate change on agricultural production. While most research has focused on the impact of C_E and T_E on crop yield, projected increases in extreme weather events could alter our predictions of the impact of climate change on agricultural production (Porter and Semenov 2005). Indeed, our results indicate that future CO_2 and temperature regimes increased yield in cotton under optimal water conditions; however, this may not be true with projected increase in extreme weather events. Relative yield loss caused by flooding and drought is likely to be greater when yield potential is higher under future CO_2 and temperature regimes. This may lead to a large year-to-year variability in yield, increasing unpredictability and instability of agricultural production. A recent meta-analysis by Challinor et al (2014) found that inter-annual variability in agricultural yield is likely to increase under climate change, yet such variability remains largely unassessed. Thus, our study provides empirical evidence and rationale for assessing the effect of extreme weather events in the context of climate change.

Appropriate adaptation has potential to avoid or even reverse yield loss expected under climate change (Howden et al. 2007; Challinor et al. 2014). For example, the negative impact of flooding on crop yield may be reduced by additional N supply (Hodgson 1982; Swarup and Sharma 1993; Singh et al. 2002) or the impact of T_E may be avoided by shifting planting date (Lobell and Field 2007). Irrigation may be the only management option for drought-affected crops; however, adequate measures to retain residual soil N in the system may benefit subsequent crop production and contribute to climate mitigation by reducing the risk of nitrous oxide emission upon re-wetting. Our results indicate that yield response depends on both plant and soil nutrient responses to extreme weather events and suggest that adaptation

582 and management practices become increasingly important as climatic variability increases
583 under future climate regimes. Recovery of both plant and soil systems is clearly important to
584 reduce the yield loss of the affected crop; however, the management practice should also
585 consider long-term implications of extreme weather events and future CO_2 and temperature
586 regimes on soils that differ in their physical and chemical characteristics. Our study therefore
587 provides a framework for future research and management guidelines to reduce the
588 vulnerability and instability of agricultural production under projected future climates.

589 *Conclusions*

590 Agricultural production is highly sensitive to climatic variability, and extreme weather events
591 are likely to cause a substantial yield loss under projected climate change. We found that the
592 magnitude of flooding and drought impact on cotton productivity was greater at future CO_2
593 and temperature regimes due to the greater yield potential under these conditions in the
594 absence of extreme weather events. C_E did not ameliorate the impact of flooding on cotton
595 yield, while flooding caused a rapid loss of N from the soil, contributing to the reduced
596 vegetative growth and yield, particularly at T_E. Flooding and drought had contrasting
597 consequences for soil N availability, with drought-induced loss of biological activity resulting
598 in a large amount of residual N in the soil. Our study demonstrated that differences in soil
599 characteristics can influence crop responses to extreme weather events, suggesting that
600 adaptation strategies should explicitly consider soil characteristics in mediating such
601 responses. This study highlights the importance of an integrated approach, considering both
602 plant and soil systems, to assist the recovery of the affected crops but also to recover the soil
603 system for the subsequent crop production. This study provides a framework for effective
604 management to ensure the long-term resilience of agricultural production under projected
605 future climates.

606 **References**

607 Aulakh MS, Doran JW, Walters DT, Power JF (1991) Legume residue and soil water effects
608 on denitrification in soils of different textures. Soil Biology & Biochemistry 23: 1161-
609 1167.

610 Bai E, Li SL, Xu WH, Li W, Dai WW, Jiang P (2013) A meta-analysis of experimental
611 warming effects on terrestrial nitrogen pools and dynamics. New Phytologist 199:
612 441-451.

613 Bange MP, Milroy SP, Thongbai P (2004) Growth and yield of cotton in response to
614 waterlogging. Field Crops Research 88: 129-142.

615 Bloom AJ, Burger M, Kimball BA, Pinter PJ (2014) Nitrate assimilation is inhibited by
616 elevated CO_2 in field-grown wheat. Nature Climate Change 4: 477-480.

617 Borenstein M, Hedges LV, Higgins JPT, Rothstein HR (2009) Introduction to Meta-Analysis.
618 John Wiley & Sons, Ltd, UK.

619 Braunack MV (2013) Cotton farming systems in Australia: factors contributing to changed
620 yield and fibre quality. Crop & Pasture Science 64: 834-844.

621 Cameron KC, Haynes RJ (1986) Retention and movement of nitrogen in soils. In: RJ Haynes
622 (ed) Mineral nitrogen in the plant-soil system. Academic Press, Inc., Orlando, Florida.

623 Cannell RQ, Belford RK (1980) Effects of waterlogging at different stages of development
624 on the growth and yield of winter oilseed rape (*Brassica napus* L.). Journal of the
625 Science of Food and Agriculture 31: 963-965.

626 Cattle SR, Field DJ (2013) A review of the soil science research legacy of the triumvirate of
627 cotton CRC. Crop & Pasture Science 64: 1076-1094.

628 Challinor AJ, Watson J, Lobell DB, Howden SM, Smith DR, Chhetri N (2014) A meta-
629 analysis of crop yield under climate change and adaptation. Nature Climate Change 4:
630 287-291.

631 Crawley MJ (2013) The R Book. 2nd edition edn. John Wiley & Sons, Ltd, West Sussex, UK.
632 de Graaff MA, van Groenigen KJ, Six J, Hungate B, van Kessel C (2006) Interactions
633 between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis.
634 Global Change Biology 12: 2077-2091.
635 Dermody O, Weltzin JF, Engel EC, Allen P, Norby RJ (2007) How do elevated CO_2,
636 warming, and reduced precipitation interact to affect soil moisture and LAI in an old
637 field ecosystem? Plant and Soil 301: 255-266.
638 Dieleman WIJ, Vicca S, Dijkstra FA, Hagedorn F, Hovenden MJ, Larsen KS, Morgan JA,
639 Volder A, Beier C, Dukes JS, King J, Leuzinger S, Linder S, Luo YQ, Oren R, de
640 Angelis P, Tingey D, Hoosbeek MR, Janssens IA (2012) Simple additive effects are
641 rare: a quantitative review of plant biomass and soil process responses to combined
642 manipulations of CO_2 and temperature. Global Change Biology 18: 2681-2693.
643 Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects,
644 mechanisms and management. Agronomy for Sustainable Development 29: 185-212.
645 Ghannoum O, Phillips NG, Conroy JP, Smith RA, Attard RD, Woodfield R, Logan BA,
646 Lewis JD, Tissue DT (2010) Exposure to preindustrial, current and future atmospheric
647 CO_2 and temperature differentially affects growth and photosynthesis in *Eucalyptus*.
648 Global Change Biology 16: 303-319.
649 Gimeno V, Diaz-Lopez L, Simon-Grao S, Martinez V, Martinez-Nicolas JJ, Garcia-Sanchez
650 F (2014) Foliar potassium nitrate application improves the tolerance of *Citrus*
651 *macrophylla* L. seedlings to drought conditions. Plant Physiology and Biochemistry
652 83: 308-315.
653 Glaz B, Lingle SE (2012) Flood duration and time of flood onset effects on recently planted
654 sugarcane. Agronomy Journal 104: 575-583.

655 Hartmann AA, Barnard RL, Marhan S, Niklaus PA (2013) Effects of drought and N-
656 fertilization on N cycling in two grassland soils. Oecologia 171: 705-717.
657 Hatfield JL, Boote KJ, Kimball BA, Ziska LH, Izaurralde RC, Ort D, Thomson AM, Wolfe D
658 (2011) Climate impacts on agriculture: implications for crop production. Agronomy
659 Journal 103: 351-370.
660 Hedges LV, Gurevitch J, Curtis PS (1999) The meta-analysis of response ratios in
661 experimental ecology. Ecology 80: 1150-1156.
662 Hodgson AS (1982) The effects of duration, timing and chemical amelioration of short-term
663 waterlogging during furrow irrigation of cotton in a cracking grey clay. Australian
664 Journal of Agricultural Research 33: 1019-1028.
665 Hovenden MJ, Newton PCD, Carran RA, Theobald P, Wills KE, Schoor JKV, Williams AL,
666 Osanai Y (2008) Warming prevents the elevated CO2-induced reduction in available
667 soil nitrogen in a temperate, perennial grassland. Global Change Biology 14: 1018-
668 1024.
669 Howden SM, Soussana JF, Tubiello FN, Chhetri N, Dunlop M, Meinke H (2007) Adapting
670 agriculture to climate change. Proceedings of the National Academy of Sciences of
671 the United States of America 104: 19691-19696.
672 Huang BR, Johnson JW, Nesmith S, Bridges DC (1994) Growth, physiological and
673 anatomical responses of two wheat genotypes to waterlogging and nutrient supply.
674 Journal of Experimental Botany 45: 193-202.
675 IPCC (2014) Managing the Risks of Extreme Events and Disasters to Advance Climate
676 Change Adaptation. A Special Report of Working Groups I and II of the
677 Intergovernmental Panel on Climate Change. In: CB Field, V. Barros, T.F. Stocker, D.
678 Qin, D.J. Dokken, K.L. Ebi, M.D. Mastrandrea, K.J. Mach, G.-K. Plattner, S.K. Allen,

679 M. Tignor, and P.M. Midgley (ed). Cambridge University Press, Cambridge, UK, and
680 New York, NY, USA.
681 Kozlowski TT (1984) Plant responses to flooding of soil. Bioscience 34: 162-167.
682 Li FS, Kang SZ, Zhang JH (2003) CO_2 enrichment on biomass accumulation and nitrogen
683 nutrition of spring wheat under different soil nitrogen and water status. Journal of
684 Plant Nutrition 26: 769-788.
685 Lobell DB, Field CB (2007) Global scale climate - crop yield relationships and the impacts of
686 recent warming. Environmental Research Letters 2.
687 Malik A, Colmer TD, Lambers H, Schortemeyer M (2001) Changes in physiological and
688 morphological traits of roots and shoots of wheat in response to different depths of
689 waterlogging. Australian Journal of Plant Physiology 28: 1121-1131.
690 McGrath JM, Lobell DB (2013) Reduction of transpiration and altered nutrient allocation
691 contribute to nutrient decline of crops grown in elevated CO_2 concentrations. Plant
692 Cell and Environment 36: 697-705.
693 Megonigal JP, Vann CD, Wolf AA (2005) Flooding constraints on tree (Taxodium
694 distichum) and herb growth responses to elevated CO2. Wetlands 25: 430-438.
695 Milroy SP, Bange MP (2013) Reduction in radiation use efficiency of cotton (*Gossypium*
696 *hirsutum* L.) under repeated transient waterlogging in the field. Field Crops Research
697 140: 51-58.
698 Naudts K, Van den Berge J, Janssens IA, Nijs I, Ceulemans R (2013) Combined effects of
699 warming and elevated CO_2 on the impact of drought in grassland species. Plant and
700 Soil 369: 497-507.
701 Porter JR, Semenov MA (2005) Crop responses to climatic variation. Philosophical
702 Transactions of the Royal Society B-Biological Sciences 360: 2021-2035.

703 R Core Team (2014) R: A language and environment for statistical computing. R Foundation
704 for Statistical Computing, Vienna, Austria.
705 Rayment GE, Lyons DJ (2010) Soil Chemical Methods - Australasia. CSIRO Publishing,
706 Melbourne, Australia.
707 Rivest D, Lorente M, Olivier A, Messier C (2013) Soil biochemical properties and microbial
708 resilience in agroforestry systems: Effects on wheat growth under controlled drought
709 and flooding conditions. Science of the Total Environment 463: 51-60.
710 Robredo A, Perez-Lopez U, de la Maza HS, Gonzalez-Moro B, Lacuesta M, Mena-Petite A,
711 Munoz-Rueda A (2007) Elevated CO_2 alleviates the impact of drought on barley
712 improving water status by lowering stomatal conductance and delaying its effects on
713 photosynthesis. Environmental and Experimental Botany 59: 252-263.
714 Robredo A, Perez-Lopez U, Miranda-Apodaca J, Lacuesta M, Mena-Petite A, Munoz-Rueda
715 A (2011) Elevated CO_2 reduces the drought effect on nitrogen metabolism in barley
716 plants during drought and subsequent recovery. Environmental and Experimental
717 Botany 71: 399-408.
718 Rochester IJ, Constable GA (2000) Denitrification and immobilisation in flood-irrigated
719 alkaline grey clays as affected by nitrification inhibitors, wheat straw, and soil texture.
720 Australian Journal of Soil Research 38: 633-642.
721 Rogers GS, Milham PJ, Thibaud MC, Conroy JP (1996) Interactions between rising CO_2
722 concentration and nitrogen supply in cotton .1. Growth and leaf nitrogen
723 concentration. Australian Journal of Plant Physiology 23: 119-125.
724 Saneoka H, Moghaieb REA, Premachandra GS, Fujita K (2004) Nitrogen nutrition and water
725 stress effects on cell membrane stability and leaf water relations in *Agrostis palustris*
726 Huds. Environmental and Experimental Botany 52: 131-138.

727 Sardans J, Penuelas J (2005) Drought decreases soil enzyme activity in a Mediterranean
728 *Quercus ilex* L. forest. Soil Biology & Biochemistry 37: 455-461.
729 Sardans J, Penuelas J (2010) Soil enzyme activity in a Mediterranean forest after six years of
730 drought. Soil Science Society of America Journal 74: 838-851.
731 Shao GC, Lan JJ, Yu SE, Liu N, Guo RQ, She DL (2013) Photosynthesis and growth of
732 winter wheat in response to waterlogging at different growth stages. Photosynthetica
733 51: 429-437.
734 Shimono H, Bunce JA (2009) Acclimation of nitrogen uptake capacity of rice to elevated
735 atmospheric CO_2 concentration. Annals of Botany 103: 87-94.
736 Shimono H, Konno T, Sakai H, Sameshima R (2012) Interactive Effects of Elevated
737 Atmospheric CO2 and Waterlogging on Vegetative Growth of Soybean (Glycine max
738 (L.) Merr.). Plant Production Science 15: 238-245.
739 Singh YV, Swarup A, Gupta SK (2002) Alleviating adverse effects of waterlogging through
740 top-dressed urea-N on growth, yield and mineral, composition of sorghum in a sodic
741 soil. Agrochimica 46: 89-99.
742 Snowden MC, Ritchie GL, Simao FR, Bordovsky JP (2014) Timing of episodic drought can
743 be critical in cotton. Agronomy Journal 106: 452-458.
744 Sogbedji JM, van Es HM, Yang CL, Geohring LD, Magdoff FR (2000) Nitrate leaching and
745 nitrogen budget as affected by maize nitrogen rate and soil type. J Environ Qual 29:
746 1813-1820.
747 Stiller WN (2008) Sicot 71BRF. Plant Varieties Journal 21: 194-197.
748 Sugimoto H, Satou T, Nishihara S, Narimatsu K (1989) Excess moisture injury of soybeans
749 cultivated in an upland field converted from paddy .3. Foliar application of urea as
750 countermeasure against excess moisture injury. Japanese Journal of Crop Science 58:
751 605-610.

752 Swarup A, Sharma DP (1993) Influence of top-dressed nitrogen in alleviating adverse-effects
753 of flooding on growth and yield of wheat in a sodic soil. Field Crops Research 35: 93-
754 100.
755 Thornton PK, Ericksen PJ, Herrero M, Challinor AJ (2014) Climate variability and
756 vulnerability to climate change: a review. Global Change Biology 20: 3313-3328.
757 Tubiello FN, Soussana JF, Howden SM (2007) Crop and pasture response to climate change.
758 Proceedings of the National Academy of Sciences of the United States of America
759 104: 19686-19690.
760 Wright GC, Hubick KT, Farquhar GD (1991) Physiological analysis of peanut cultivar
761 response to timing and duration of drought stress. Australian Journal of Agricultural
762 Research 42: 453-470.
763 Yaduvanshi NPS, Setter TL, Sharma SK, Singh KN, Kulshreshtha N (2012) Influence of
764 waterlogging on yield of wheat (*Triticum aestivum*), redox potentials, and
765 concentrations of microelements in different soils in India and Australia. Soil
766 Research 50: 489-499.

767

Tables

Table 1 Results of analysis of variance (ANOVA) showing the immediate impact of flooding and drought on leaf physiology, leaf nitrogen and soil nitrate (flooding only) and their consequences on the post-flowering growth rate, seed cotton yield and yield components of cotton plants and residual soil nitrate (drought only) between the two soils under the current CO_2 and temperature regime (C_AT_A). Values are probability with significant results (P<0.05) shown in bold, marginally significant results (P<0.1) in italic and non-significant results (P>0.1) as n.s.

	Immediate impact				Consequences				
	Photosynthetic rate	Stomatal conductance	Leaf N	Soil nitrate	Post-flower growth rate	Seed cotton yield	Boll number	Boll size	Residual soil nitrate
Flooding treatment									
Flood	**0.03**	n.s.	**0.01**	**<0.0001**	**0.003**	n.s.	**0.01**	n.s.	-
Soil	n.s.	n.s.	n.s.	**0.03**	n.s.	**0.0001**	n.s.	**<0.0001**	-
Flood x Soil	n.s.	*0.08*	n.s.	**0.03**	n.s.	n.s.	**0.04**	*0.08*	-
Drought treatment									
Drought	**<0.0001**	**<0.0001**	**0.0002**	-	**0.0001**	**0.0003**	**0.01**	**0.002**	**<0.0001**
Soil	n.s.	n.s.	n.s.	-	n.s.	**0.001**	**0.04**	**0.0005**	n.s.
Drought x Soil	n.s.	n.s.	n.s.	-	*0.08*	n.s.	n.s.	n.s.	n.s.

Table 2 Results of analysis of variance (ANOVA) showing the immediate impact of flooding and its interactions with CO_2, temperature and soil on leaf physiology, leaf nitrogen and soil nitrate and their consequences on the post-flooding growth rate, seed cotton yield and yield components of cotton plants. Values are probability with significant results ($P<0.05$) shown in bold, marginally significant results ($P<0.1$) in italic and non-significant results ($P>0.1$) as n.s.

	Immediate impact				Consequences			
Factors	Photosynthetic rate	Stomatal conductance	Leaf N	Soil nitrate	Post-flood growth rate	Seed cotton yield	Boll number	Boll size
Flooding effects								
Flood	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.01**	**0.0002**	n.s.
Flood x Soil	n.s.	n.s.	*0.07*	**0.03**	n.s.	n.s.	n.s.	n.s.
Flood x CO_2	**0.01**	**0.02**	**<0.0001**	n.s.	**0.01**	*0.07*	*0.09*	n.s.
Flood x Temp	*0.08*	*0.07*	n.s.	**0.003**	**0.0005**	**0.01**	**<0.0001**	n.s.
Flood x Soil x CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	**0.03**	**0.01**	n.s.
Flood x Soil x Temp	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*0.10*	n.s.
Flood x CO_2 x Temp	n.s.	n.s.	**<0.0001**	n.s.	**0.01**	n.s.	**0.02**	*0.07*
Flood x Soil x CO_2 x Temp	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Non-flooding effects								
Soil	**0.002**	**0.02**	n.s.	*0.05*	n.s.	**<0.0001**	**0.04**	**<0.0001**
CO_2	**<0.0001**	n.s.	**0.01**	n.s.	n.s.	**0.01**	**0.0002**	n.s.
Temp	**0.01**	**<0.0001**	**<0.0001**	**<0.0001**	n.s.	**<0.0001**	n.s.	**<0.0001**
Soil x CO_2	n.s.	n.s.	n.s.	**0.02**	n.s.	n.s.	**0.01**	**0.04**
Soil x Temp	n.s.	n.s.	n.s.	n.s.	n.s.	**<0.0001**	**0.05**	**<0.0001**
CO_2 x Temp	**<0.0001**	**<0.0001**	n.s.	n.s.	**<0.0001**	n.s.	**0.0001**	n.s.
Soil x CO_2 x Temp	n.s.	**0.04**	**0.04**	n.s.	n.s.	n.s.	**0.004**	n.s.

781 **Table 3** The effect of drought on leaf physiology at six days into the treatment and N concentrations of cotton plants at 13 days into the
782 treatment grown on grey vertosol and black vertosol under the four climate change treatments. The effect is expressed in response ratios
783 calculated from comparisons between well-watered plants and drought plants. Values are mean response ratios. Asterisks indicate significant
784 effect based on ± 95% confidence intervals.

Soil	Grey				Black			
Climate treatment	C_AT_A	C_AT_E	C_ET_A	C_ET_E	C_AT_A	C_AT_E	C_ET_A	C_ET_E
Photosynthetic rate	0.19 *	0.97	0.70 *	0.94	0.06 *	1.03	0.83 *	0.83 *
Stomatal conductance	0.14 *	0.39 *	0.31 *	0.72 *	0.14 *	0.61 *	0.61 *	0.57 *
Leaf N	0.90 *	0.96 *	0.92 *	0.93 *	0.82 *	0.96 *	0.91 *	0.88 *

785

Table 4 Results of analysis of variance (ANOVA) showing the immediate impact of drought and its interactions with CO_2, temperature and soil on leaf physiology and leaf nitrogen and their consequences on the post-drought growth rate, seed cotton yield, yield components of cotton plants and residual soil nitrate. Values are probability with significant results ($P<0.05$) shown in bold, marginally significant results ($P<0.1$) in italic and non-significant results ($P>0.1$) as n.s.

	Immediate impact			Consequences				
Parameters	Photosynthetic rate	Stomatal conductance	Leaf N	Post-drought growth rate	Seed cotton yield	Boll number	Boll size	Residual soil nitrate
Drought effects								
Drought	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Drought x Soil	n.s.	n.s.	n.s.	**0.01**	n.s.	n.s.	n.s.	n.s.
Drought x CO_2	n.s.	n.s.	n.s.	n.s.	**<0.0001**	**0.002**	**0.0002**	**0.02**
Drought x Temp	**<0.0001**	n.s.	n.s.	**<0.0001**	**<0.0001**	**0.05**	**<0.0001**	n.s.
Drought x Soil x CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	**0.01**	**0.01**	n.s.
Drought x Soil x Temp	n.s.	n.s.	n.s.	n.s.	*0.07*	n.s.	n.s.	n.s.
Drought x CO_2 x Temp	**<0.0001**	n.s.	**0.04**	**0.005**	n.s.	**0.004**	n.s.	**0.001**
Drought x Soil x CO_2 x Temp	**0.04**	*0.09*	n.s.	n.s.	n.s.	*0.10*	n.s.	n.s.
Non-drought effects								
Soil	**0.02**	**0.01**	n.s.	n.s.	**0.03**	n.s.	**<0.0001**	**0.03**
CO_2	**<0.0001**	n.s.	**<0.0001**	**<0.0001**	*0.08*	**0.003**	n.s.	**0.0003**
Temp	**<0.0001**	**<0.0001**	**<0.0001**	*0.06*	**<0.0001**	**0.0003**	**<0.0001**	**<0.0001**
Soil x CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	**0.001**	n.s.	n.s.
Soil x Temp	**0.02**	**0.005**	n.s.	n.s.	**0.01**	n.s.	**0.01**	
CO_2 x Temp	**<0.0001**	**<0.0001**	**<0.0001**	*0.08*	n.s.	**<0.0001**	**0.001**	**<0.0001**
Soil x CO_2 x Temp	n.s.	n.s.	n.s.	n.s.	n.s.	*0.07*	n.s.	n.s.

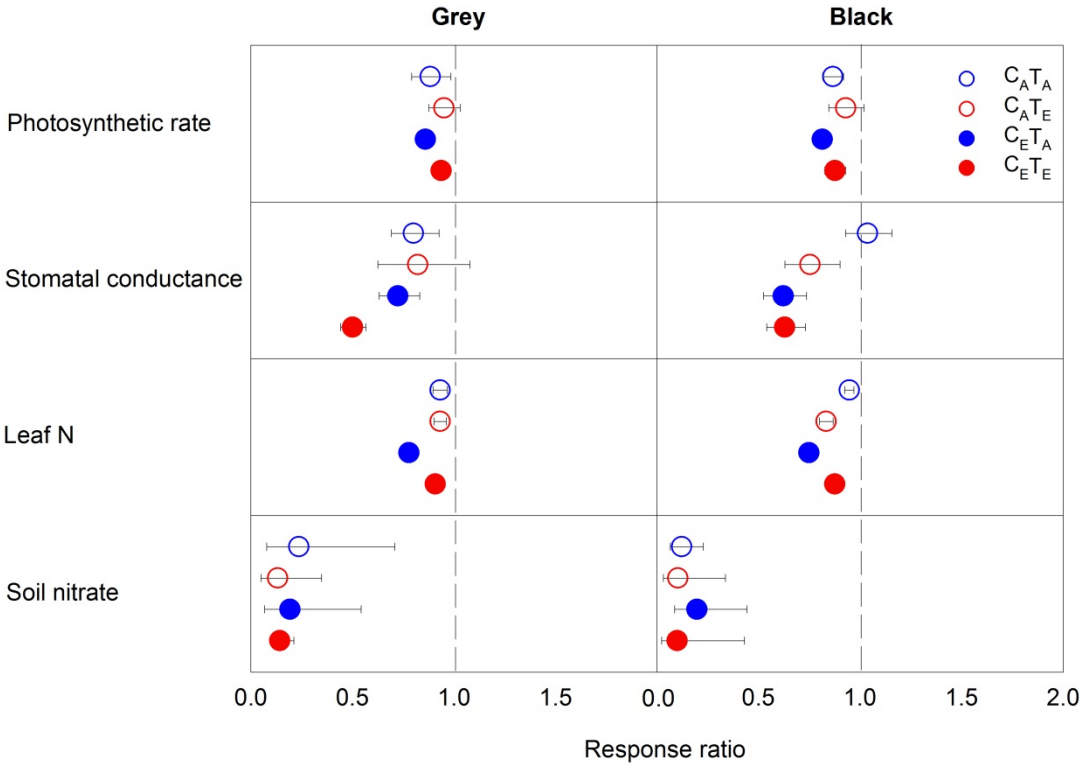
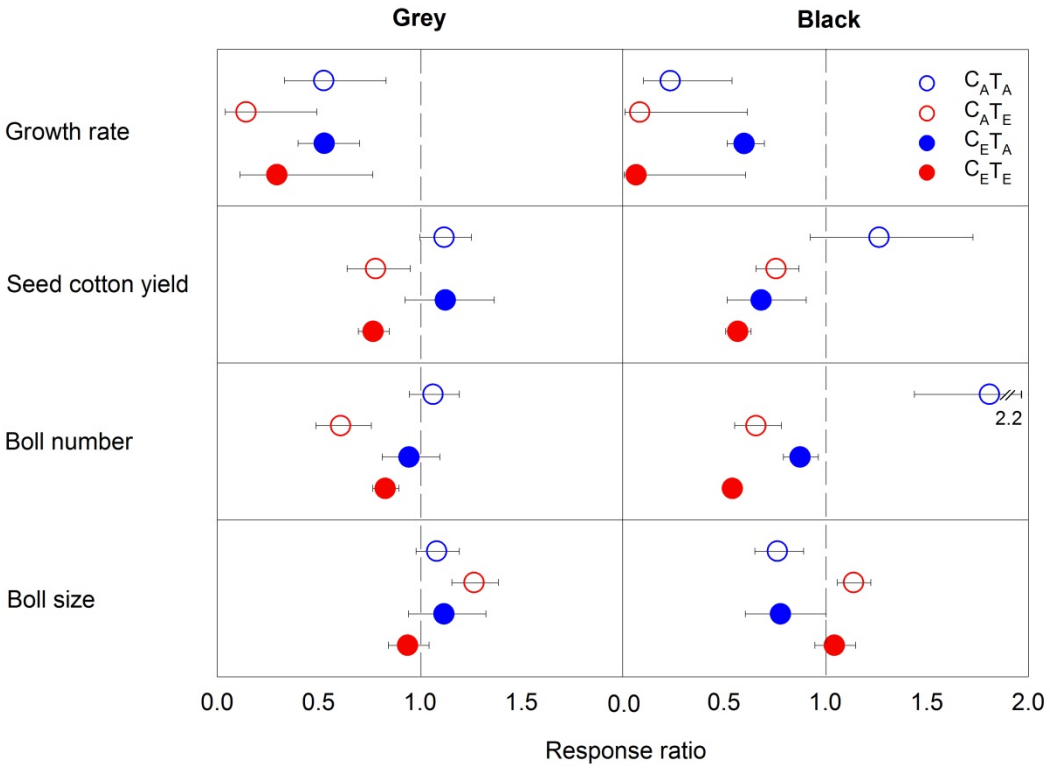


Fig. 1 The effect of flooding on leaf physiology at the end of flooding treatment and leaf and soil N status at 7 days after the end of flooding period from grey vertosol and black vertosol planted with cotton under the four climate treatments. The effect is expressed in response ratios calculated from comparisons between well-watered plants and flooded plants. Values are mean response ratios with ± 95% confidence intervals (CI). Values >1 and <1 indicate positive and negative responses, respectively. Effects are considered significant when CI does not overlap 1.



802

803 **Fig. 2** The impact of flooding on vegetative growth rate, seed cotton yield and yield
804 components of cotton plants grown on grey vertosol and black vertosol under the four climate
805 treatments. Response ratios were calculated from comparisons between well-watered plants
806 and flooded plants. Values are mean response ratios with ± 95% confidence intervals (CI).
807 Values >1 and <1 indicate positive and negative responses, respectively. Effects are
808 considered significant when CI does not overlap 1.

809

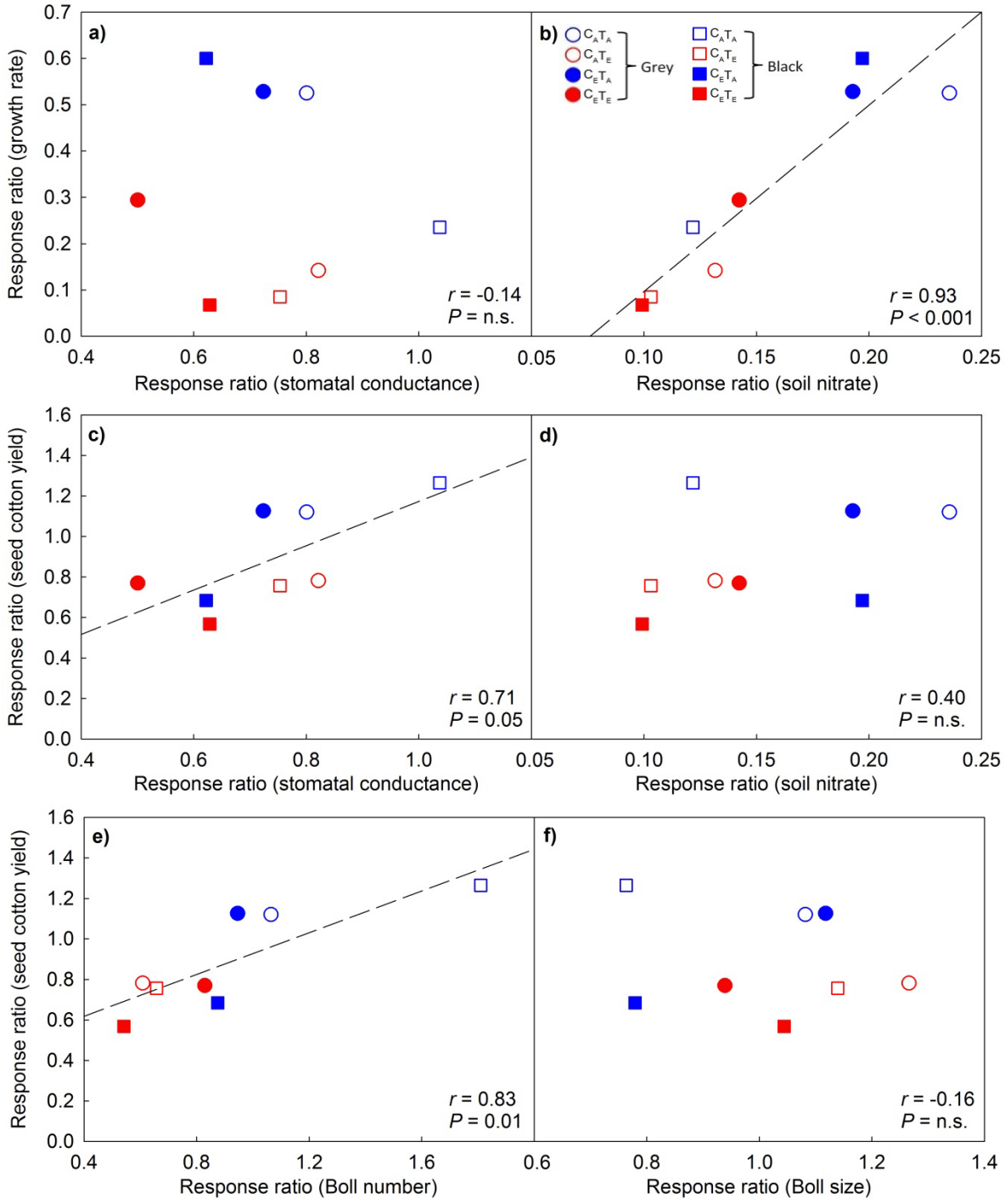


Fig. 3 Relationship between the flooding impact on post-flooding growth rate and stomatal conductance (a) and soil nitrate (b), seed cotton yield and stomatal conductance (c), soil nitrate (d), boll number (e) and boll size of cotton plants grown on grey vertosol and black vertosol under the four climate treatments.

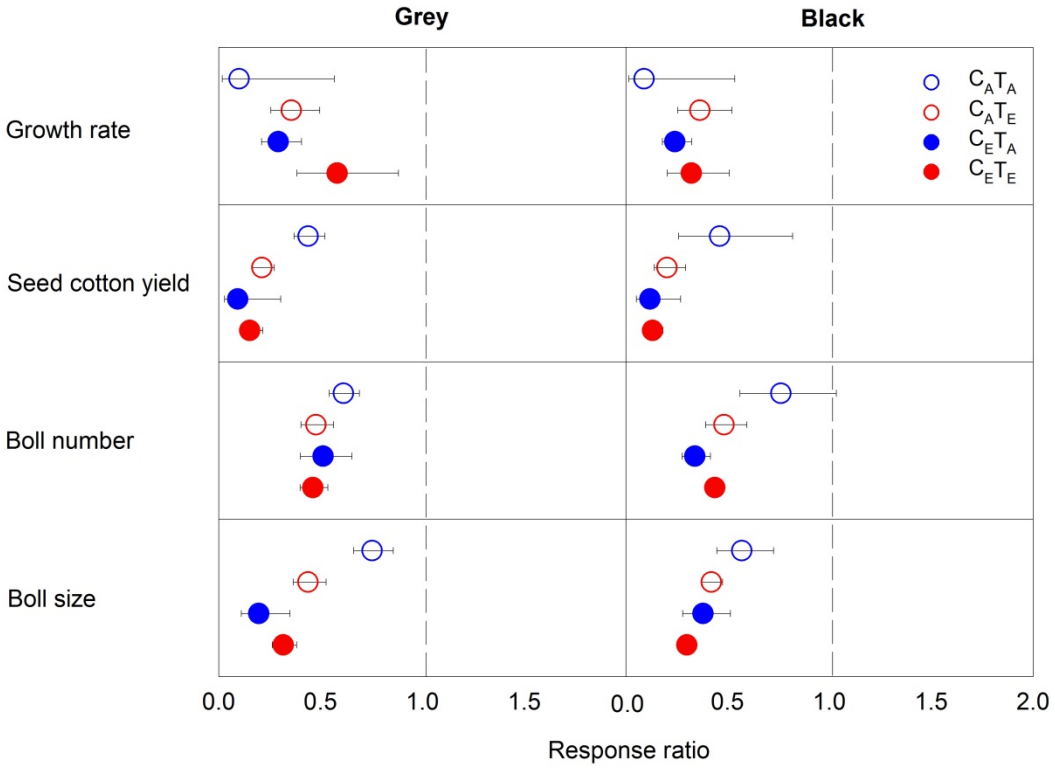
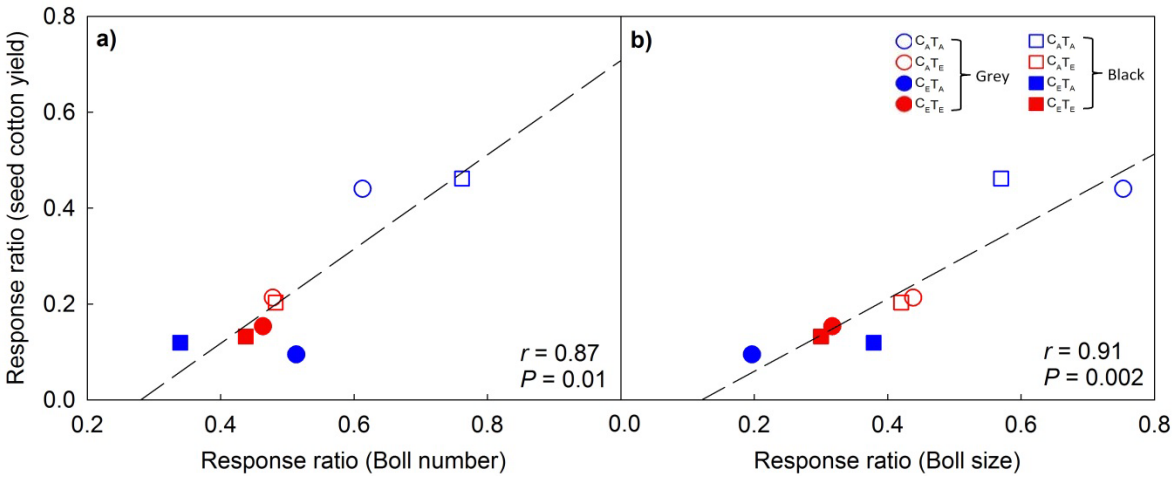


Fig. 4 The impact of drought on vegetative growth rate during the drought treatment, seed cotton yield and yield components of cotton plants grown on grey vertosol and black vertosol under the four climate treatments. Response ratios were calculated from comparisons between well-watered plants and drought plants. Values are mean response ratios with ± 95% confidence intervals (CI). Values >1 and <1 indicate positive and negative responses, respectively. Effects are considered significant when CI does not overlap 1.



823

824 **Fig. 5** Relationship between the drought impact on seed cotton yield and boll number (a) and
825 boll size (b) of cotton plants grown on grey vertosol and black vertosol under the four climate
826 treatments.

Supplementary information

Table S1 Physical and chemical characteristics of top-soil (0-20cm) and sub-soil (20-40cm) of grey vertosol and black vertosol before planting (before fertiliser application). Values are means with standard errors in parenthesis. The results of analysis of variance is also presented showing probability values with significant values (P<0.05) in bold, marginally significant values (P<0.1) in italic and non-significant results (P>0.1) as n.s.

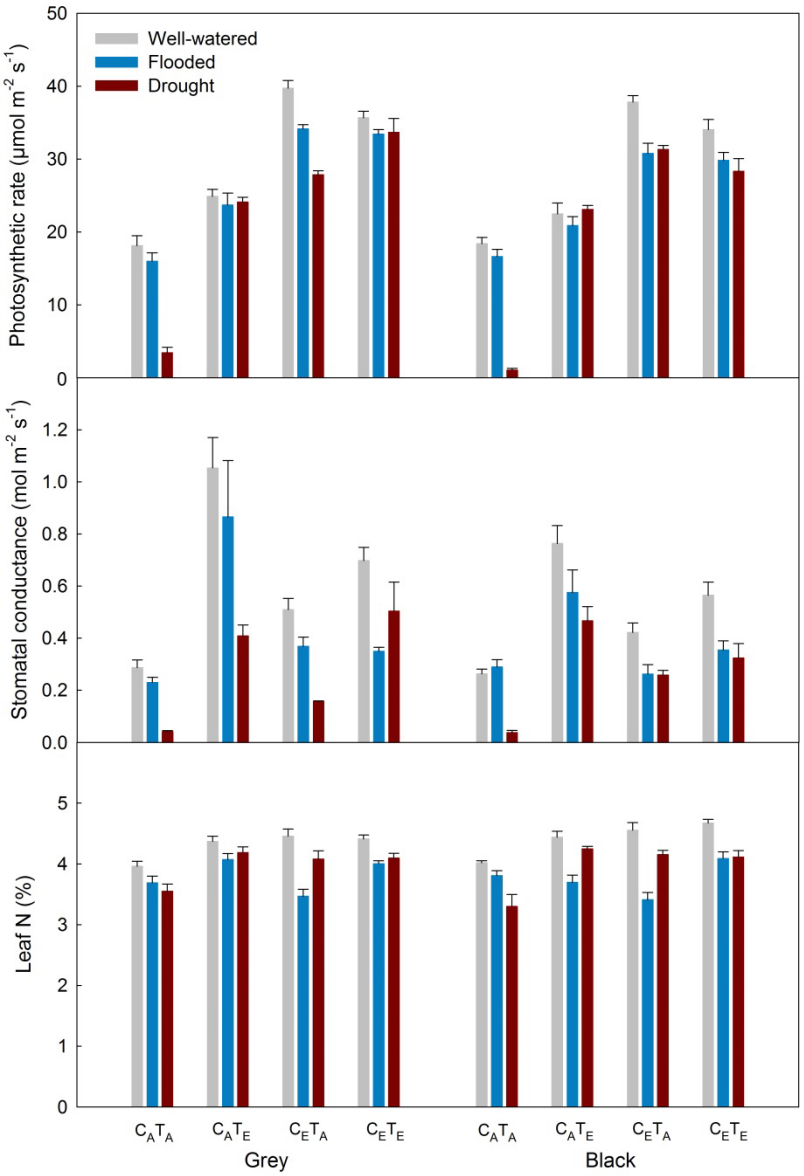
	Grey vertosol		Black vertosol		*P* values		
	Top-soil	Sub-soil	Top-soil	Sub-soil	Soil	Depth	S x D
Particle size							
% Clay	44.0 (2.0)	48.7 (0.7)	47.0 (4.4)	54.3 (0.9)	n.s.	**0.04**	n.s.
% Silt	22.7 (1.3)	20.3 (0.3)	7.7 (2.7)	9.7 (0.3)	**<0.0001**	n.s.	n.s.
% Sand (Fine)	26.0 (2.1)	26.7 (1.3)	30.7 (0.3)	23.7 (0.3)	n.s.	**0.04**	**0.02**
% Sand (Coarse)	7.3 (1.5)	4.7 (0.3)	15.3 (2.2)	12.7 (1.3)	**0.001**	n.s.	n.s.
Chemical properties							
pH (Water)	7.9 (0.07)	8.3 (0.06)	8.5 (0.10)	8.8 (0.03)	**<0.0001**	**0.001**	n.s.
EC (dS/m)	0.1 (0.01)	0.1 (0.00)	0.2 (0.00)	0.2 (0.01)	**<0.0001**	**0.001**	**0.002**
ECSE (dS/m)	0.6 (0.03)	0.9 (0.03)	1.2 (0.00)	1.2 (0.03)	**<0.0001**	**0.0004**	**0.002**
PBI	88.0 (3.61)	96.3 (3.67)	160.0 (10)	173.3 (6.67)	**<0.0001**	n.s.	n.s.
CEC	29.2 (1.08)	34.4 (0.76)	53.7 (2.55)	56.5 (1.07)	**<0.0001**	**0.03**	n.s.
Macronutrients							
Nitrate (mg/kg)	16.3 (3.4)	10.4 (1.7)	23.3 (2.9)	15.3 (0.9)	**0.04**	**0.02**	n.s.
Ammonium (mg/kg)	4.2 (2.4)	1.4 (0.7)	3.5 (1.2)	2.2 (0.8)	n.s.	n.s.	n.s.
Potassium (%)	4.3 (0.24)	2.5 (0.25)	1.2 (0.35)	0.6 (0.04)	**<0.0001**	**0.001**	**0.04**
Potassium (cmol(+)/kg)	1.3 (0.03)	0.9 (0.06)	0.6 (0.17)	0.4 (0.03)	**0.0003**	**0.01**	n.s.
Available potassium (mg/kg)	490 (5.8)	333 (23.3)	250 (69.3)	140 (11.6)	**0.0004**	**0.01**	n.s.
Calcium (%)	70.7 (0.67)	74.7 (0.88)	70.3 (2.33)	63.3 (2.40)	**0.01**	n.s.	**0.01**
Calcium (cmol(+)/kg)	20.7 (0.88)	25.7 (0.88)	37.7 (0.88)	35.7 (0.88)	**<0.0001**	n.s.	**0.004**
Magnesium (%)	24.7 (0.33)	22.3 (0.88)	27.3 (2.60)	34.7 (1.86)	**0.002**	n.s.	**0.02**
Magnesium (cmol(+)/kg)	7.2 (0.24)	7.7 (0.07)	15.0 (2.08)	19.7 (1.33)	**<0.0001**	*0.07*	n.s.
Phosphate (mg/kg)	65.7 (1.2)	40.3 (3.0)	19.3 (5.8)	7.7 (0.3)	**<0.0001**	**0.001**	*0.07*
Sulfate (mg/kg)	3.4 (0.4)	2.9 (0.1)	12.0 (1.0)	10.7 (1.4)	**<0.0001**	n.s.	n.s.
Micronutrients							
Iron (mg/kg)	11.7 (0.4)	9.3 (0.9)	9.3 (0.1)	8.6 (0.2)	**0.01**	**0.01**	n.s.
Manganese (mg/kg)	6.8 (0.4)	5.3 (0.7)	4.4 (1.0)	2.9 (0.2)	**0.01**	**0.05**	n.s.
Zinc (mg/kg)	0.4 (0.06)	0.2 (0.05)	0.5 (0.20)	0.1 (0.00)	n.s.	**0.02**	n.s.
Copper (mg/kg)	1.3 (0.1)	1.3 (0.1)	1.4 (0.0)	1.5 (0.0)	**0.02**	n.s.	n.s.
Boron (mg/kg)	1.1 (0.0)	1.1 (0.0)	2.2 (0.6)	3.3 (0.6)	**0.003**	n.s.	n.s.
Chloride (mg/kg)	<10 (0.0)	<10 (0.0)	13.3 (1.2)	15.3 (1.9)	**0.004**	n.s.	n.s.
Others							
Organic carbon (%)	1.1 (0.0)	0.9 (0.02)	1.1 (0.16)	0.8 (0.05)	n.s.	**0.02**	n.s.
Sodium (%)	0.3 (0.02)	0.7 (0.06)	0.8 (0.06)	1.5 (0.34)	**0.01**	**0.02**	n.s.
Sodium (cmol(+)/kg)	0.1 (0.03)	0.2 (0.02)	0.4 (0.04)	0.8 (0.21)	**0.002**	**0.02**	n.s.
Water holding capacity (%)	55.2 (2.9)	33.1 (1.8)	71.3 (1.6)	42.8 (1.0)	**0.001**	**0.002**	n.s.

EC=electrical conductivity, EC_{SE}=electrical conductivity of saturated soil extract, PBI=phosphorus buffer index, CEC=cation exchange capacity.

836 **Table S2** Results of analysis of variance (ANOVA) showing the effect of sampling (pre- and
837 post-extreme weather treatment), soil, CO_2 and temperature on nitrate concentrations of well-
838 watered control soils and flooded/drought soils planted with cotton under the four climate
839 change treatments. Flooding effect was examined by comparing changes in nitrate
840 concentrations at pre-flooding treatment (at early flowering) and post-flooding treatment (at 7
841 days after the end of flooding period) for well-watered and flooded soils. Drought effect was
842 examined by comparing changes in nitrate concentrations at pre-drought treatment (at early
843 flowering) and post-drought treatment (at harvest) for well-watered and drought soils. Values
844 are probability with significant results (P<0.05) shown in bold, marginally significant results
845 (P<0.1) in italic and non-significant results (P>0.1) as n.s.

846

	Flooding effect		Drought effect	
	Well-watered	Flooded	Well-watered	Drought
Sampling (pre vs post)				
Sampling	**0.001**	**<0.0001**	**<0.0001**	n.s.
Sampling x Soil	n.s.	*0.09*	**<0.0001**	n.s.
Sampling x CO_2	n.s.	n.s.	n.s.	n.s.
Sampling x Temp	n.s.	**<0.0001**	**0.001**	n.s.
Sampling x Soil x CO_2	n.s.	n.s.	n.s.	n.s.
Sampling x Soil x Temp	n.s.	n.s.	n.s.	*0.08*
Sampling x CO_2 x Temp	n.s.	n.s.	*0.06*	**0.01**
Sampling x Soil x CO_2 x Temp	n.s.	n.s.	n.s.	n.s.
Others				
Soil	**<0.0001**	*0.09*	**<0.0001**	**0.001**
CO_2	n.s.	n.s.	n.s.	n.s.
Temp	**<0.0001**	**<0.0001**	**0.0004**	**<0.0001**
Soil x CO_2	n.s.	n.s.	n.s.	*0.08*
Soil x Temp	n.s.	n.s.	n.s.	n.s.
CO_2 x Temp	n.s.	n.s.	n.s.	**0.03**
Soil x CO_2 x Temp	n.s.	n.s.	n.s.	n.s.

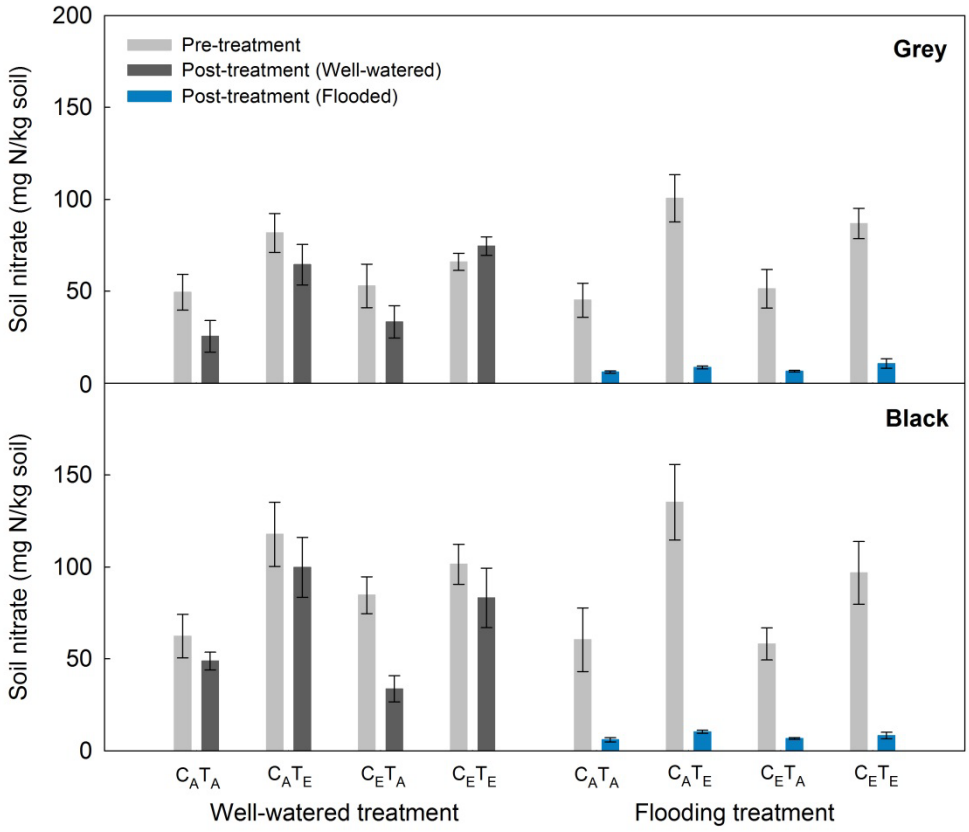


847

848 **Fig. S1** Photosynthetic rate, stomatal conductance and leaf N concentrations of well-watered,

849 flooded and drought cotton plants grown on grey vertosol and black vertosol under the four

850 climate treatments measured at the end of flooding and early drought period.



851

852 **Fig. S2** Soil nitrate concentrations of grey vertosol and black vertosol planted with cotton

853 under climate treatments at pre-water treatment (at early flowering) and post-water treatment

854 (at 7 days after the end of flooding period) for well-watered and flooded soils.

855

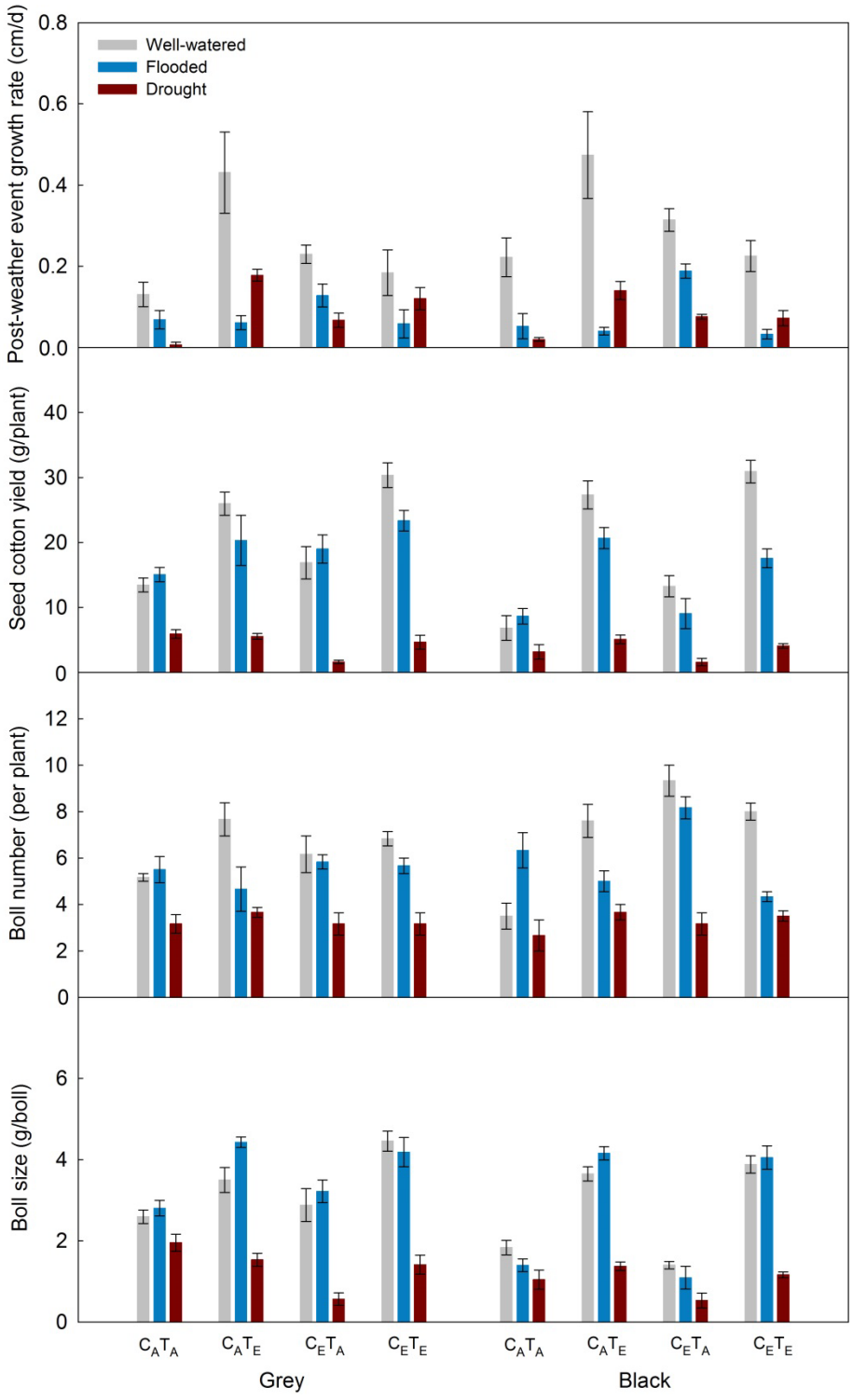
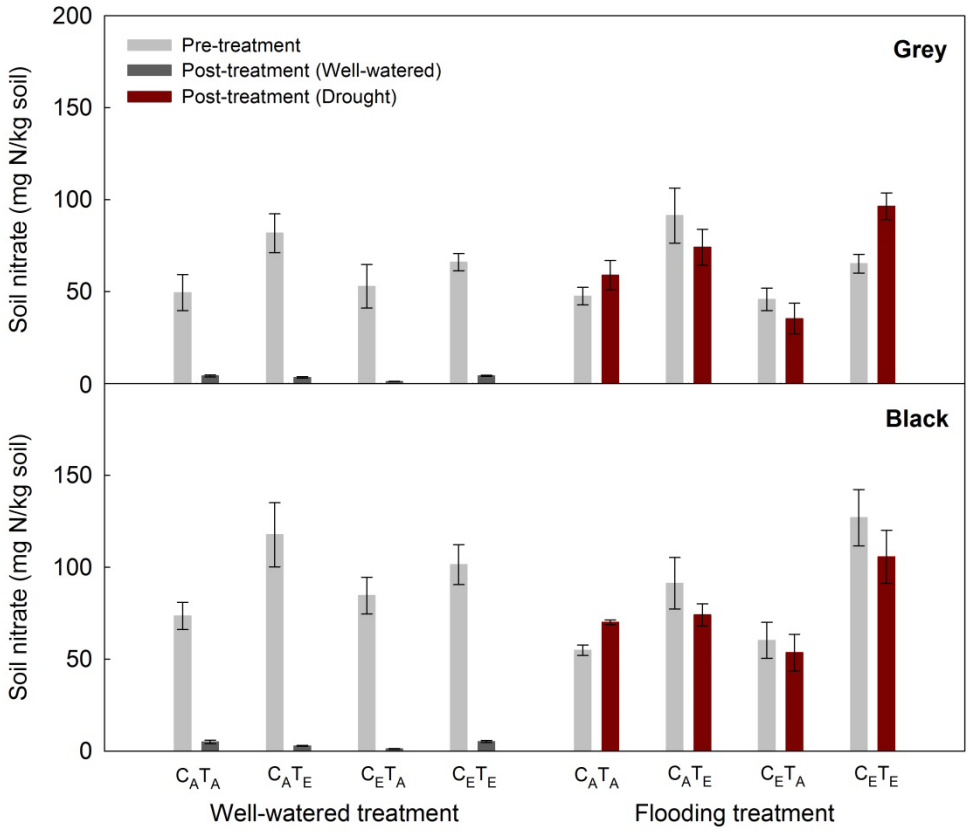
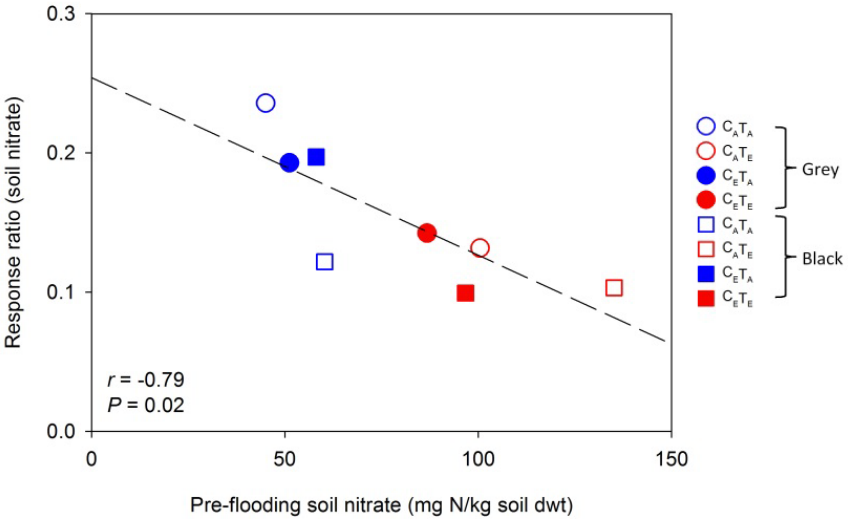


Fig. S3 Post-flooding/drought vegetative growth rate, seed cotton yield, boll number and boll size of well-watered and flooded/drought cotton plants grown on grey vertosol and black vertosol under the four climate treatments.



861

862 **Fig. S4** Soil nitrate concentrations of grey vertosol and black vertosol planted with cotton
863 under climate treatments at pre-water treatment (at early flowering) and post-water treatment
864 (at harvest) for well-watered and drought soils.



865

866 **Fig. S5** The relationship between response ratio of soil nitrate to the flooding treatment and

867 soil nitrate concentrations at pre-flooding treatment.