



# 1 Investigating the impacts of biochar on water fluxes in 2 tropical agriculture using stable isotopes

3 Benjamin M. C. Fischer<sup>1,2,3</sup>, Laura Morillas<sup>4</sup>, Johanna Rojas Conejo<sup>5</sup>, Ricardo Sánchez-Murillo<sup>6</sup>,  
4 Andrea Suárez Serrano<sup>5</sup>, Jay Frentress<sup>7, 8</sup>, Chih-Hsin Cheng<sup>9</sup>, Monica Garcia<sup>10</sup>, Stefano Manzoni<sup>1,3</sup>,  
5 Mark S. Johnson<sup>11,12</sup>, and Steve W. Lyon<sup>1,3,13</sup>

6 [1] Department of Physical Geography, Stockholm University, Stockholm, Sweden.

7 [2] Department of Earth Sciences, Uppsala University, Uppsala, Sweden

8 [3] Bolin Centre for Climate Research, Stockholm University, Stockholm, Sweden

9 [4] Centre for Sustainable Food Systems, The University of British Columbia, Vancouver, British  
10 Columbia V6T 1Z4, Canada

11 [5] Water Resources Center for Central America and the Caribbean (HIDROCEC-UNA),  
12 Universidad Nacional de Costa Rica, Guanacaste,

13 [6] Stable Isotopes Research Group and Water Resources Management Laboratory, Universidad  
14 Nacional, Heredia, Costa Rica

15 [7] Free University of Bolzano, Italy

16 [8] Water Resources, Ramboll Sverige AB, Stockholm, Sweden

17 [9] School of Forestry and Resource Conservation, National Taiwan University, Taipei, Taiwan

18 [10] Department of Environmental Engineering, Technical University of Denmark, 2800 Kgs.  
19 Lyngby, Denmark

20 [11] Department of Earth, Ocean and Atmospheric Sciences, The University of British Columbia,  
21 Vancouver, British Columbia V6T 1Z4 Canada

22 [12] Institute for Resources, Environment and Sustainability, The University of British Columbia,  
23 Vancouver, British Columbia V6T 1Z4, Canada

24 [13] School of Environment and Natural Resources, Ohio State University, Ohio, USA

25 *Correspondence to:* B. M. C. Fischer (benjamin.fischer@natgeo.su.se)

26 **Keywords:** biochar, stable isotopes of water, soil and plant water, soil water retention curves,  
27 plant water uptake



## 28 Abstract

29 Amending soils with biochar, a pyrolyzed organic material, is an emerging practice to potentially  
30 increase plant available water. However, it is not clear (1) to what extent biochar amendments increase  
31 soil water storage relative to non-amended soils and (2) whether plants grown in biochar amended soils  
32 access different pools of water compared to those grown in non-amended soils. To investigate these  
33 questions, we set up an upland rice field experiment in a tropical seasonally dry region in Costa Rica,  
34 with plots treated with two different biochar amendments and control plots, from where we collected  
35 hydrometric and isotopic data ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  from rain, soil, groundwater and rice plants). Our results  
36 show that the soil water retention curves for biochar treated soils shifted, indicating that rice plants had  
37 2 % to 7 % more water available throughout the growing season relative to the control plots. In addition,  
38 we observed a within treatment variability in the soil water retention curves which was in the same  
39 order of magnitude as one would expect from responses due to differences in biochar application rates  
40 or due to differences in biochar typologies. The stable water isotope composition of plant water showed  
41 that the rice plants across all plots preferentially utilized the more variable soil water from the top 20  
42 cm of the soil instead of using the deeper and less variable sources of water. Our results indicated that  
43 rice plants in biochar amended soils could access larger stores of water more consistently and thus could  
44 withstand dry spells of seven extra days relative to rice grown in non-treated soils. Though supplemental  
45 irrigation was required to facilitate plant growth during extended dry periods. Therefore, biochar  
46 amendments can complement, but not necessarily replace, other water management strategies.



## 47 1. Introduction

48 Rainfed agriculture provides food for the growing world population (Fraiture et al., 2009; Fraiture and  
49 Wichelns, 2010) without over-exploiting groundwater resources (Famiglietti, 2014; Jasechko et al.,  
50 2017). However, the spatial and temporal variability of rainfall makes rainfed agriculture vulnerable to  
51 droughts (Fischer et al., 2013) and poses a risk for food security (Fraiture and Wichelns, 2010). Extreme  
52 weather events such as El Niño-Southern Oscillation (ENSO) influence global precipitation patterns  
53 and can bring prolonged dry spells that limit rainfed agriculture production. This is especially true in  
54 the tropics, where rainfall regimes are changing and will continue to change (Feng et al., 2013; Giorgi,  
55 2006; Knutson et al., 2006), leading to more frequent long-term droughts (i.e. periods of more than 10  
56 years with limited rainfall; Hidalgo et al., 2019). Climate projections for the Mesoamerican tropics  
57 suggest (1) decreases in rainfall during the wet season (May-November) of 10 % to 25 %; (2) expansion  
58 of the areas affected by mid-summer droughts; and (3) increases in temperature and extreme dry spells  
59 – all of which result in a net decrease of water availability (Imbach et al., 2018). Such a decrease in  
60 water availability could have significant impacts on rainfed agricultural production and food security  
61 globally. Therefore, to reduce societal exposure to risk, it becomes necessary to make rainfed agriculture  
62 more resilient to current and future climate variability.

63 Agricultural innovations can offer a pathway forward. Common innovations considered capturing rain  
64 (Biazin et al., 2012) or flood water (Castelli et al., 2018), plant and soil water conservation measures  
65 (Enfors and Gordon, 2007; Makurira et al., 2007; Vico and Brunsell, 2018) or introducing  
66 supplementary irrigation (Mutiro et al., 2006). Amending soils with biochar is an emerging practice in  
67 agriculture that could be useful for improving resilience to climate variability (Fischer et al., 2018).  
68 Biochar is a collective name for organic material (e.g. woody or herbaceous vegetation, crop residues  
69 or waste material) that is pyrolyzed in low-tech (Sundberg et al., 2020) or high-tech furnaces (Liu et al.,  
70 2016). The result is a charcoal with different material properties (e.g. particle size, pore structure,  
71 surface area and hydrophobicity) from the original feedstock. Biochar can be applied on the soil surface  
72 or incorporated in the soil where it alters the original soil matrix thereby changing the infiltration  
73 capacity (Blanco-Canqui, 2017; Lim and Spokas, 2018; Sun and Lu, 2014) and creating a multilayer



74 soil profile. The altered soil physical characteristics increase the soil water holding capacity and more  
75 in general the amount of soil water stored at a given soil matric potential (Omondi et al., 2016).  
76 However, despite documented positive effects of biochar amendments on agricultural productivity  
77 (Kätterer et al., 2019; Novak et al., 2016), also negligible or no effects have also been observed (Fischer  
78 et al., 2018; Jeffery et al., 2015, 2017; Nelissen et al., 2015; Reyes-Cabrera et al., 2017). These diverging  
79 findings might be due to different biochar typologies (Fischer et al., 2018), but also to the fact that many  
80 of the available studies are based on laboratory and pot experiments unable to mimic the variety of  
81 processes occurring in agroecosystems at field scale (Agegnehu et al., 2017; Blanco-Canqui, 2017;  
82 Zhang et al., 2016).

83 At the agroecosystem scale, soil water depends not only on the storage characteristics of the soil, but  
84 also on variability of vertical fluxes resulting from rainfall and irrigation, evaporation, leakage and  
85 runoff (Falkenmark, 1997; Rockström, 1999; Vico and Porporato, 2015). Thus, biochar impacts could  
86 manifest themselves across the myriad pathways by which water can move through the soil-plant-  
87 atmosphere continuum. Stable water isotopes can be a powerful tool to study how biochar additions  
88 modify water stores and fluxes in agroecosystems. As part of the water molecule itself, the stable  
89 isotopes of the water ( $^{18}\text{O}$  and  $^2\text{H}$ ) in combination with hydrometric data, are a proven tool to trace flow  
90 pathways of water from rainfall (Fischer et al., 2017b) to evaporation (Benettin et al., 2018; Gonfiantini,  
91 1986), through the (un)saturated zone (Jasechko et al., 2017; Koeniger et al., 2016; Sánchez-Murillo  
92 and Birkel, 2016; Saxena, 1987), catchments (Fischer et al., 2017a; Klaus and McDonnell, 2013) and  
93 more recently in the soil-plant-atmosphere continuum (Allen et al., 2019; Brooks et al., 2010; Dawson  
94 and Ehleringer, 1991; McDonnell, 2014; Penna et al., 2018; Rothfuss and Javaux, 2017; Sprenger et  
95 al., 2016).

96 Root water resembles the isotopic composition from the absorbed soil water from a specific location in  
97 the soil profile (Berry et al., 2018), while, xylem water in the plant stem represents the isotopic  
98 composition of all the soil profile within the root network (Dawson and Ehleringer, 1991; Penna et al.,  
99 2018). To identify which water stores are available to vegetation, various potential water sources -e.g.,  
100 rain (Fischer et al., 2019; Prechsl et al., 2014), soil water (Sprenger et al., 2015) and groundwater (Beyer



101 et al., 2016) are collected and analyzed for their stable isotope composition. The stable isotope  
102 composition of the different collected water has allowed researchers to develop new theories whether  
103 plants use soil-bound vs. mobile soil water pools (Brooks et al., 2010) or consume water from specific  
104 soil layers that change over time (Berry et al., 2018; Beyer et al., 2016; Goldsmith et al., 2012; Koeniger  
105 et al., 2016; Muñoz-Villers et al., 2020). Amin et al (2020) compared results from different stable  
106 isotopes studies performed in natural catchments and deduced that plants in dry tropical climates  
107 consume water from soil layers deeper than 50 cm. Beyond investigating natural ecosystems, stable  
108 isotopes offer opportunities to study the sources of water in agroecosystems and quantifying the  
109 efficiency of agricultural innovations.

110 Despite that stable isotopes have been used to a lesser extent in agricultural systems than in natural  
111 systems to investigate plant water sources (Penna et al., 2020), there are successful studies done in  
112 coffee (Muñoz-Villers et al., 2020), maize, wheat (Stumpp et al., 2009) and rice cultures  
113 (Mahindawansha et al., 2018; Shen et al., 2015). In the case of rice, Shen et al. (2015) observed that  
114 flooded rice consumed soil water from 0-15 cm deep, while Mahindawansha et al. (2018) found that  
115 upland rice in dry conditions mostly consumed soil water from up to 50 cm deep except during the  
116 maturing stage, when plants shifted to use water from the 10-30 cm soil depth. Based on this evidence,  
117 we hypothesized that amending biochar into the top 10-30 cm of the soil, as it is commonly done, could  
118 increase resilience to climate variability of upland rice in the tropics.

119 Our study seeks to test this hypothesis explicitly in a field experiment with upland rice in soil amended  
120 with two different biochar types vs. a control treatment (no biochar) in a tropical seasonally dry region  
121 in northwestern Costa Rica. We use a combination of hydrometric and isotopic data ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  of  
122 rain, soil, groundwater and rice plants) to target 1) to what extent do biochar amendments increase the  
123 soil water storage relative to non-amended soils during the growing period of rice? and 2) do rice plants  
124 grown in biochar amended soils access different pools of water compared to those grown in non-  
125 amended soils?



## 126 2. Study site and experimental design

### 127 2.1 Study site

128 The biochar rice experiment was conducted at the Enrique Jiménez Núñez Experimental Station  
129 (EEEJN) from the Instituto Nacional de Innovación y Transferencia en Tecnología Agropecuaria  
130 (INTA) near the city of Cañas in the Guanacaste province of Costa Rica (Figure 1a). Soils at the  
131 experimental site are loamy vertosols (Table A1) typically more than 2 m deep (Diogenes Cubero and  
132 Maria José Elizondo, 2014). Guanacaste province is part of the Dry Corridor of Central America  
133 (Sánchez-Murillo et al., 2020) and characterized by a seasonally dry tropical climate with marked dry  
134 and wet seasons and limited temperature variability over a year (Birkel et al., 2017). The annual average  
135 temperature at EEEJN-INTA is 27.4 °C. The dry season typically spans from mid-November to April  
136 with virtually no rainfall. Wet season precipitation exhibits a bi-modal distribution dominated by the  
137 influence of the Intertropical Convergence Zone with peaks occurring in May/June and  
138 September/October. The moderate dry period between these two peaks is usually referred to as the mid-  
139 summer drought (Magaña et al., 1999). The average annual rainfall in the area is approximately 1,547  
140  $\pm 473$  mm yr<sup>-1</sup> based on a 100-year observation record from a meteorological station ~10 km distance  
141 of the experimental site (Figure 2a). The annual average actual evapotranspiration is around 1,100 mm  
142 yr<sup>-1</sup> (Sánchez-Murillo and Birkel, 2016). In the last century, 70 % of the driest years in this region (i.e.,  
143 years with less than 1,153 mm yr<sup>-1</sup> of rainfall, which is the 25<sup>th</sup> percentile of annual rainfall), occurred  
144 during warm ENSO years. Based on the Standardized Precipitation Index (SPI; Naresh Kumar et al.,  
145 2009), recurrent below average rainfall has been observed in this region since 1960s (Figure 2b) with a  
146 significant periodicity of severe (SPI<-1.5) and sustained droughts of around 10 years (Hidalgo et al.,  
147 2019).

### 148 2.2 Experimental design

149 For this experiment two types of biochar were tested to represent a more locally-produced biochar and  
150 a more industrially-processed biochar, respectively. Biochar 1 (BC1) was made of locally sourced  
151 bamboo (*Guadua angustifolia*) and produced at the Costa Rica Institute of Technology (TEC, Cartago,  
152 CR; Table A1). The feedstock consisted of wood pieces up to 30 cm in length from construction waste,  
153 which were pyrolyzed using a pyrolysis furnace under a temperature ranging 450-480 °C. A second



154 biochar, biochar 2 (BC2) was produced from sugarcane filter cake collected from the Huwei Sugar Mill  
155 (Taiwan Sugar Corporation, Taipei, Taiwan). For the industrial processing of BC2, the filter cake was  
156 pelletized into pellets with 7.6 mm diameter and 20-30 mm long and pyrolyzed at 600 °C under a  
157 controlled nitrogen-rich atmosphere. Pyrolyzed pellets were crushed and sieved to  $\leq 2$  mm prior to field  
158 application.

159 Within the EEEJN-INTA experimental station, an area of approximately 160 m<sup>2</sup> was delineated and  
160 divided into three sections of 40 m<sup>2</sup> each for treatments. The three different treatment sections, one for  
161 each biochar type (BC1 and BC2) and a control treatment (C) with no biochar added, were subdivided  
162 into three plots each to create three independent monitoring replicates of each treatment (Figure 1b).  
163 The BC1 and C plots were 7 m<sup>2</sup> each (5 m long x 1.4 m wide) in area while the BC2 plots were 3.5 m<sup>2</sup>  
164 each (2.5 m long x 1.4 m wide) in area. This difference in areas between biochar treatments was due to  
165 a lower amount of BC2 being available (shortage of feedstock at the biochar supplier) while securing a  
166 similar application rate (1 kg m<sup>-2</sup>) across biochar treatments. For the biochar treatments, the  $\leq 2$  mm  
167 particle size biochar was mechanically worked into the top 20 cm of the field prior to planting. It should  
168 be noted that BC1 was incorporated into the field about six months earlier than BC2 due to logistical  
169 constraints. BC1 addition was followed by an irrigated melon crop on the treated plot prior to our rice  
170 experiment.

171 After the treatment sections were prepared, an upland rice variety Palmar 18 (*Oryza sativa* L.) was sown  
172 simultaneously on the three sections on 18 July 2018 indicating the start of the experiment. For sowing,  
173 5 cm deep longitudinal rills were created in all plots with a spacing of 25 cm. In each rill, rice seeds  
174 were sown by hand of about 1 seed cm<sup>-1</sup>, equivalent to 20 g m<sup>-2</sup>. After sowing, the rills were covered  
175 with soil. During the growing season, rice plants were primarily rainfed which is the standard procedure  
176 for the predominant upland rice grown in the region. In some cases, where water sources for irrigation  
177 are available, sporadic support irrigation is used by local farmers to support crops and avoid wither.  
178 Due to prolonged dry spells that occurred during the study period, all experimental plots were irrigated  
179 with 7 L m<sup>-2</sup> on July 22 and August 25 to assist germination and avoid plant drought damage  
180 respectively on each date. Following typical regional crop management practices, fertilizer (100 g m<sup>-2</sup>



181 consisting of 10 % N, 30 % P, 10 % K in combination of 11 ml MEGAFOL® and 11 g magnesium  
182 sulphate) and insecticide/herbicide (2 ml Muralla® Delta; 50 ml Garlon and 20 ml bispiribac sodium)  
183 were applied to all experimental plots using 2 L m<sup>-2</sup> irrigation water on each treatment date (August 10,  
184 September 6, and November 5) to support plant growth. At monthly intervals, manual weed control was  
185 performed in all plots. Harvest took place on 21 November 2018 and indicated the end of the  
186 experiment.

## 187 2.3 Instrumentation and sampling

### 188 2.3.1 Meteorological and hydrometric observations

189 A meteorological station (Vaisala WT520; 1.5 m height) was used to continuously monitor  
190 precipitation, wind speed and direction, air temperature, relative humidity and atmospheric pressure at  
191 the site during the entire study period (Figure 1b and c). Each experimental plot was instrumented with  
192 one sensor installed at 15 cm depth to monitor volumetric soil water content, soil electrical conductivity  
193 and soil temperature (model GS3, Decagon Devices, Inc., Pullman USA), and one additional sensor at  
194 same depth to monitor soil matric potential and soil temperature (model MPS6, Decagon Devices). Both  
195 sensors were between rice rows in each plot (Figure 1c). Additionally, soil samples were collected at  
196 15 cm soil depth from each plot at the beginning of the experiment and after harvest to determine the  
197 gravimetric soil moisture content. These data were used to perform a two-point calibration of the  
198 volumetric soil water content measurements derived from the sensors at each plot during the entire time  
199 series.

200 Depth of groundwater levels was measured using a groundwater well (groundwater well A) installed  
201 between the BC1 and C treatment sections (Figure 1b). The well consisted of screened PVC tube  
202 instrumented with a sensor to continuously monitor groundwater level, electrical conductivity and water  
203 temperature (model CTD, Decagon Devices). Manual water level measurements were also made every  
204 other week during the study period to calibrate the continuous sensor data. All sensors were connected  
205 to a datalogger (Campbell CR1000 logger and an AM416 Relay Multiplexer) and programmed to record  
206 at 30-minute intervals.





207 2.3.2 Water and plant sample collection

208 Water samples from different pools of water (namely, rainwater, irrigation water, soil water and  
209 groundwater) were collected for isotopic analysis. Rainwater was collected using a funnel connected  
210 with tubing to a PET bottle (1.5 liter) wrapped in aluminum foil similar to Prechsl et al. (2014). In each  
211 plot, lysimeters (Soilmoisture equipment corp., Santa Barbara, USA) were installed in the soil reaching  
212 to 15 cm and 40 cm soil depth respectively to sample soil water. Groundwater samples were collected  
213 from a second groundwater well (groundwater well B) installed near the BC2 treatment section (Figure  
214 1b).

215 Rainwater samples were collected daily at 7:00 AM. Water from additional application sources such as  
216 irrigation (to supplement rainfall) and fertilizer/pesticide/herbicide applications were sampled as a grab  
217 sample using a PE bottle during each application. Soil water and groundwater samples were collected  
218 approximately biweekly (every other week) after plant germination from 31 July 2018 until the harvest  
219 day on 21 November 2018, resulting in 11 sampling days. Soil water was collected from lysimeters by  
220 applying an 800-mbar vacuum for 2 minutes. Groundwater was sampled by purging the well and  
221 waiting 1 hour before collecting the groundwater sample. All water samples were collected in 30 ml PE  
222 bottles, which were capped and sealed with Parafilm® for transport and cold storage (5 °C) until  
223 analysis. At the end of each sampling day, all excess water from all sampler tubing, bottles, and suction  
224 lysimeters was removed to prevent inter-sampling contamination.

225 Plant material from the rice plants was also collected on each of the 11 biweekly sampling dates at  
226 around 12:00 noon. For plant material sampling, six rice plants were randomly selected within each  
227 plot. The plant height from the soil to the plant tip was measured and recorded before sampling. To  
228 avoid loss of biomass on sampled plants, the plants were extracted using a small knife which was  
229 carefully wiggled into the soil. The roots, stems and leaves of the extracted plants were separated  
230 immediately and transferred into double re-sealable zipper storage bag. To minimize post-sampling  
231 transpiration, storage bags were directly placed in a cooler with ice. All plant material was stored in the  
232 laboratory freezer (-80 °C) before extracting the plant water for isotopic analysis.



### 233 3. Laboratory methods and data analysis

#### 234 3.1 Plant water extraction

235 Plant water was extracted from the stem (xylem water) of the different rice plants to infer which sources  
236 of water the rice plants used. We used the cryogenic vacuum extraction technique described by  
237 Koeniger et al., (2011) to extract the plant water for stable isotope analysis. The method uses a heated  
238 vial and a cold trap vial (Exetainer® vial with standard cap and rubber septum, Labco Ltd, Lampeter,  
239 United Kingdom) connected with stainless-steel capillary tubing. About 3 g of plant material from the  
240 rice stem was placed in the heated vial before the system was evacuated to 85 kPa with a vacuum hand  
241 pump (Mityvac). The heated vial was heated for 1 hour at 100°C using a test tube heater (HI839800  
242 COD Test Tube Heater; Hanna instruments) while the cold trap vial rested in a Dewar flask containing  
243 liquid nitrogen at about -196°C. After the extraction was stopped, the cold trap vial was sealed with  
244 Parafilm and left to thaw. After thawing, the extracted liquid water was pipetted into 2 ml vials (32 x  
245 11.6 mm screw neck vials with cap and PTFE/silicone/PTFE septa) and stored cold (5 °C) until stable  
246 isotope analysis. On average 86±5 % plant water was extracted from xylem.

#### 247 3.2 Isotope analysis

248 All non-plant water samples were filtered (0.45 µm filter 13 mm PTFE Syringe Filter, Fisher scientific)  
249 and pipetted in vials (2 mL into a 1.5 mL 32 × 11.6 mm screw neck vials with cap and  
250 PTFE/silicone/PTFE septa) prior to analysis. Water stable isotopes analysis was conducted at the Stable  
251 Isotopes Research Group facilities of the Universidad Nacional of Costa Rica using a water isotope  
252 analyzer LWIA-45P (Los Gatos Research Inc., USA). All data were normalized and corrected for drift  
253 and memory effects. The analytical long-term error was ± 0.5 (‰) (1σ) for δ<sup>2</sup>H and ± 0.1 (‰) (1σ) for  
254 δ<sup>18</sup>O.

255 Plant water stable isotopes analysis was conducted at the Swedish University of Agricultural Sciences  
256 (SLU) Stable Isotope Laboratory (SSIL) in Umeå using an Isotope Ratio Mass Spectrometer (TC/EA-  
257 IRMS; DeltaV Advantage, Thermo Fisher Scientific, Bremen, Germany; High Temperature Conversion  
258 Elemental Analyzer, Thermo Fisher Scientific, Bremen, Germany and an AI 1310 Autosampler,  
259 Thermo Fisher Scientific, Bremen, Germany). All water samples were injected into a glassy carbon



260 reactor containing glassy carbon chips at 1,400°C and converted to H<sub>2</sub> and CO gases which were  
261 separated on a column and analyzed on a mass spectrometer. All data were corrected for drift and  
262 memory. The analytical precision and accuracy were ± 2 (‰) (1σ) for δ<sup>2</sup>H and ± 0.15 (‰) (1σ) for  
263 δ<sup>18</sup>O.

264 All stable isotope compositions are presented as delta notations (δ) in ‰, relating the ratios (R) of  
265 <sup>18</sup>O/<sup>16</sup>O and <sup>2</sup>H/<sup>1</sup>H, relative to the VSMOW-SLAP scale. The Global Meteoric Water Line (GMWL)  
266 was defined as δ<sup>2</sup>H = 8 · δ<sup>18</sup>O + 10 by Craig (1961). The Local Meteoric Water Line (LMWL) was derived  
267 as δ<sup>2</sup>H = 7.4 · δ<sup>18</sup>O + 5.5 using the long term isotopic data from the rain sampler at the Water Resources  
268 Center for Central America and the Caribbean (Sánchez-Murillo et al., in review) located ~50 km  
269 distance of the experimental site. In addition, the deuterium excess (*d*-excess) was defined as *d*-excess  
270 = δ<sup>2</sup>H – 8 · δ<sup>18</sup>O (Dansgaard, 1964).

### 271 3.3 Evapotranspiration and soil water retention impacts

272 Daily evapotranspiration rates (*ET*) from the experimental area were estimated by the crop coefficient  
273 method ( $ET = K_c \cdot ET_{ref}$ ) or FAO56 Penman-Monteith method (Allen et al., 1998). We used site specific  
274 meteorological observations to estimate daily reference *ET* ( $ET_{ref}$ ) and experimentally derived crop  
275 coefficient ( $K_c$ ) values for the three different stages of the crop growth (initial, mid-season, and late-  
276 season). Instead of using globally averaged values of  $K_c$  for rice (Allen et al., 1998), we used region-  
277 specific  $K_c$  values experimentally derived from a nearby field experimental site equipped with an Eddy  
278 Covariance (EC) tower where the same variety of upland rice is grown (Morillas et al., 2019). Daily  $K_c$   
279 values from the EC site were derived as the ratio of daily measured *ET* and site-specific  $ET_{ref}$ , and then  
280 averaged for the three stationary crop growth stages ( $K_c$  initial = 0.7,  $K_c$  mid-season = 0.9 and  $K_c$  late  
281 season = 0.5). The length of each crop growth stage was also calibrated for this region by observing the  
282 pattern of daily measured *ET* over the whole growing season (initial ≈ 25 days, development ≈ 20 days,  
283 mid-season ≈ 50 days, late-season ≈ 23 days for an average growing season of 120 days).

284 Field derived 30-minute records of all meteorological and hydrometric observations (precipitation,  
285 volumetric soil water content, soil matric potential and groundwater level) were aggregated to daily  
286 averages. Accumulated precipitation and evapotranspiration were also derived from daily



287 measurements and estimates respectively for the entire experimental period (July 18-November 21).  
288 Average volumetric soil water content and soil matric potential for each treatment (BC1, BC2 and C)  
289 were calculated by averaging the observations in the three replicated plots per treatment.  
290 Treatment specific volumetric soil water content ( $\theta$ ) and soil matric potential ( $\psi$ ) were linked through  
291 soil water retention curves using the Van Genuchten model (Van Genuchten, 1980) (Eq. 1)

$$\theta = \theta_r + \frac{\theta_s - \theta_r}{[1 + (\alpha \psi)^n]^m} \quad (1)$$

292 where  $\theta_r$  [%],  $\alpha$  [-] and  $n$  [-] represent residual, and the fitted scale and shape parameters, respectively;  
293 parameter and  $m = 1 - 1/n$  [-] while saturation soil moisture ( $\theta_s$ ) is based on field observations. To  
294 examine the effect of biochar on soil physical and hydraulic properties, we compared the indicators  $\theta_{WP}$ ;  
295  $\theta_{FC}$  and van Genuchten parameter  $\alpha$  and  $n$  estimated for the biochar amended treatments (BC1 and  
296 BC2) with the same indicators for the unamended treatment (C) using response ratios ( $RR$ ) as in Fischer  
297 et al. (2018). For this study,  $RR$  represents the ratio of the variable of interest in the treatment to the  
298 same property in the control such that  $RR > 1$  or  $R < 1$  indicates that the treatment has a positive or  
299 respectively negative effect.

### 300 3.4 Plant water source estimation

301 The isotopic composition of the water samples was represented in the dual isotope space  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$   
302 to infer which sources of water rice plants consumed. To represent a potential plant water source under  
303 rainfed conditions, the isotope composition of rainfall was considered as the volume weighted isotope  
304 composition of rainfall collected in the two-week period before a given plant water sampling day. Since  
305 residual rainfall can evaporate while in the soil (simplified assumption not accounting of mixing with  
306 pre-event water), the isotopic composition of the residual rainfall for each water sampling day was  
307 estimated following (Gonfiantini, 1986) and (Benettin et al., 2018)

$$\delta_{PR} = (\delta_P - \delta^*)(1 - f_E)^U + \delta^* \quad (2)$$

308 where  $\delta_{PR}$  [‰],  $\delta_P$  [‰], and  $f_E$  [-] represent the isotopic compositions of the residual rainfall, the  
309 volume weighted isotope composition of rainfall collected in the two-week period before a sampling



310 day, and the fraction of rainfall that fell in the two-week period before a sampling day and that has  
 311 evaporated on the sampling day, respectively. The variables  $\delta^*$  [‰] and  $U$  [-] represents the limiting  
 312 isotopic composition and the temporal enrichment slope, which were determined using equation 3 and  
 313 4 respectively

$$\delta^* = \frac{R_H \delta_A + \varepsilon_k + \frac{\varepsilon^+}{\alpha^+}}{R_H - 10^{-3} \left( \varepsilon_k + \frac{\varepsilon^+}{\alpha^+} \right)} \quad (3)$$

$$U = \frac{R_H - 10^{-3} \left( \varepsilon_k + \frac{\varepsilon^+}{\alpha^+} \right)}{1 - R_H \varepsilon_k} \quad (4)$$

314 where  $R_H$  [-] represents the average relative humidity of the two-week period before a sampling day,  $\delta_A$   
 315 [‰] the approximation of the isotopic composition of the atmospheric vapor (equation 5 following  
 316 Gibson et al., (2016)),  $\varepsilon_k$  [‰] the simplified kinetic fractionation factor (Eq. 6) and  $\varepsilon^+$  [‰] and  $\alpha^+$  [-] the  
 317 two equilibrium fractionation factors (Eq. 7 and 8).

$$\delta_A = \frac{\delta_p - \varepsilon^+}{\alpha^+} \quad (5)$$

$$\varepsilon_k = (1 - R_H)(1 - S_{18O \text{ or } 2H})10^3 \quad (6)$$

$$\begin{aligned} & 10^3 \ln(\alpha^+ \delta^{2H}) \\ &= 1158.8 \frac{T^3}{10^9} - 1620.1 \frac{T^2}{10^6} + 794.84 \frac{T}{10^3} - 161.04 \\ &+ 2.9992 \frac{10^9}{T^3} \end{aligned}$$

or

$$10^3 \ln(\alpha^+ \delta^{18O}) = 0.3504 \frac{10^9}{T^3} - 1.6664 \frac{10^6}{T^2} + 6.7123 \frac{10^3}{T} - 7.685 \quad (7)$$

$$\varepsilon^+ = (\alpha^+ - 1)10^3 \quad (8)$$



318 where  $S_{18O} = 0.9755$  and  $S_{2H} = 0.9723$  (Merlivat, 1978) and  $T$  [K] represents the average  
319 temperature of the two-week period before a sampling day. The volume-weighted isotope composition  
320 of rainfall before each sampling day, which was generally near the GMWL and LMWL, and the  
321 corresponding estimated isotopic composition of the residual rainfall, which was generally off the  
322 GMWL and LMWL, provided the start and end point of a theoretical evaporation line in dual isotope  
323 space. Similarly, an evaporation line for the median sampled soil water of a period was developed. Such  
324 evaporation lines map the evolution of the soil water available from residual rainfall or evaporated soil  
325 water for plants to be consumed between sampling days allowing us to track which stores of water the  
326 rice plants interact with across the treatments. In addition, the within treatment variability defined as  
327 difference between the minimum and maximum observed isotopic composition of plant water within a  
328 treatment on any given sampling day were calculated.

## 329 4. Results

### 330 4.1 Hydrometric variability

331 Based on the temporal variability of rainfall, we identified three distinct periods within the overall study  
332 period (Figure 3). Period I (18 July to 20 September) was characterized with alternating wet and dry  
333 days, Period II (20 September to 9 November) presented consistent high daily rainfall inputs, and Period  
334 III (10 November to 21 November) was characterized by a long dry spell ending with rice harvest.  
335 Throughout the study period, daytime air temperatures were around  $26.7^{\circ}\text{C}$  (standard deviation =  $3^{\circ}\text{C}$ )  
336 and evapotranspiration rates on average  $3.1\text{ mm day}^{-1}$  (standard deviation =  $0.7\text{ mm day}^{-1}$ ).

337 During Period I (germination and vegetative phase), the rice in the different plots grew to a height of  
338 50 cm in all experimental plots (standard deviation  $<2.5\text{ cm}$ ). This period was characterized by  
339 intermittent dry and wet spells with accumulated precipitation slightly higher than evapotranspiration  
340 ( $P_{\text{cum}} = 240\text{ mm}$  and  $ET_{\text{cum}} = 191\text{ mm}$  over the 64-day period; Figure 3b). During this period, the  
341 maximum recorded volumetric soil water contents were 40 %, 43 %, and 35 %, and decreased to the  
342 minimum values 30 %, 25 %, and 23 % in the BC1, BC2, and control treatment, respectively (Figure  
343 3c). Regarding soil matric potential ( $\psi$ ) during this period, it surpassed field capacity ( $\psi_{FC} = -0.05$   
344 MPa) with a maximum of  $-0.008\text{ MPa}$  during rain events and decreased to a minimum of  $-0.32\text{ MPa}$



345 observed in all treatments a few days after the third sampling day as a result of the driest spell of Period  
346 I (Figure 3d). Generally, the soil matric potential in the biochar treatments was 0.002 MPa higher than  
347 in the control treatment and never reached the wilting point ( $\psi_{WP} = -1.5$  MPa). The groundwater level  
348 was generally 0.7 m below the surface, rising after sampling day 1 to less than 0.6 m below the surface  
349 before sampling day 4, and to less than 0.5 m below the surface in response to the largest rainfall of  
350 Period I (Figure 3e).

351 During Period II (vegetative and reproductive phase), rice plants attained their maximum heights of  
352 around 100 cm (standard deviation <5 cm), across all three plots (Period II was the wettest period with  
353 15 out of 42 rain days with intensities greater than 20 mm d<sup>-1</sup> of rainfall (and one day with 93 mm d<sup>-1</sup>)  
354 (Figure 3a and b). This wet condition lead to cumulative precipitation being much greater than  
355 cumulative evapotranspiration during the period ( $P_{cum} = 570$  mm and  $ET_{cum} = 147$  mm; over the 50-day  
356 period). The volumetric soil water content over Period II was generally higher than in Period I, with  
357 multiple peaks driven by rainfall events and then a decrease towards the end of the period. After rain  
358 events, the volumetric soil water contents rose from 28 % to 40 %, from 24 % to 45 %, and from 23 %  
359 to 36 % in BC1, BC2, and control treatment, respectively. Soil moisture then decreased in the three  
360 treatments to 32 %, 38 %, and 32 % during the last part of the period. The soil matric potential during  
361 Period II remained largely above field capacity except by the end of the period when it decreased (before  
362 sampling day 8) to a minimum of -0.23 MPa in BC1 and -0.16 MPa in BC2 and C. The groundwater  
363 level increased multiple times during this period from 0.7 m below the surface to reach the soil surface  
364 the rainiest day of the study period. Between Sampling days 6 and 7, groundwater level remained no  
365 lower than 0.4 m below the surface.

366 During the final experimental period, Period III (ripening phase), rice plants maintained their maximum  
367 height acquired by the end of Period II. This period was characterized by a 12 day long dry spell such  
368 that cumulative evapotranspiration was greater than cumulative precipitation ( $P_{cum} = 2$  mm and  $ET_{cum} =$   
369 63 mm; 12-day period). By the end of Period III, the volumetric soil water content in the BC1 and BC2  
370 treatments converged to the lowest observed value of ~21 %. It is relevant that the control treatment  
371 reached this value about seven days earlier than the biochar amended plots, and the control plots



372 continued decreasing to reach a minimum value of 18 % (Figure 3c). The soil matric potential for all  
373 three plots decreased from above the field capacity to near the wilting point by the end of Period III.  
374 The groundwater level also decreased from 0.4 m to 0.8 m below the surface (i.e. the sampling well  
375 went dry).

#### 376 4.2 Impact of biochar on soil water retention curves

377 The soil water retention curves from the different treatments showed different shapes and different  
378 volumetric water content at a given soil matric potential (Figure 4). Comparing the different soil water  
379 retention curves across the different plots of the different treatments shows a within treatment  
380 variability, i.e., range of different volumetric soil moisture contents relative to the observed soil matric  
381 potentials (Figure 4). Comparing the different soil water retention curves across the periods shows that  
382 biochar treatments increased volumetric soil moisture content relative to the control treatment  
383 consistently across the ranges of observed soil matric potentials in all three periods (Figure 4, Table  
384 A2). The soil water retention curves estimated for Period III were shifted to lower volumetric water  
385 contents relative to the other periods and ranged from close to field capacity to wilting point.

386 The effect of biochar on the soil water retention curve can also be quantified by the response ratios of  
387 the wilting point, field capacity and the van Genuchten parameters  $\alpha$  and  $n$ . Most of these ratios were  
388 found to be larger than one (Table 1), which indicates increased soil water content for a given water  
389 potential value.

#### 390 4.3 Isotopic variability

391 Overall, the  $\delta^{18}\text{O}$  and  $d$ -excess of rainfall was between -15.7 ‰ and -0.2 ‰ ( $S_D = 3.4$  ‰) and 0 ‰ and  
392 +18 ‰ ( $S_D = 4.6$  ‰) respectively ( $\delta^{18}\text{O}$  see Figure 5a,  $d$ -excess see Figure A2a and A3). The  $\delta^{18}\text{O}$  and  
393  $d$ -excess of soil water and groundwater collected on the different sampling days was between -7.5 ‰  
394 and -4.5 ‰ ( $S_D = 1.3$  ‰) and -1.1 ‰ and +9.7 ‰ ( $S_D = 4.9$  ‰) respectively ( $\delta^{18}\text{O}$  see Figure 5b-d,  $d$ -  
395 excess see Figure A2b-d and A3). The within treatment variability in isotopic composition of soil water  
396 samples for each sample day was  $<1$  ‰ for  $\delta^{18}\text{O}$  and  $<6$  ‰ for  $d$ -excess (Figure 6). The  $\delta^{18}\text{O}$  and  $d$ -  
397 excess of plant water was between -8.7 ‰ and -2.7 ‰ ( $S_D = 3.7$  ‰) and -14.6 ‰ to +3.2 ‰ ( $S_D = 11.4$   
398 ‰) respectively ( $\delta^{18}\text{O}$  see Figure 5b-d,  $d$ -excess see Figure A2b-d and A3). The within treatment





399 variability in isotopic composition of plant water samples on each sample day  $>3\text{‰}$  for  $\delta^{18}\text{O}$  and  $>8\text{‰}$   
400 for  $d$ -excess (Figure 6). The within treatment variability was smaller for the biochar amended treatments  
401 relative to the within treatment variability in the control treatment (Figure 6).

402 During Period I, the isotopic composition of rainfall varied between  $-5.6\text{‰}$  to  $-0.2\text{‰}$  for  $\delta^{18}\text{O}$  (Figure  
403 5a) and from  $-1.1\text{‰}$  to  $+9\text{‰}$  for  $d$ -excess (Figure A2). On rainy days when rainfall intensities were  
404 below  $10\text{ mm d}^{-1}$ , sub-cloud evaporation may exert an important control on rainfall enrichment  
405 (Sánchez-Murillo et al., 2016, 2017) and potentially also the low amount of rain water collected in  
406 relation to the bottle volume causing water to evaporated water in the sampler. For example, the  
407 observed fractionated isotopic compositions of these rain samples were often recorded to be  $<5\text{‰}$  with  
408 regard to  $d$ -excess. The average isotopic composition of plant water in the different treatments decreased  
409 from roughly from  $+3.2\text{‰}$  to  $-4\text{‰}$  for  $\delta^{18}\text{O}$  and increased from roughly  $-40\text{‰}$  to  $+18\text{‰}$  for  $d$ -excess  
410 during Period I (Figures 5 and A2). In Period II, the isotopic composition of rainfall varied between -  
411  $3.7\text{‰}$  to  $-12.7\text{‰}$  for  $\delta^{18}\text{O}$  (Figure 5a) and  $+6\text{‰}$  to  $+11.8\text{‰}$  for  $d$ -excess (Figure A2). The average  
412 isotopic composition of plant water varied in all treatments to between  $-7\text{‰}$  to  $-2\text{‰}$  for  $\delta^{18}\text{O}$  and  $-11.8$   
413  $\text{‰}$  to  $+9.2\text{‰}$  for  $d$ -excess. It should be noted that there was a change from negative to positive  $d$ -excess  
414 for the plant water isotopic compositions between sampling day five and seven, indicating a change  
415 from highly fractionated isotopic compositions to compositions similar to that of rainfall. During the  
416 dry spell of Period III no rainfall occurred and hence no rainwater was collected. Also, no soil water  
417 could be extracted from lysimeters sampling water from 15 below the surface on sampling day 10 and  
418 day 11. The average isotopic composition of plant water varied between  $-7\text{‰}$  to  $-6\text{‰}$  for  $\delta^{18}\text{O}$  and  $-7$   
419  $\text{‰}$  to  $-2\text{‰}$  for  $d$ -excess, showing a high fractionation signature (Figures 5, A2 and A3).

#### 420 4.4 Using dual isotope space to characterize plant water sources

421 Rainfall isotopic compositions fell along the GMWL and LMWL for our experimental site (Figure 7).  
422 The soil water and ground water isotopic samples from Period I were more fractionated, i.e. they  
423 deviated from the GMWL, compared to soil water isotopic samples from the wet Period II and III which  
424 fell more along the GMWL (Figure 8). The plant water isotopic compositions from the different  
425 treatments and sampling periods were somewhat different from each other in terms of absolute values



426 but showed a similar temporal evolution (Figure 7). In Period I, plant water samples from all treatments  
427 deviated from the GMWL and moved primarily along the modeled evaporation lines of the sampled  
428 soil water (Figure 7 a, d and g). The plant water thus resembled soil water with a strong evaporation  
429 signature in Period I.

430 In Period II, which was much wetter than Period I, the plant water samples fell on or were close to the  
431 GMWL independent of the treatment and moved from sampling day to sampling day along the GMWL.  
432 It is likely that plant water responded to the replenished soil water that acquired the signature of rainfall  
433 during this period. At the end of Period II, plant water samples from BC1 and the control treatment  
434 showed a more fractioned signature and fell on the modelled evaporation line indicating that plant water  
435 resembled soil water with signature from evaporated rain from day 8 (Figure 7 b and h). Plant water  
436 samples in the BC2 treatment, however, showed the signature from soil water more similar to original  
437 rainfall (Figure 7 e and e2). During the dry Period III, all plant water samples deviated from the GMWL  
438 and fell along modeled evaporation lines with signature of residual rainfall that had fallen in Period II  
439 (depicted in blue in Figure 7 c, f, and i).

## 440 5. Discussion

### 441 5.1 Variable effect of biochar on the soil hydraulic properties

442 Incorporating two different types of biochar in plots planted with rice affected the soil hydraulic  
443 properties. The soil water retention curves of the biochar amended treatments showed higher soil water  
444 contents at similar matric potential relative to the control treatment, leading to more plant water  
445 available under similar conditions (Figure 4, Table 1). The soil water retention curve of the BC1  
446 treatment became more similar to the curves found in finer grained soils, which indicates increased  
447 water retention, a common expected impact of biochar additions (Fischer et al., 2018; Sun and Lu,  
448 2014). Conversely, the soil water retention curve for the BC2 treatment became more similar to the  
449 curves associated with coarser soils indicating enhanced water flows, which has also been described as  
450 a potential impact of biochar additions (Fischer et al., 2018; Liu et al., 2017).

451 The overall soil response to biochar amendments in our experiment had a within treatment variability  
452 but was comparable to the response found in other tropical soils where a lower range of  $\theta_{WP}$  and  $\theta_{FC}$



453 was found (Obia et al., 2016). But the soil water retention curves we found were more irregular shaped  
454 compared to laboratory derived soil water retention curves reported in the literature (Iiyama, 2016;  
455 Morgan et al., 2001) which usually present one single continuous drying curve (e.g. Batool et al., 2015;  
456 Gląb et al., 2016 or Obia et al., 2016). Instead the field-data derived soil water retention curves in the  
457 present study were field derived and the result of temporally variable atmospheric forcing. Specifically,  
458 our observed within treatment variability in the soil water retention curves was a same order of  
459 magnitude as the responses due to differences in biochar application rates or due to differences in  
460 biochar typologies reported in laboratory studies (e.g. Batool et al., 2015; Gląb et al., 2016 or Obia et  
461 al., 2016). Laboratory studies may overestimate the volumetric soil moisture content at a given soil  
462 matric potential compared to field-derived soil water retention curves (Iiyama, 2016; Morgan et al.,  
463 2001).

464 Although the two biochar types tested were produced in different ways, their experimental application  
465 was similar (i.e. same application rate, similar particle size, application amount, depth, site  
466 characteristics and climate). One key distinction between the two biochar treatments was the application  
467 date, which may be important because aging can change the physical and chemical characteristics of  
468 biochar (Blanco-Canqui, 2017). Due to some logistical constraints, biochar was introduced to the BC1  
469 plot about six months before the BC2 plot. This allowed the biochar to age in situ and for the disturbed  
470 soils to settle under the BC1 treatment. Thus, the BC2 soil likely had relatively larger macropores that  
471 could have increased the connectivity of the 20 cm soil layer where biochar was applied with deeper  
472 soil layers. This difference in application timing may have influenced the hydraulic differences in  
473 results observed between the two biochar treatments (Figure 4) and amplified the differences due to the  
474 contrasting production methods. Clearly, the interplay of all the possible biochar variables with all the  
475 possible site-specific heterogeneities makes it challenging to isolate the biochar effect in  
476 agroecosystems. Taken altogether, these differences in biochar treatment responses and the relative  
477 impacts of both B1 and B2 biochar treatments compared to the control plot highlights the potential for  
478 variability in biochar responses – which has been documented in the literature (Fischer et al., 2018) and  
479 creates ambiguity around predicting the response of biochar amendments at field scale. This further



480 highlighting the difficulty to transfer laboratory-scale results to the field scale where management  
481 decisions are made.

## 482 5.2 Temporally variable soil water fluxes

483 The isotopic composition of different water samples was useful to infer how water fluxes varied through  
484 time. The isotopic composition of soil water sampled at two different depths across the plots was rather  
485 stable over time compared to the temporally variable isotopic composition of rainfall (Figure 5). In  
486 addition, the temporal variability of isotopic composition of soil water from our experiment was less  
487 than the spatial variability or change in isotopic composition with depth reported in previous biochar  
488 studies (e.g. Beyer et al., 2016; Koeniger et al., 2016; Saxena, 1987 and Sprenger et al., 2016). When  
489 comparing our findings with other tropical systems, the  $d$ -excess of the soil water we found during dry  
490 spells (Figure A2) had a smaller variation range than observed in a coffee plantation in Mexico by  
491 Muñoz-Villers et al. (2020) and was generally less variable than observations made by Jiménez-  
492 Rodríguez et al. (2020) in a tropical wet forest in Costa Rica. The low  $d$ -excess values and ranges of  
493 the soil water observed in this study indicate high evaporative processes in the top soil layer (Amin et  
494 al., 2020; Sprenger et al., 2016). This is consistent with our high estimated evapotranspiration rates  
495 (average  $3.1 \text{ mm day}^{-1}$  up to  $6 \text{ mm day}^{-1}$ ) which are typical for the Dry Corridor of Central America  
496 characterized by high solar radiation and air temperatures (Morillas et al., 2019).

497 During Period I, when rice plants were small and sparse, leaving much bare soil, the evaporation  
498 occurring from the soil across the different treatments was homogenous, creating a low  $d$ -excess signal  
499 in the soil water. During wet spells in Period II, the  $d$ -excess increased slightly, indicating mixing of  
500 rainfall with soil water. At the end of Period II and throughout Period III, the  $d$ -excess remained higher  
501 despite high evaporation, which might be due to a more homogenous crop cover creating a consistent  
502 microclimate as described by Sprenger et al. (2017). The isotopic composition of groundwater (1) had  
503  $d$ -excess values similar to that of meteoric water during dry spells and (2) decreased during wet spells  
504 showing a high evaporative signal (Figure A2). Such observed changes in  $d$ -excess are generally not  
505 found in temperate zones (Sprenger et al., 2016), but indicate that rainfall flushed the fractionated soil  
506 water downwards promoting mixing with groundwater (Gat and Airey (2006).



507 5.3 Temporally variable plant water sources

508 The studied rice plants had different water sources available during different periods of the experiment,  
509 but what water did they consume?

510 It is likely that the fractionation observed in the plant water collected in this study represents fractionated  
511 soil water that was consumed by the plants. This is consistent with results observed in previous studies  
512 using stable water isotopes to map out plant water sources (Brooks et al., 2010; Penna et al., 2020;  
513 Sprenger et al., 2016). Further, this interpretation of plant water composition is supported by plant water  
514 samples falling along the theoretical evaporation lines estimating how soil water would evolves  
515 isotopically due to evaporation. Therefore, it is likely that during Period I, the young rice plants (with  
516 shallow root system <20 cm as reported by Mahindawansa et al. 2018) consumed the fractionated soil  
517 water (Figure 7) which was not sampled with the lysimeters at 15 cm and 40 cm below the surface.

518 During Period II, plants grew to their maximum heights with roots reaching deeper soil layers (length  
519 >60 cm as reported by Mahindawansa et al. 2018). This means that the rice plants, similar to larger  
520 vegetation e.g. trees (Allen et al., 2019), would have had access to deeper and more-stable pools of  
521 water with a distinct lower  $d$ -excess signature. However, the isotopic composition of plant water during  
522 this period followed the GMWL (Figure 7 b, e and h), indicating that plants consumed largely shallow  
523 soil water from recent rainfall. In Period III, it became increasingly difficult to extract water from  
524 lysimeters at 15 cm below the surface and the isotopic composition of plant water drifted from the  
525 GMWL, along the theoretical evaporation line of residual rainfall which fell in Period II. With the  
526 experiment being held in the tropics and based on Amin et al (2020) one would expect that the rice  
527 plants with their longer roots would accessed access the more stable and older water stores in deeper  
528 subsurface zones below 60 cm. Instead, the rice plants in the different treatments preferably consumed  
529 the temporally variable and isotopically labeled newer surface soil water similarly to what has been  
530 documented in natural ecosystems (e.g. van der Velde et al., 2015) and temperate grasslands (Bachmann  
531 et al., 2015).

532 By mixing biochar in the top soil, a multi-layer soil profile was created and based on studies in natural  
533 catchments, e.g. Penna et al. (2018) or Sprenger et al. (2016), these different layers could store not only



534 different quantities of water but also water characterized by different ages. Performing additional  
535 isotopic experiments (Beyer et al., 2016), higher temporal resolution sampling of plant water (Marshall  
536 et al., 2020; Volkmann et al., 2016) and spatiotemporal soil water (Sprenger et al., 2015) or including  
537 interception, transpiration and atmospheric processes into the experimental analysis (Jiménez-  
538 Rodríguez et al., 2020) would allow to not only distinguish in more detail whether the rice plants prefer  
539 bounded or mobile water (Berry et al., 2018; Brooks et al., 2010; McDonnell, 2014) but also to quantify  
540 the fraction of water sources (Muñoz-Villers et al., 2020). Consequently, this would also allow to  
541 indicate how long the soil water resides in the different soil layers before it is consumed by plants. In  
542 addition to the aforementioned vertical processes also the lateral water fluxes (Sprenger and Allen,  
543 2020) need to be considered to assess the field-scale responses to biochar amendments (Fischer et al.,  
544 2018). These analyses are beyond the scope of this initial investigation; however, our results indicate  
545 that rice plants growing in biochar amended soils not only had access to more water (Figure 4) but also  
546 had a more stable source of green water (i.e. soil moisture from rainfall) and thus could withstand dry  
547 spells seven days longer (Figure 3). Regardless of the potential advantages, as stated by Fischer et al.  
548 (2018), it must be noted that biochar as water management tool does not adhere to a one size fits all  
549 approach but needs fine tuning in accordance with climate, site and plant characteristics to obtain stable  
550 and optimal yields.

## 551 6. Conclusions

552 Amending soils with biochar is an emerging and promising practice to improving resilience of rainfed  
553 agriculture to climate variability by increasing the soil water and plant available water. Using an  
554 experimental field study, we observed biochar amendments to create generally 2 % to 7 % higher soil  
555 water content and therefore more plant water relative to the control treatment, despite differing impacts  
556 between biochar treatments depending on the type of biochar and timing of application. In addition, we  
557 observed a within treatment variability in the soil water retention curves which was in the same order  
558 of magnitude as one would expect from responses due to differences in biochar application rates or due  
559 to differences in biochar typologies. Further, we were able to trace the effect of biochar on the soil water  
560 storage to investigate which water plants consume. The isotopic composition of soil water sampled in



561 two distinct depths in the different plots was rather stable in time compared to the temporal variable  
562 isotopic composition of rainfall. The stable isotope composition of plant water instead showed that the  
563 rice plants preferably consumed the temporal variable soil water comprised of residual rainfall the  
564 experienced evaporation in the top 20 cm of the soil. When comparing the different treatments, our  
565 results indicated that rice plants grown in biochar amended soils not only had more water available but  
566 also had a more stable source of green water. Thus, these rice plants in biochar amended soils could  
567 withstand dry spells of up to an extra seven days. Despite these positive effects of biochar amendment,  
568 it still seems necessary to provide additional irrigation to facilitate optimal plant growth if extended dry  
569 periods occur during certain growing stages to have optimal yields. So, while our study highlights some  
570 of the usefulness of combining hydrometric and isotopic data to map out how biochar additions impact  
571 plant-water interactions in the field, we acknowledge more work is needed to fully characterize the  
572 influence biochar additions may have at scale on agroecosystems. This further understanding is  
573 important given the need of more specific management recommendations to ensure biochar additions  
574 in agricultural landscapes result in net benefits for both farmers and the environment.

### 575 Data availability

576 Upon acceptance, all of the research data that were required to create the plots will be available from  
577 the Bolin Center for Climate Research.

### 578 Author contribution

579 BF, LM, MG, SM, MJ, AS and SL designed the experiment, and BF, JR carried it out. CC provided  
580 BC2, RS analyzed the stable isotope composition of the collected water. BF performed the data analysis  
581 and prepared the paper with contributions from all co-authors.

### 582 Competing interests

583 The authors declare that they have no conflict of interest.

### 584 Acknowledgements

585 We thank all the people who helped in the field and the laboratory, particularly Sharon Arce, Johnny  
586 Arriola and Eduardo Rodríguez and all the HIDROCEC team of Universidad Nacional, Liberia, Costa



587 Rica. The authors would also like to thank the collaboration from the Stable Isotopes Research Group  
588 & Water Resources Management Laboratory (Universidad Nacional, Heredia) on helping with the  
589 lysimeters and wells installation as well as water stable isotopes. Especially Edwin Quirós Ramos,  
590 Roberto Ramírez, Juan Carlos Jiménez Vargas and all technical staff from the EEEJN-INTA who help  
591 develop the experimental design and advised about regional crop management practices and Dr. Jaime  
592 Quesada from TEC for providing the biochar national used in this study.

### 593 Financial support

594 This research was conducted as part of the Agricultural Water Innovations in the Tropics (AgWIT)  
595 project funded by the Joint Call of the Water Joint Programming Initiative (Water JPI) and the Joint  
596 Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI) of the  
597 European Union and partner countries. Stefano Manzoni and Steve Lyon acknowledge partial support  
598 from the Swedish Research Agencies Vetenskapsrådet, Formas, and Sida through the joint call on  
599 Sustainability and resilience-Tackling climate and environmental changes (grant VR 2016-06313), and  
600 the Bolin Centre for Climate Research (Research Area 7). Ricardo Sánchez-Murillo acknowledges the  
601 financial support from the International Atomic Energy Agency (IAEA) grants COS/7/005, RC-19747  
602 (CRP-F31004), RC-22760 (CRP-F33024) which were fundamental to conduct the water stable isotope  
603 analysis in Costa Rica.





## 604 References

- 605 Agegnehu, G., Srivastava, A. K. and Bird, M. I.: The role of biochar and biochar-compost in improving  
606 soil quality and crop performance: A review, *Applied Soil Ecology*, 119, 156–170,  
607 doi:10.1016/j.apsoil.2017.06.008, 2017.
- 608 Allen, R. G., Pereira, L. S., Raes, D. and Smith, M.: *Crop evapotranspiration-Guidelines for computing*  
609 *crop water requirements*, FAO, Rome., 1998.
- 610 Allen, S. T., Kirchner, J. W., Braun, S., Siegwolf, R. T. W. and Goldsmith, G. R.: Seasonal origins of  
611 soil water used by trees, *Hydrol. Earth Syst. Sci.*, 23(2), 1199–1210, doi:10.5194/hess-23-1199-  
612 2019, 2019.
- 613 Amin, A., Zuecco, G., Geris, J., Schwendenmann, L., McDonnell, J. J., Borga, M. and Penna, D.: Depth  
614 distribution of soil water sourced by plants at the global scale: A new direct inference approach,  
615 *Ecohydrology*, 13(2), e2177, doi:10.1002/eco.2177, 2020.
- 616 Bachmann, D., Gockele, A., Ravenek, J. M., Roscher, C., Strecker, T., Weigelt, A. and Buchmann, N.:  
617 No Evidence of Complementary Water Use along a Plant Species Richness Gradient in  
618 Temperate Experimental Grasslands, *PLOS ONE*, 10(1), 1–14,  
619 doi:10.1371/journal.pone.0116367, 2015.
- 620 Batool, A., Taj, S., Rashid, A., Khalid, A., Qadeer, S., Saleem, A. R. and Ghufuran, M. A.: Potential of  
621 soil amendments (Biochar and Gypsum) in increasing water use efficiency of *Abelmoschus*  
622 *esculentus* L. Moench, *Frontiers in Plant Science*, 6(September), 1–13,  
623 doi:10.3389/fpls.2015.00733, 2015.
- 624 Benettin, P., Volkman, T. H. M., von Freyberg, J., Frentress, J., Penna, D., Dawson, T. E. and  
625 Kirchner, J. W.: Effects of climatic seasonality on the isotopic composition of evaporating soil  
626 waters, *Hydrology and Earth System Sciences*, 22(5), 2881–2890, doi:10.5194/hess-22-2881-  
627 2018, 2018.
- 628 Berry, Z. C., Evaristo, J., Moore, G., Poca, M., Steppe, K., Verrot, L., Asbjornsen, H., Borma, L. S.,  
629 Brefeld, M., Hervé-Fernández, P., Seyfried, M., Schwendenmann, L., Sinacore, K., De  
630 Wispelaere, L. and McDonnell, J.: The two water worlds hypothesis: Addressing multiple  
631 working hypotheses and proposing a way forward, *Ecohydrology*, 11(3), e1843,  
632 doi:10.1002/eco.1843, 2018.
- 633 Beyer, M., Koeniger, P., Gaj, M., Hamutoko, J. T., Wanke, H. and Himmelsbach, T.: A deuterium-  
634 based labeling technique for the investigation of rooting depths, water uptake dynamics and  
635 unsaturated zone water transport in semiarid environments, *Journal of Hydrology*, 533, 627–  
636 643, doi:10.1016/j.jhydrol.2015.12.037, 2016.
- 637 Biazin, B., Sterk, G., Temesgen, M., Abdulkedir, A. and Stroosnijder, L.: Rainwater harvesting and  
638 management in rainfed agricultural systems in sub-Saharan Africa – A review, *Physics and*  
639 *Chemistry of the Earth, Parts A/B/C*, 47–48, 139–151, doi:10.1016/j.pce.2011.08.015, 2012.
- 640 Birkel, C., Brenes, A. and Sánchez-Murillo, R.: The Tempisque-Bebedero catchment system: energy-  
641 water-food consensus in the seasonally dry tropics of northwestern Costa Rica, in *Nexus*  
642 *Outlook: assessing resource use challenges in the water, energy and food nexus*, TH-Koeln,  
643 University of Applied Sciences. [online] Available from: [25](https://www.water-energy-</a></p></div><div data-bbox=)



- 644 food.org/fileadmin/user\_upload/files/documents/others/Outlook-  
645 Nexus\_Assessing\_Resource\_Use\_Challenges.pdf, 2017.
- 646 Blanco-Canqui, H.: Biochar and Soil Physical Properties, *Soil Science Society of America Journal*,  
647 81(4), 687, doi:10.2136/sssaj2017.01.0017, 2017.
- 648 Brooks, R. J., Barnard, H. R., Coulombe, R. and McDonnell, J. J.: Ecohydrologic separation of water  
649 between trees and streams in a Mediterranean climate, *Nature Geoscience*, 3(2), 100–104,  
650 doi:10.1038/ngeo722, 2010.
- 651 Castelli, G., Bresci, E., Castelli, F., Hagos, E. Y. and Mehari, A.: A participatory design approach for  
652 modernization of spate irrigation systems, *Agricultural Water Management*, 210, 286–295, doi:  
653 10.1016/j.agwat.2018.08.030, 2018.
- 654 Craig, H.: Isotopic Variations in Meteoric Waters, *Science*, 133(3465), 1702–1703,  
655 doi:10.1126/science.133.3465.1702, 1961.
- 656 Dansgaard, W.: Stable isotopes in precipitation, *Tellus*, 16(4), 436–468, doi:10.1111/j.2153-  
657 3490.1964.tb00181.x, 1964.
- 658 Dawson, T. E. and Ehleringer, J. R.: Streamside trees that do not use stream water, *Nature*, 350(6316),  
659 335–337, doi:10.1038/350335a0, 1991.
- 660 Diogenes Cubero, F. and Maria José Elizondo, A.: Estudio detallado de suelos y capacidad de uso de  
661 las tierras de estación experimental Enrique Jiménez Núñez (Detailed study of soils and capacity  
662 of use of the lands of experimental station Enrique Jiménez Núñez), Instituto Nacional de  
663 Innovación y Transferencia en Tecnología Agropecuaria, Cañas., 2014.
- 664 Enfors, E. I. I. and Gordon, L. J. J.: Analysing resilience in dryland agro-ecosystems: a case study of  
665 the Makanya catchment in Tanzania over the past 50 years, *Land Degradation & Development*,  
666 18(6), 680–696, doi:10.1002/ldr.807, 2007.
- 667 Falkenmark, M.: Society’s interaction with the water cycle: a conceptual framework for a more holistic  
668 approach, *Hydrological Sciences Journal*, 42(4), 451–466, doi:10.1080/02626669709492046,  
669 1997.
- 670 Famiglietti, J. S.: The global groundwater crisis, *Nature Climate Change*, 4(11), 945–948,  
671 doi:10.1038/nclimate2425, 2014.
- 672 Feng, X., Porporato, A. and Rodriguez-Iturbe, I.: Changes in rainfall seasonality in the tropics, *Nature*  
673 *Climate Change*, 3(9), 811–815, doi:10.1038/nclimate1907, 2013.
- 674 Fischer, B. M. C., Mul, M. L. and Savenije, H. H. G.: Determining spatial variability of dry spells: a  
675 Markov-based method, applied to the Makanya catchment, Tanzania, *Hydrology and Earth*  
676 *System Sciences*, 17(6), 2161–2170, doi:10.5194/hess-17-2161-2013, 2013.
- 677 Fischer, B. M. C., Stähli, M. and Seibert, J.: Pre-event water contributions to runoff events of different  
678 magnitude in pre-alpine headwaters, *Hydrology Research*, 48(1), 28–47,  
679 doi:10.2166/nh.2016.176, 2017a.
- 680 Fischer, B. M. C., van Meerveld, H. J. (Ilja) and Seibert, J.: Spatial variability in the isotopic  
681 composition of rainfall in a small headwater catchment and its effect on hydrograph separation,  
682 *Journal of Hydrology*, 547, 755–769, doi:10.1016/j.jhydrol.2017.01.045, 2017b.



- 683 Fischer, B. M. C., Manzoni, S., Morillas, L., Garcia, M., Johnson, M. S. and Lyon, S. W.: Improving  
684 agricultural water use efficiency with biochar – A synthesis of biochar effects on water storage  
685 and fluxes across scales, *Science of The Total Environment*, doi:  
686 10.1016/j.scitotenv.2018.11.312, 2018.
- 687 Fischer, B. M. C., Aemisegger, F., Graf, P., Sodemann, H. and Seibert, J.: Assessing the Sampling  
688 Quality of a Low-Tech Low-Budget Volume-Based Rainfall Sampler for Stable Isotope  
689 Analysis, *Frontiers in Earth Science*, 7, 244, doi:10.3389/feart.2019.00244, 2019.
- 690 Fraiture, C. de and Wichelns, D.: Satisfying future water demands for agriculture, *Agricultural Water  
691 Management*, 97(4), 502–511, doi: 10.1016/j.agwat.2009.08.008, 2010.
- 692 Fraiture, C. de, Karlberg, L. and Rockström, J.: Can rainfed agriculture feed the world? An assessment  
693 of potentials and risk, in *Rainfed agriculture: Unlocking the potential.*, pp. 124–132, CAB  
694 International, London., 2009.
- 695 Gat, J. R. and Airey, P. L.: Stable water isotopes in the atmosphere/biosphere/lithosphere interface:  
696 Scaling-up from the local to continental scale, under humid and dry conditions, *Global and  
697 Planetary Change*, 51(1), 25–33, doi:10.1016/j.gloplacha.2005.12.004, 2006.
- 698 Gibson, J. J., Birks, S. J. and Yi, Y.: Stable isotope mass balance of lakes: a contemporary perspective,  
699 *Quaternary Science Reviews*, 131, 316–328, doi:10.1016/j.quascirev.2015.04.013, 2016.
- 700 Giorgi, F.: Climate change hot-spots, *Geophysical Research Letters*, 33(8),  
701 doi:10.1029/2006GL025734, 2006.
- 702 Głab, T., Palmowska, J., Zaleski, T. and Gondok, K.: Effect of biochar application on soil hydrological  
703 properties and physical quality of sandy soil, *Geoderma*, 281, 11–20,  
704 doi:10.1016/j.geoderma.2016.06.028, 2016.
- 705 Goldsmith, G. R., Muñoz-Villers, L. E., Holwerda, F., McDonnell, J. J., Asbjornsen, H. and Dawson,  
706 T. E.: Stable isotopes reveal linkages among ecohydrological processes in a seasonally dry  
707 tropical montane cloud forest, *Ecohydrology*, 5(6), 779–790, doi:10.1002/eco.268, 2012.
- 708 Gonfiantini, R.: Chapter 3 - environmental isotopes in lake studies, in *The Terrestrial Environment*, B,  
709 edited by P. Fritz and J. C. Fontes, pp. 113–168, Elsevier, Amsterdam., 1986.
- 710 Hidalgo, H. G., Alfaro, E. J., Amador, J. A. and Bastidas, Á.: Precursors of quasi-decadal dry-spells in  
711 the Central America Dry Corridor, *Climate Dynamics*, 53(3), 1307–1322, doi:10.1007/s00382-  
712 019-04638-y, 2019.
- 713 Iiyama, I.: Differences between field-monitored and laboratory-measured soil moisture characteristics,  
714 *Soil Science and Plant Nutrition*, 62(5–6), 416–422, doi:10.1080/00380768.2016.1242367,  
715 2016.
- 716 Imbach, P., Chou, S. C., Lyra, A., Rodrigues, D., Rodriguez, D., Latinovic, D., Siqueira, G., Silva, A.,  
717 Garofolo, L. and Georgiou, S.: Future climate change scenarios in Central America at high  
718 spatial resolution, *PLOS ONE*, 13(4), e0193570, doi:10.1371/journal.pone.0193570, 2018.
- 719 Jasechko, S., Perrone, D., Befus, K. M., Bayani Cardenas, M., Ferguson, G., Gleeson, T., Luijendijk,  
720 E., McDonnell, J. J., Taylor, R. G., Wada, Y. and Kirchner, J. W.: Global aquifers dominated  
721 by fossil groundwaters but wells vulnerable to modern contamination, *Nature Geoscience*,  
722 10(6), 425–429, doi:10.1038/ngeo2943, 2017.



- 723 Jeffery, S., Meinders, M. B. J., Stoof, C. R., Bezemer, T. M., van de Voorde, T. F. J., Mommer, L. and  
724 van Groenigen, J. W.: Biochar application does not improve the soil hydrological function of a  
725 sandy soil, *Geoderma*, 251–252, 47–54, doi:10.1016/j.geoderma.2015.03.022, 2015.
- 726 Jeffery, S., Abalos, D., Prodana, M., Bastos, A. C., Van Groenigen, J. W., Hungate, B. A. and Verheijen,  
727 F.: Biochar boosts tropical but not temperate crop yields, *Environmental Research Letters*,  
728 12(5), doi:10.1088/1748-9326/aa67bd, 2017.
- 729 Jiménez-Rodríguez, C. D., Coenders-Gerrits, M., Wenninger, J., Gonzalez-Angarita, A. and Savenije,  
730 H.: Contribution of understory evaporation in a tropical wet forest during the dry season,  
731 *Hydrol. Earth Syst. Sci.*, 24(4), 2179–2206, doi:10.5194/hess-24-2179-2020, 2020.
- 732 Kätterer, T., Roobroeck, D., Andrén, O., Kimutai, G., Karlton, E., Kirchmann, H., Nyberg, G.,  
733 Vanlauwe, B. and Röing de Nowina, K.: Biochar addition persistently increased soil fertility  
734 and yields in maize-soybean rotations over 10 years in sub-humid regions of Kenya, *Field Crops*  
735 *Research*, 235, 18–26, doi:10.1016/j.fcr.2019.02.015, 2019.
- 736 Klaus, J. and McDonnell, J. J.: Hydrograph separation using stable isotopes: Review and evaluation,  
737 *Journal of Hydrology*, 505, 47–64, doi:10.1016/j.jhydrol.2013.09.006, 2013.
- 738 Knutson, T. R., Delworth, T. L., Dixon, K. W., Held, I. M., Lu, J., Ramaswamy, V., Schwarzkopf, M.  
739 D., Stenchikov, G. and Stouffer, R. J.: Assessment of Twentieth-Century Regional Surface  
740 Temperature Trends Using the GFDL CM2 Coupled Models, *J. Climate*, 19(9), 1624–1651,  
741 doi:10.1175/JCLI3709.1, 2006.
- 742 Koeniger, P., Marshall, J. D., Link, T. and Mulch, A.: An inexpensive, fast, and reliable method for  
743 vacuum extraction of soil and plant water for stable isotope analyses by mass spectrometry,  
744 *Rapid Communications in Mass Spectrometry*, 25(20), 3041–3048, doi:10.1002/rcm.5198,  
745 2011.
- 746 Koeniger, P., Gaj, M., Beyer, M. and Himmelsbach, T.: Review on soil water isotope-based  
747 groundwater recharge estimations, *Hydrological Processes*, 30(16), 2817–2834,  
748 doi:10.1002/hyp.10775, 2016.
- 749 Lim, T.-J. and Spokas, K.: Impact of Biochar Particle Shape and Size on Saturated Hydraulic Properties  
750 of Soil, *Korean Journal of Environmental Agriculture*, 37(1), 1–8, doi:KJEA-37-1, 2018.
- 751 Liu, C., Wang, H., Tang, X., Guan, Z., Reid, B. J., Rajapaksha, A. U., Ok, Y. S. and Sun, H.: Biochar  
752 increased water holding capacity but accelerated organic carbon leaching from a sloping  
753 farmland soil in China, *Environmental Science and Pollution Research*, 23(2), 995–1006,  
754 doi:10.1007/s11356-015-4885-9, 2016.
- 755 Liu, Z., Dugan, B., Masiello, C. A. and Gonnermann, H. M.: Biochar particle size, shape, and porosity  
756 act together to influence soil water properties, *PLOS ONE*, 12(6), 1–19,  
757 doi:10.1371/journal.pone.0179079, 2017.
- 758 Magaña, V., Amador, J. A. and Medina, S.: The Midsummer Drought over Mexico and Central  
759 America, *J. Climate*, 12(6), 1577–1588, doi:10.1175/1520-  
760 0442(1999)012<1577:TMDOMA>2.0.CO;2, 1999.
- 761 Mahindawansa, A., Orłowski, N., Kraft, P., Rothfuss, Y., Racela, H. and Breuer, L.: Quantification of  
762 plant water uptake by water stable isotopes in rice paddy systems, *Plant and Soil*, 429(1), 281–  
763 302, doi:10.1007/s11104-018-3693-7, 2018.



- 764 Makurira, H., Mul, M. L., Vyagusa, N. F., Uhlenbrook, S. and Savenije, H. H. G.: Evaluation of  
765 community-driven smallholder irrigation in dryland South Pare Mountains, Tanzania: A case  
766 study of Manoo micro dam, *Physics and Chemistry of the Earth, Parts A/B/C*, 32(15–18), 1090–  
767 1097, doi: 10.1016/j.pce.2007.07.020, 2007.
- 768 Marshall, J., Cuntz, M., Beyer, M., Dubbert, M. and Kuehnhammer, K.: Borehole Equilibration: Testing  
769 a New Method to Monitor the Isotopic Composition of Tree Xylem Water in situ, *Frontiers in*  
770 *Plant Science*, 11, doi:10.3389/fpls.2020.00358, 2020.
- 771 McDonnell, J. J.: The two water worlds hypothesis: ecohydrological separation of water between  
772 streams and trees?, *WIREs Water*, 1(4), 323–329, doi:10.1002/wat2.1027, 2014.
- 773 Merlivat, L.: Molecular diffusivities of H<sub>2</sub> 16O, HD16O, and H<sub>2</sub> 18O in gases, *J. Chem. Phys.*, 69(6),  
774 2864–2871, doi:10.1063/1.436884, 1978.
- 775 Morgan, K. T., Parsons, L. R. and Adair Wheaton, T.: Comparison of laboratory- and field-derived soil  
776 water retention curves for a fine sand soil using tensiometric, resistance and capacitance  
777 methods, *Plant and Soil*, 234(2), 153–157, doi:10.1023/A:1017915114685, 2001.
- 778 Morillas, L., Hund, S. V. and Johnson, M. S.: Water Use Dynamics in Double Cropping of Rainfed  
779 Upland Rice and Irrigated Melons Produced Under Drought-Prone Tropical Conditions, *Water*  
780 *Resources Research*, 55(5), 4110–4127, doi:10.1029/2018WR023757, 2019.
- 781 Muñoz-Villers, L. E., Geris, J., Alvarado-Barrientos, M. S., Holwerda, F. and Dawson, T.: Coffee and  
782 shade trees show complementary use of soil water in a traditional agroforestry ecosystem,  
783 *Hydrology and Earth System Sciences*, 24(4), 1649–1668, doi:10.5194/hess-24-1649-2020,  
784 2020.
- 785 Mutiro, J., Makurira, H., Senzanje, A. and Mul, M. L.: Water productivity analysis for smallholder  
786 rainfed systems: A case study of Makanya catchment, Tanzania, *Physics and Chemistry of the*  
787 *Earth, Parts A/B/C*, 31(15–16), 901–909, doi:10.1016/j.pce.2006.08.019, 2006.
- 788 Narseh Kumar, M., Murthy, C. S., Sesha Sai, M. V. R. and Roy, P. S.: On the use of Standardized  
789 Precipitation Index (SPI) for drought intensity assessment, *Meteorological Applications*, 16(3),  
790 381–389, doi:10.1002/met.136, 2009.
- 791 Nelissen, V., Ruysschaert, G., Manka Abusi, D., D’Hose, T., De Beuf, K., Al-Barri, B., Cornelis, W.  
792 and Boeckx, P.: Impact of a woody biochar on properties of a sandy loam soil and spring barley  
793 during a two-year field experiment, *European Journal of Agronomy*, 62, 65–78,  
794 doi:10.1016/j.eja.2014.09.006, 2015.
- 795 Novak, J., Ro, K., Ok, Y. S., Sigua, G., Spokas, K., Uchimiya, S. and Bolan, N.: Biochars  
796 multifunctional role as a novel technology in the agricultural, environmental, and industrial  
797 sectors, *Chemosphere*, 142, 1–3, doi:10.1016/j.chemosphere.2015.06.066, 2016.
- 798 Obia, A., Mulder, J., Martinsen, V., Cornelissen, G. and Børresen, T.: In situ effects of biochar on  
799 aggregation, water retention and porosity in light-textured tropical soils, *Soil and Tillage*  
800 *Research*, 155, 35–44, doi:10.1016/j.still.2015.08.002, 2016.
- 801 Omondi, M. O., Xia, X., Nahayo, A., Liu, X., Korai, P. K. and Pan, G.: Quantification of biochar effects  
802 on soil hydrological properties using meta-analysis of literature data, *Geoderma*, 274, 28–34,  
803 doi:10.1016/j.geoderma.2016.03.029, 2016.



- 804 Penna, D., Hopp, L., Scandellari, F., Allen, S. T., Benettin, P., Beyer, M., Geris, J., Klaus, J., Marshall,  
805 J. D., Schwendenmann, L., Volkmann, T. H. M., von Freyberg, J., Amin, A., Ceperley, N.,  
806 Engel, M., Frentress, J., Giambastiani, Y., McDonnell, J. J., Zuecco, G., Llorens, P., Siegwolf,  
807 R. T. W., Dawson, T. E. and Kirchner, J. W.: Ideas and perspectives: Tracing terrestrial  
808 ecosystem water fluxes using hydrogen and oxygen stable isotopes – challenges and  
809 opportunities from an interdisciplinary perspective, *Biogeosciences*, 15(21), 6399–6415,  
810 doi:10.5194/bg-15-6399-2018, 2018.
- 811 Penna, D., Geris, J., Hopp, L. and Scandellari, F.: Water sources for root water uptake: Using stable  
812 isotopes of hydrogen and oxygen as a research tool in agricultural and agroforestry systems,  
813 *Agriculture, Ecosystems & Environment*, 291, 106790, doi:10.1016/j.agee.2019.106790, 2020.
- 814 Prechsl, U. E., Gilgen, A. K., Kahmen, A. and Buchmann, N.: Reliability and quality of water isotope  
815 data collected with a low-budget rain collector, *Rapid Communications in Mass Spectrometry*,  
816 28(8), 879–885, doi:10.1002/rcm.6852, 2014.
- 817 Reyes-Cabrera, J., Erickson, J. E., Leon, R. G., Silveira, M. L., Rowland, D. L., Sollenberger, L. E. and  
818 Morgan, K. T.: Converting bahiagrass pasture land to elephantgrass bioenergy production  
819 enhances biomass yield and water quality, *Agriculture, Ecosystems and Environment*,  
820 248(July), 20–28, doi:10.1016/j.agee.2017.07.021, 2017.
- 821 Rockström, J.: On-farm green water estimates as a tool for increased food production in water scarce  
822 regions, *Physics and Chemistry of the Earth, Part B: Hydrology, Oceans and Atmosphere*,  
823 24(4), 375–383, doi:10.1016/S1464-1909(99)00016-7, 1999.
- 824 Rothfuss, Y. and Javaux, M.: Reviews and syntheses: Isotopic approaches to quantify root water uptake:  
825 a review and comparison of methods, *Biogeosciences*, 14(8), 2199–2224, doi:10.5194/bg-14-  
826 2199-2017, 2017.
- 827 Sánchez-Murillo, R. and Birkel, C.: Groundwater recharge mechanisms inferred from isoscapes in a  
828 complex tropical mountainous region, *Geophysical Research Letters*, 43(10), 5060–5069,  
829 doi:10.1002/2016GL068888, 2016.
- 830 Sánchez-Murillo, R., Esquivel-Hernández, G., Birkel, C., Correa, A., Welsh, K., Durán-Quesada, A.  
831 M., Sánchez-Gutiérrez, R. and Poça, M.: Tracing water sources and fluxes in a dynamic tropical  
832 environment: from observations to modeling, *Frontiers in Earth Science*, in review.
- 833 Sánchez-Murillo, R., Birkel, C., Welsh, K., Esquivel-Hernández, G., Corrales-Salazar, J., Boll, J.,  
834 Brooks, E., Rouspard, O., Sáenz-Rosales, O., Katchan, I., Arce-Mesén, R., Soulsby, C. and  
835 Araguás-Araguás, L. J.: Key drivers controlling stable isotope variations in daily precipitation  
836 of Costa Rica: Caribbean Sea versus Eastern Pacific Ocean moisture sources, *Quaternary  
837 Science Reviews*, 131, 250–261, doi:10.1016/j.quascirev.2015.08.028, 2016.
- 838 Sánchez-Murillo, R., Durán-Quesada, A. M., Birkel, C., Esquivel-Hernández, G. and Boll, J.: Tropical  
839 precipitation anomalies and d-excess evolution during El Niño 2014-16, *Hydrological  
840 Processes*, 31(4), 956–967, doi:10.1002/hyp.11088, 2017.
- 841 Sánchez-Murillo, R., Esquivel-Hernández, G., Corrales-Salazar, J. L., Castro-Chacón, L., Durán-  
842 Quesada, A. M., Guerrero-Hernández, M., Delgado, V., Barberena, J., Montenegro-Rayó, K.,  
843 Calderón, H., Chevez, C., Peña-Paz, T., García-Santos, S., Ortiz-Roque, P., Alvarado-Callejas,  
844 Y., Benegas, L., Hernández-Antonio, A., Matamoros-Ortega, M., Ortega, L. and Terzer-



- 845 Wassmuth, S.: Tracer hydrology of the data-scarce and heterogeneous Central American  
846 Isthmus, *Hydrological Processes*, 34(11), 2660–2675, doi:10.1002/hyp.13758, 2020.
- 847 Saxena, R.: Oxygen-18 fractionation in nature and estimation of groundwater recharge [Rayleigh  
848 distillation formula, reverse Rayleigh process, throughfall], Report-University of Uppsala,  
849 Department of Physical Geography, Hydrological Division Series A (Sweden), 1987.
- 850 Shen, Y. J., Zhang, Z. B., Gao, L. and Peng, X.: Evaluating contribution of soil water to paddy rice by  
851 stable isotopes of hydrogen and oxygen, *Paddy and Water Environment*, 13(1), 125–133,  
852 doi:10.1007/s10333-013-0414-y, 2015.
- 853 Sprenger, M. and Allen, S. T.: What Ecohydrologic Separation Is and Where We Can Go With It, *Water  
854 Resources Research*, 56(7), e2020WR027238, doi:10.1029/2020WR027238, 2020.
- 855 Sprenger, M., Herbstritt, B. and Weiler, M.: Established methods and new opportunities for pore water  
856 stable isotope analysis, *Hydrological Processes*, 29(25), 5174–5192, doi:10.1002/hyp.10643,  
857 2015.
- 858 Sprenger, M., Leister, H., Gimbel, K. and Weiler, M.: Illuminating hydrological processes at the soil-  
859 vegetation-atmosphere interface with water stable isotopes, *Reviews of Geophysics*, 54(3),  
860 674–704, doi:10.1002/2015RG000515, 2016.
- 861 Sprenger, M., Tetzlaff, D. and Soulsby, C.: Soil water stable isotopes reveal evaporation dynamics at  
862 the soil–plant–atmosphere interface of the critical zone, *Hydrol. Earth Syst. Sci.*, 21(7), 3839–  
863 3858, doi:10.5194/hess-21-3839-2017, 2017.
- 864 Stumpp, C., Maloszewski, P., Stichler, W. and Fank, J.: Environmental isotope ( $\delta^{18}\text{O}$ ) and hydrological  
865 data to assess water flow in unsaturated soils planted with different crops: Case study lysimeter  
866 station “Wagna” (Austria), *Journal of Hydrology*, 369(1), 198–208,  
867 doi:10.1016/j.jhydrol.2009.02.047, 2009.
- 868 Sun, F. and Lu, S.: Biochars improve aggregate stability, water retention, and pore-space properties of  
869 clayey soil, *Journal of Plant Nutrition and Soil Science*, 177(1), 26–33,  
870 doi:10.1002/jpln.201200639, 2014.
- 871 Sundberg, C., Karlton, E., Gitau, J. K., Kätterer, T., Kimutai, G. M., Mahmoud, Y., Njenga, M., Nyberg,  
872 G., Roing de Nowina, K., Roobroeck, D. and Sieber, P.: Biochar from cookstoves reduces  
873 greenhouse gas emissions from smallholder farms in Africa, *Mitigation and Adaptation  
874 Strategies for Global Change*, doi:10.1007/s11027-020-09920-7, 2020.
- 875 Van Genuchten, M. T.: A closed-form equation for predicting the hydraulic conductivity of unsaturated  
876 soils, *Soil science society of America journal*, 44(5), 892–898, 1980.
- 877 van der Velde, Y., Heidbüchel, I., Lyon, S. W., Nyberg, L., Rodhe, A., Bishop, K. and Troch, P. A.:  
878 Consequences of mixing assumptions for time-variable travel time distributions: Mixing  
879 assumptions and time-variable travel time distributions, *Hydrological Processes*, 29(16), 3460–  
880 3474, doi:10.1002/hyp.10372, 2015.
- 881 Vico, G. and Brunzell, N. A.: Tradeoffs between water requirements and yield stability in annual vs.  
882 perennial crops, *Advances in Water Resources*, 112, 189–202, doi:  
883 10.1016/j.advwatres.2017.12.014, 2018.



- 884 Vico, G. and Porporato, A.: Ecohydrology of Agroecosystems: Quantitative Approaches Towards  
885 Sustainable Irrigation, *Bulletin of Mathematical Biology*, 77(2), 298–318, doi:10.1007/s11538-  
886 014-9988-9, 2015.
- 887 Volkmann, T. H. M., Kühnhammer, K., Herbstritt, B., Gessler, A. and Weiler, M.: A method for in situ  
888 monitoring of the isotope composition of tree xylem water using laser spectroscopy, *Plant, Cell  
889 & Environment*, 39(9), 2055–2063, doi:10.1111/pce.12725, 2016.
- 890 Zhang, D., Yan, M., Niu, Y., Liu, X., van Zwieten, L., Chen, D., Bian, R., Cheng, K., Li, L., Joseph,  
891 S., Zheng, J., Zhang, X., Zheng, J., Crowley, D., Filley, T. R. and Pan, G.: Is current biochar  
892 research addressing global soil constraints for sustainable agriculture?, *Agriculture, Ecosystems  
893 & Environment*, 226, 25–32, doi:10.1016/j.agee.2016.04.010, 2016.





894 Table

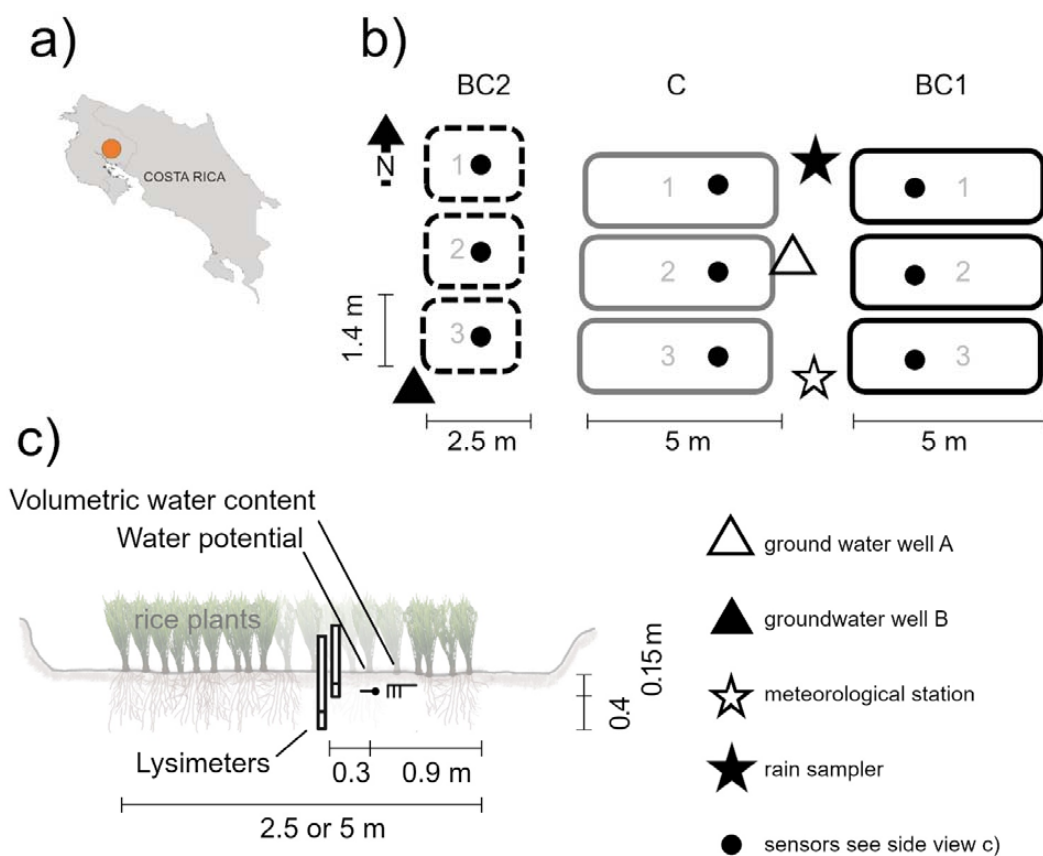
895 **Table 1** Response ratios for wilting point ( $\theta_{WP}$ ), minimum observed average volumetric soil moisture contents ( $\theta_{min}$ ) field  
 896 capacity ( $\theta_{FC}$ ), and for the van Genuchten parameters  $\alpha$  and  $n$  (Equation 1) for BC1 and BC2. Parameters are  
 897 derived for the average soil water retention curve of figure 4 for Periods I-III. A response ratio  $RR > 1$  indicates  
 898 that biochar has a positive effect on a soil water content while a  $RR \approx 1$  indicates that biochar has no effect,  
 899 while  $RR < 1$  indicates a negative response for the variable of interest.

	BC	Period I	Period II	Period III
$\theta_{WP\ BC} \theta_{WP\ C}^{-1}$	1	1.36	1.46	1.18
	2	1.16	1.32	1.03
$\theta_{min\ BC} \theta_{min\ C}^{-1}$	1	1.12	1.16	1.17
	2	1.08	1.03	1.11
$\theta_{FC\ BC} \theta_{FC\ C}^{-1}$	1	1.08	1.14	1.04
	2	1.13	1.13	0.88
$\alpha_{BC} \alpha_C^{-1}$	1	1.29	0.50	0.68
	2	3.21	1.34	1.08
$n_{BC} n_C^{-1}$	1	1.00	0.89	1.47
	2	1.00	0.92	1.06

900



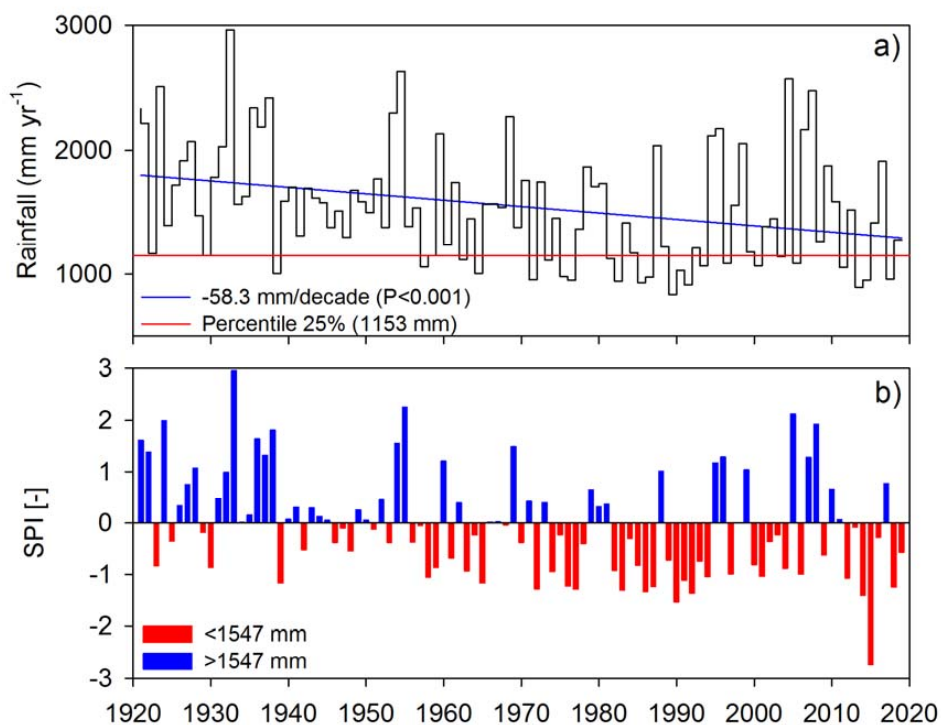
901 Figures



902

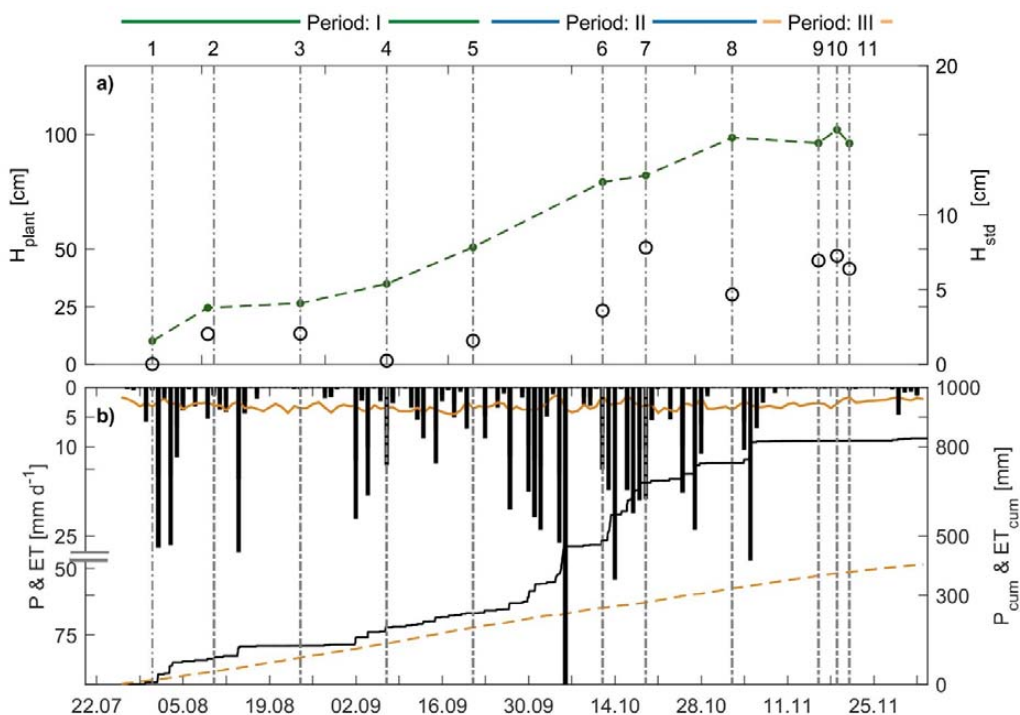
903 **Figure 1** (a) Map of Costa Rica with location of the experimental site (orange circle), (b) schematic top view of the rice  
 904 experiment with the three different treatment sections, BC1, BC2 and C. Symbols indicate the different  
 905 instruments: rain sampler for stable isotope samples (filled star), meteorological station (open star), continuous  
 906 groundwater level measurements in well A (open triangle), groundwater well B for stable isotope samples  
 907 (closed triangle) and (c) a schematic side view of a plot with suction lysimeters for stable isotope samples 15 cm  
 908 and 40 cm below the surface, the water potential and volumetric water content sensors.

909



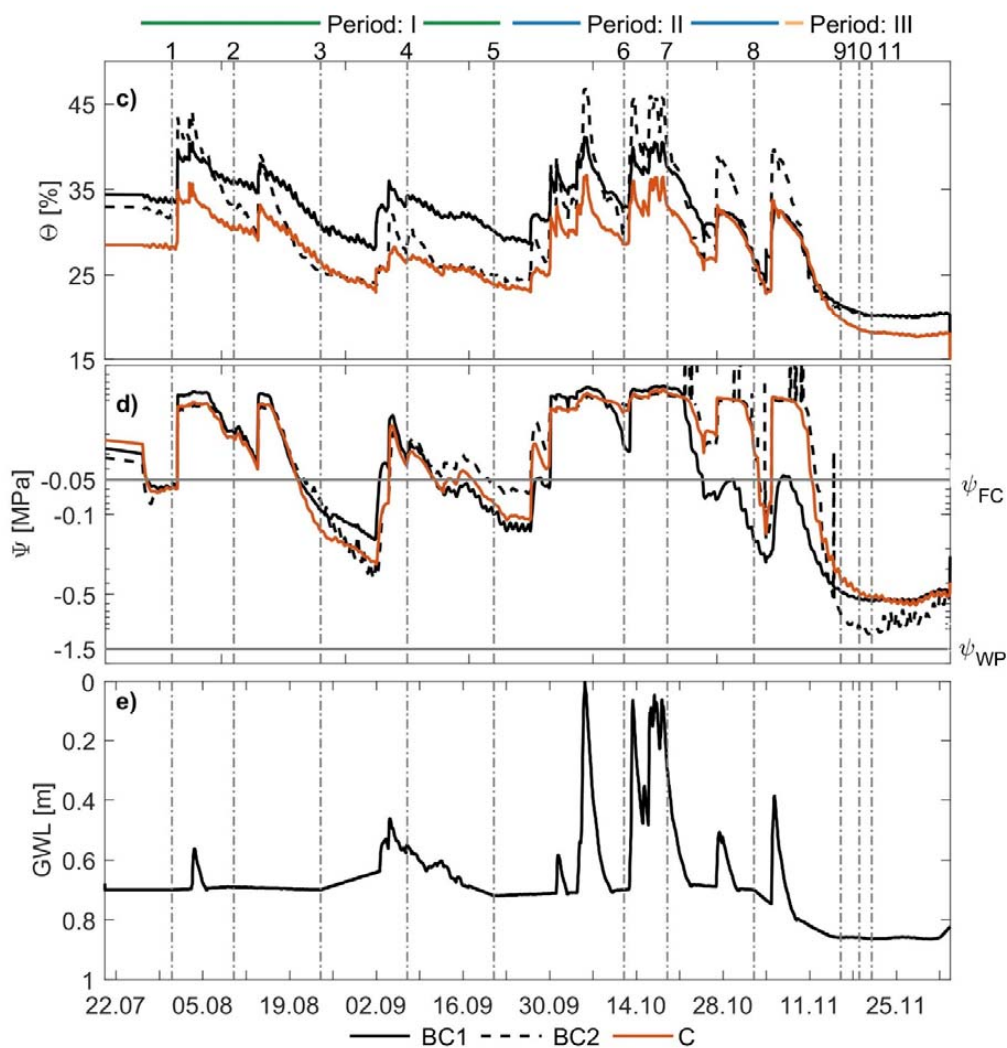
910

911 **Figure 2** (a) Long-term rainfall (mm yr<sup>-1</sup>) including a significant rainfall decrease of -53 mm per decade (blue line) and  
912 25% percentile of 1153 mm (red line as reference) and b) Standardized Precipitation Index (SPI) within the  
913 lowlands of Guanacaste between 1921-2019 (Long-term rainfall average=1547±473 mm yr<sup>-1</sup>)(Rainfall data  
914 source: Ing. Werner Hagnauer, Cañas, Guanacaste).



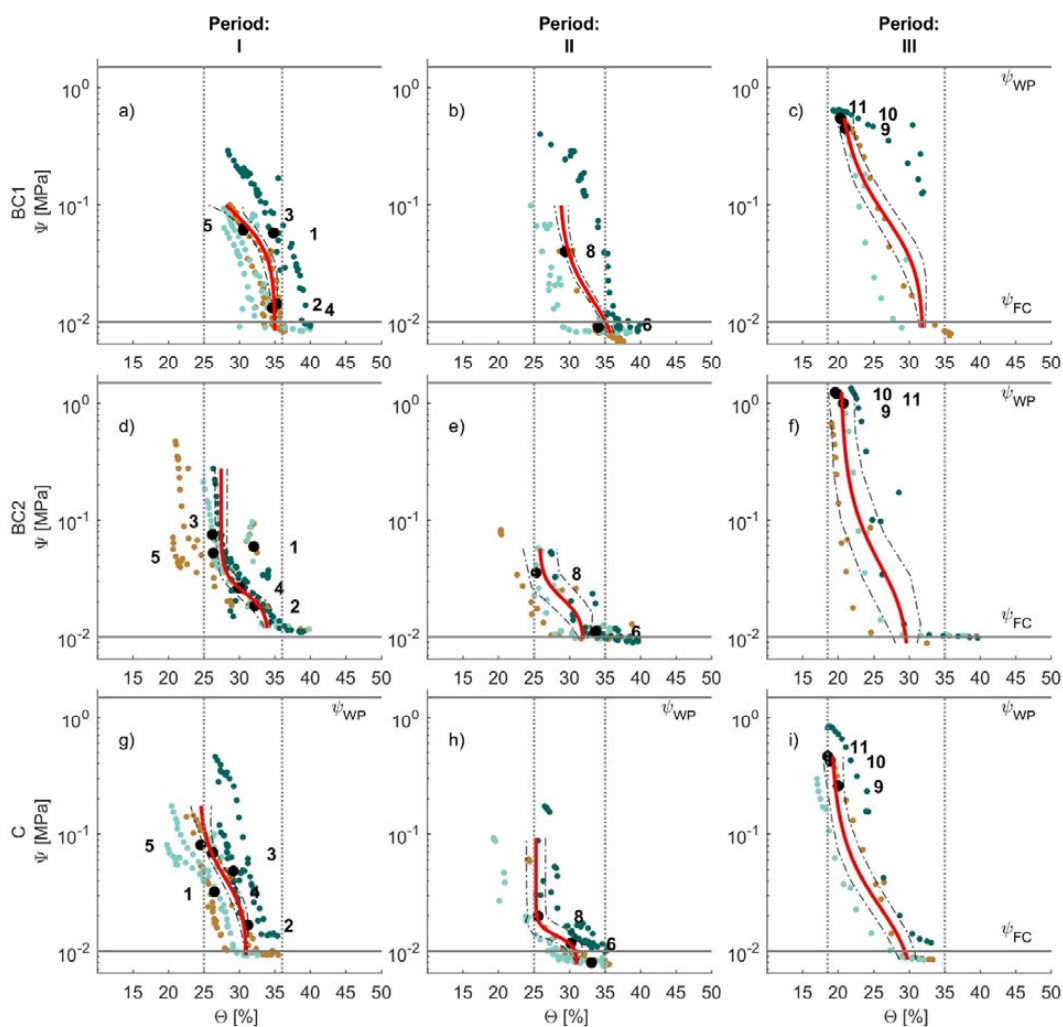
915

916 **Figure 3** Time series of (a) rice plant average height ( $H_{plant}$ ) of the rice plants (filled green circles and dashed line) and  
 917 the standard deviation the plant height (open black circles); b) precipitation (P, black bars), estimated  
 918 evapotranspiration (ET, solid orange line), accumulated P (solid black line) and accumulated ET (orange  
 919 dashed line). The different water sampling days 1-11 are indicated in each panel as vertical dashed lines and  
 920 numbered on top of panel a and the date are given on the x-axis of panel b as dd.mm. Period I, II and III are  
 921 indicated on the top of panel c.

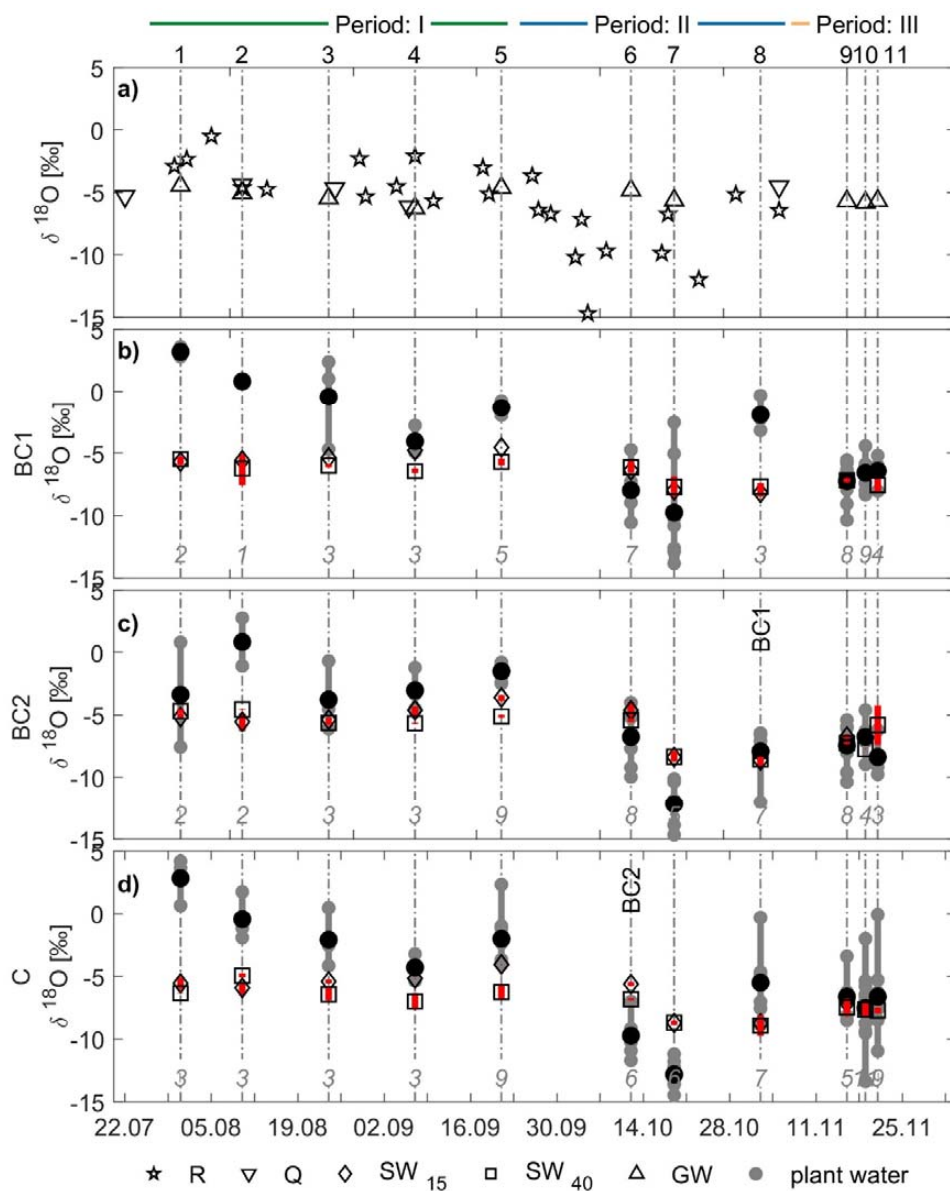


922

923 *Figure 3 (continued) Time series of: (c) average volumetric water content and (d) the average water potential for each*  
924 *treatment; (e) measured groundwater level. The different water sampling days 1-11 are indicated in each panel*  
925 *as vertical dashed lines and numbered on top of panel c and the date are given on the x-axis of panel e as*  
926 *dd.mm. Period I, II and III are indicated on the top of panel c.*

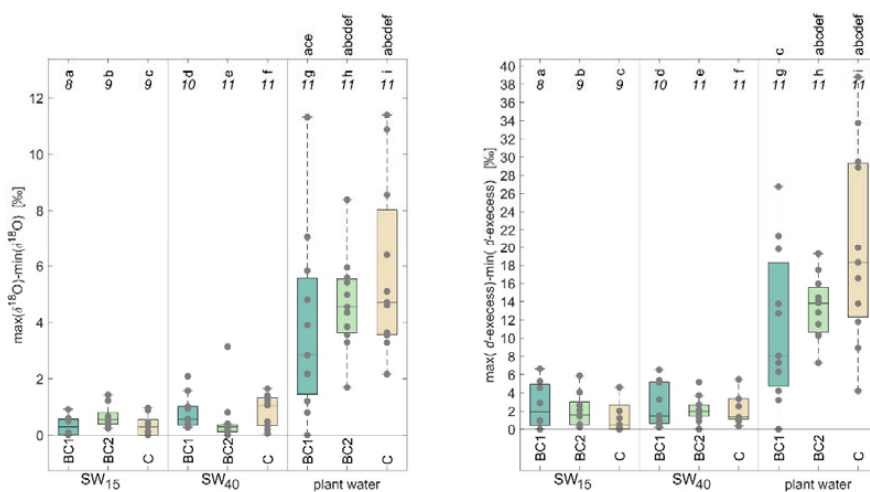


928 **Figure 4** The soil matric potential represented as a function of average soil water content of the different plots (colors)  
 929 for the treatments BC1 (a-c), BC2 (d-f) and C (g-i) and the Periods I, II and III (columns). The fitted average  
 930 soil water retention curves within a treatment using equation 1 (red line) including the 95% confidence interval  
 931 (dashed line). Black circles indicate the soil water content and soil matric potential on the sampling days  
 932 indicated by numbers.



933

934 **Figure 5** Time series of (a)  $\delta^{18}\text{O}$  in rainfall, irrigation water, ground water and (b-d) soil water sampled at 15 cm ( $\text{SW}_{15}$ )  
 935 and 40 cm ( $\text{SW}_{40}$ ), ranges of  $\delta^{18}\text{O}$  of SW (red line). The  $\delta^{18}\text{O}$  of plant water (grey circle) and its average (black  
 936 circle) are shown for the BC1 (b), BC2 (c) and control treatment (d), for sampling days 1-11 (indicated in each  
 937 panel as vertical dashed lines and numbered on top of panel a). Period I, II and III are indicated on the top of  
 938 panel a. Italic numbers in panels b-d indicate the numbers of plants samples. Significant differences among the  
 939 average plant water values (per treatment  $n > 3$ ) of each sampling day are on the vertical dashed lines as letter  
 940 of the treatment e.g. BC1, BC2 or C (Tukey's honestly significant difference criterion  $\alpha = 0.05$ ).

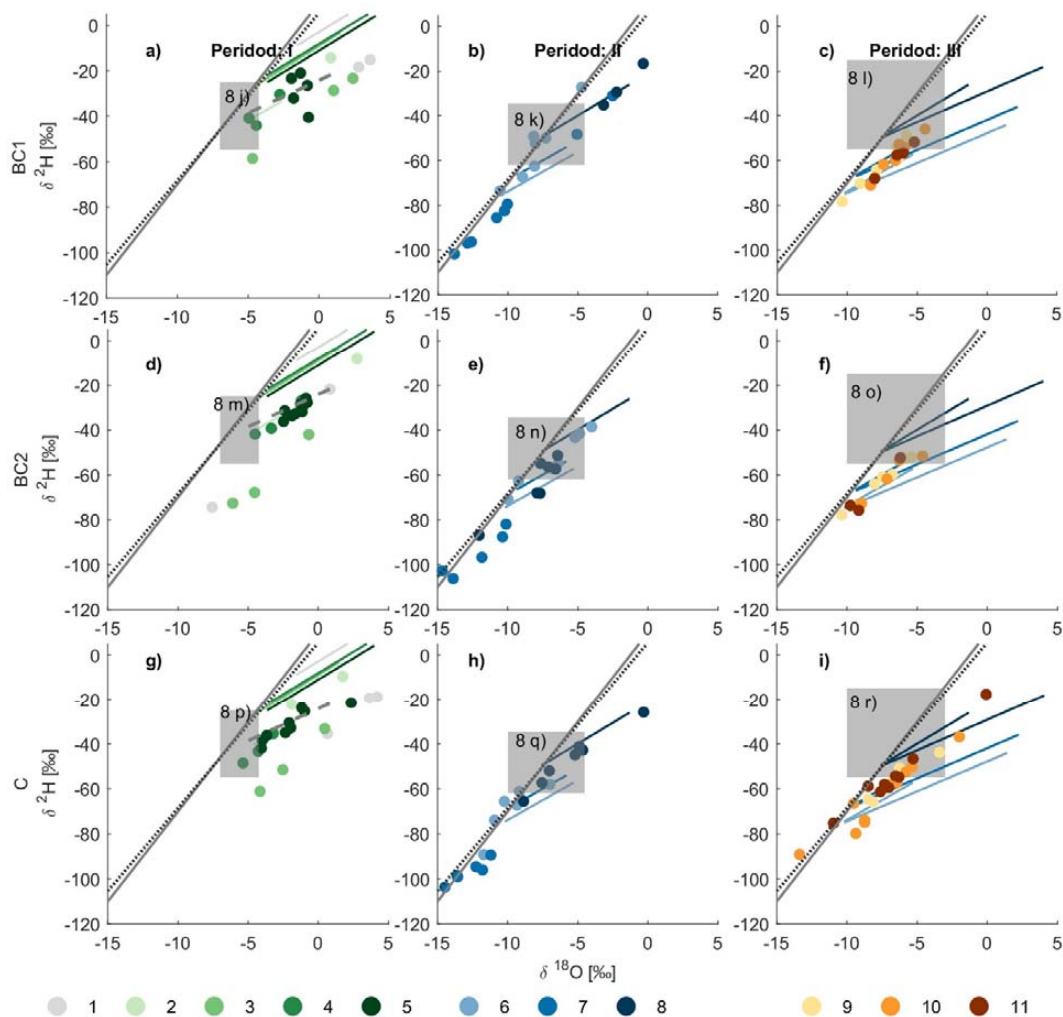


94

942 **Figure 6** The variability in stable isotope composition  $\delta^{18}O$  (left) and d-excess (right) expressed as range (maximum-  
 943 minimum observed isotopic composition) for the soil water collected at 15 cm (SW<sub>15</sub>) and 40 cm (SW<sub>40</sub>) below  
 944 surface, and plant water in the BC1, BC2 and control treatment. The boxes show the range of values for  
 945 different sample groups (showing the median and the interquartile range, with whiskers indicating 10<sup>th</sup> and 90<sup>th</sup>  
 946 percentiles). Circles indicate the data points. Numbers above each box indicate the number of samples available.  
 947 Letters on top of each box indicate significant differences among the average values of the different groups  
 948 (Tukey's honestly significant difference criterion  $\alpha = 0.05$ ).

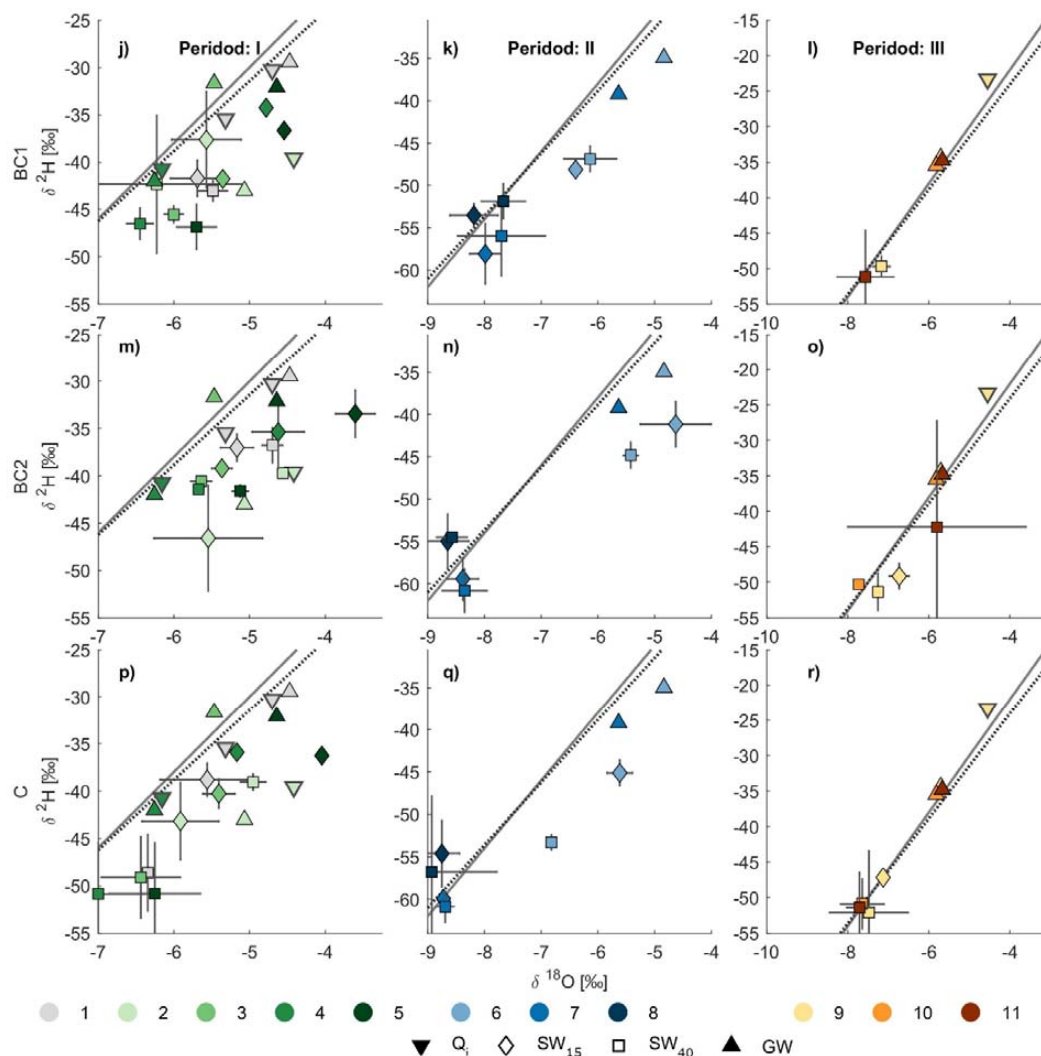
949





950

951 **Figure 7** The dual isotope space with the isotopic composition of plant water samples (circles), the calculated evaporation  
 952 lines of residual rainfall and sampled soil water for the treatments BC1 (a-c), BC2 (d-f) and C (g-i) and periods  
 953 I-III (columns). Colors indicate the different sampling days (note that lines in period III are blue because they  
 954 have been obtained from samples taken in period II). The local meteoric line (black dotted line) and global  
 955 meteoric water line (grey solid line) are indicated in all panels. The grey dashed lines (panel a, d and g) indicate  
 956 the evaporation line of median soil water. Isotopic compositions of irrigation, soil water and groundwater vary  
 957 within the grey shaded squares indicated as 8 j-8 r, and enlarged in figure 8 j-r.



958

959 **Figure 8** The dual isotope space with the isotopic composition of irrigation (down facing triangle), soil water collected at  
 960 15 cm ( $SW_{15}$ , diamond) and 40 cm ( $SW_{40}$ , square) and groundwater (upward facing triangle). The local  
 961 meteoric line (black dotted line) and global meteoric water line (grey solid line) are indicated in all panels. The  
 962 different treatments BC1 (j-l), BC2 (m-o) and C (p-r) and different periods I-III (columns) indicated in grey  
 963 panels of Figure 7 a-i. Colors indicate the different sampling days.



964 **Appendix**

965 *Table A1 Soil characteristics of the experimental site.*

	BC1	BC2	C
Soil (0-20 cm) texture sand/silt/clay	34/30/36		
Infiltration capacity Wet / Dry season [mm h <sup>-1</sup> ]	15 / 30	15 / 40	8/40
pH	6.5	6.3	6.4
Ca [mol kg <sup>-1</sup> ]	11.77	12.43	11.77
Mg [mol kg <sup>-1</sup> ]	2.60	2.63	2.47
K [mol kg <sup>-1</sup> ]	0.87	0.97	0.80
P [mg L <sup>-1</sup> ]	22.3	29.0	21.6
Zn [mg L <sup>-1</sup> ]	3.2	3.3	3.1
Mn [mg L <sup>-1</sup> ]	24.0	30.6	22.0
Cu [mg L <sup>-1</sup> ]	9.3	11.0	9.6
Fe [mg L <sup>-1</sup> ]	43.00	57.33	45.00
Organic C [%]	2.29	2.18	2.16
Total N [%]	0.15		

966

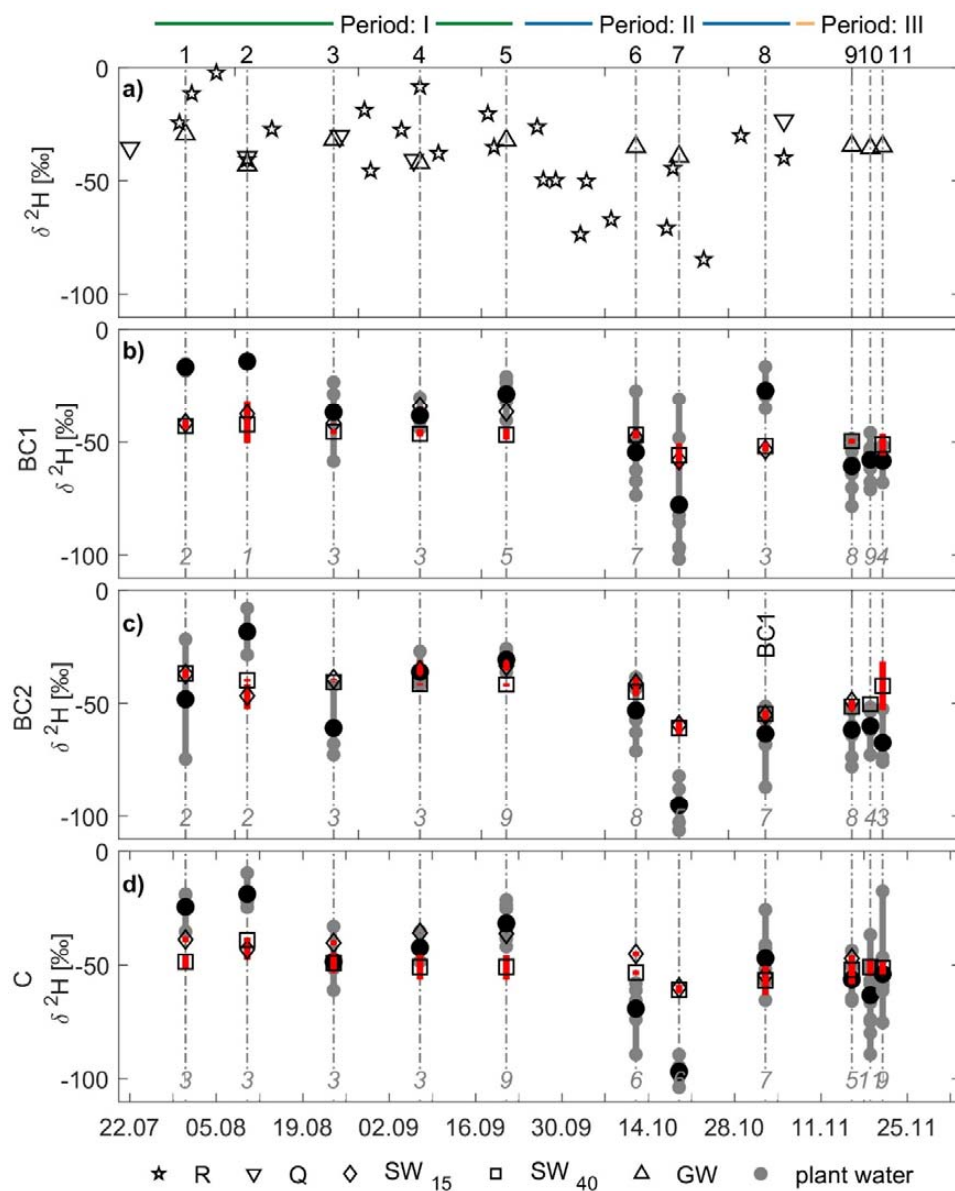
967



968 **Table A2** *The fitted parameters  $\theta$ ,  $\alpha$  and  $n$  the average soil water retention curves of the different treatments (BC1, BC2*  
 969 *and C) and the Periods I-III of equation 1 with the 95% confidence interval in brackets.*

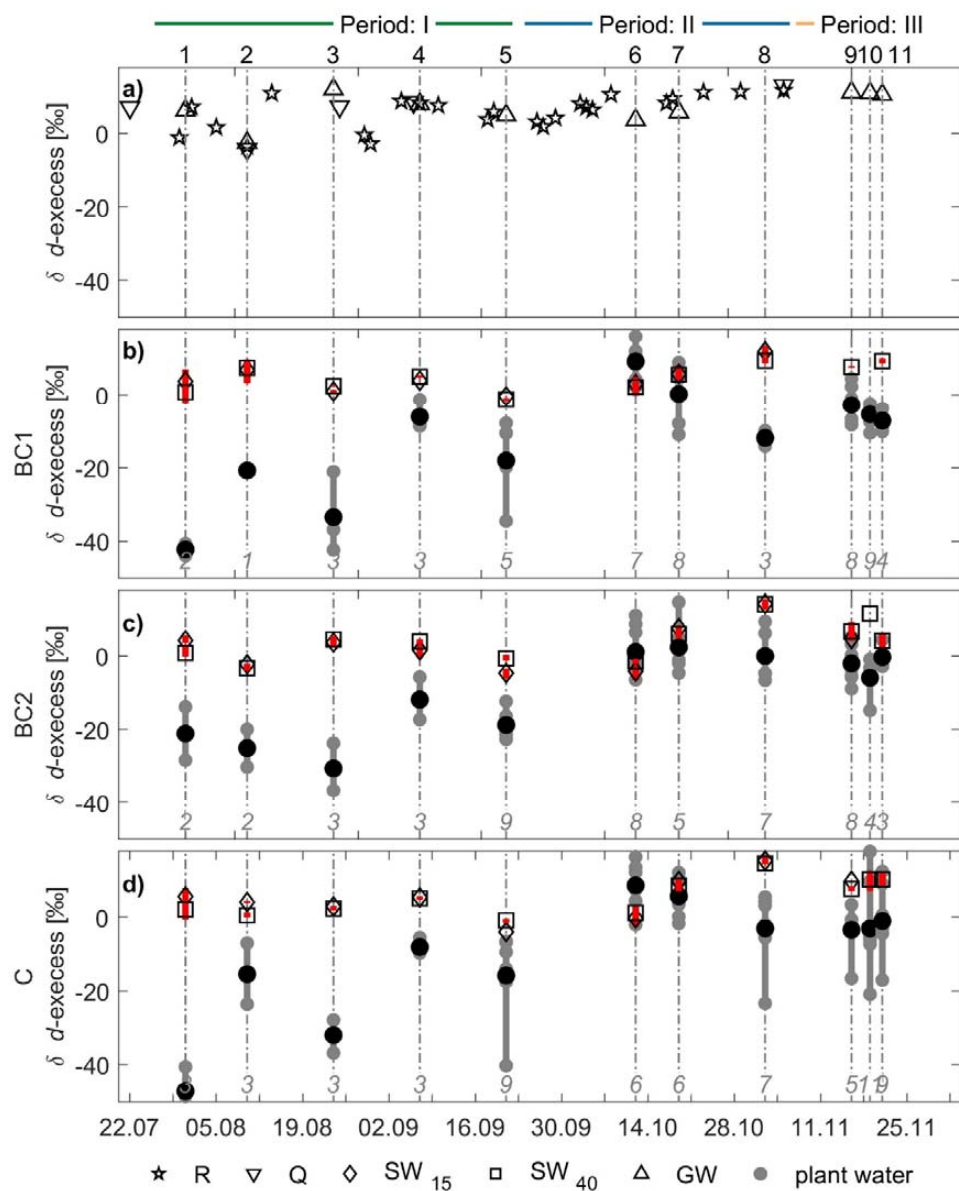
	BC1			BC2			C		
	Period			Period			Period		
	I	II	III	I	II	III	I	II	III
$\theta$	0.2 (-0.2,0.6)	0.3 (0.3,0.3)	0.2 (0.2,0.2)	0.3 (0.3,0.3)	0.3 (0.2,0.3)	0.2 (0.2,0.2)	0.2 (0.2,0.3)	0.3 (0.2,0.3)	0.2 (0.2,0.2)
$\alpha$	13 (-6.7,33)	78 (65,90)	18 (8.1,29)	44 (36,52)	49 (29,70)	34 (-14,81)	27 (20,34)	75 (61,88)	58 (30,85)
$n$	2.5 (-0.3,5.4)	2.6 (1.8,3.4)	2 (0.9,3.1)	5.7 (1.4,10)	5.1 (-0.2,12)	2 (0.2,3.8)	3 (1.2,4.8)	10 (-0.4,24)	2 (1.2,2.9)

970



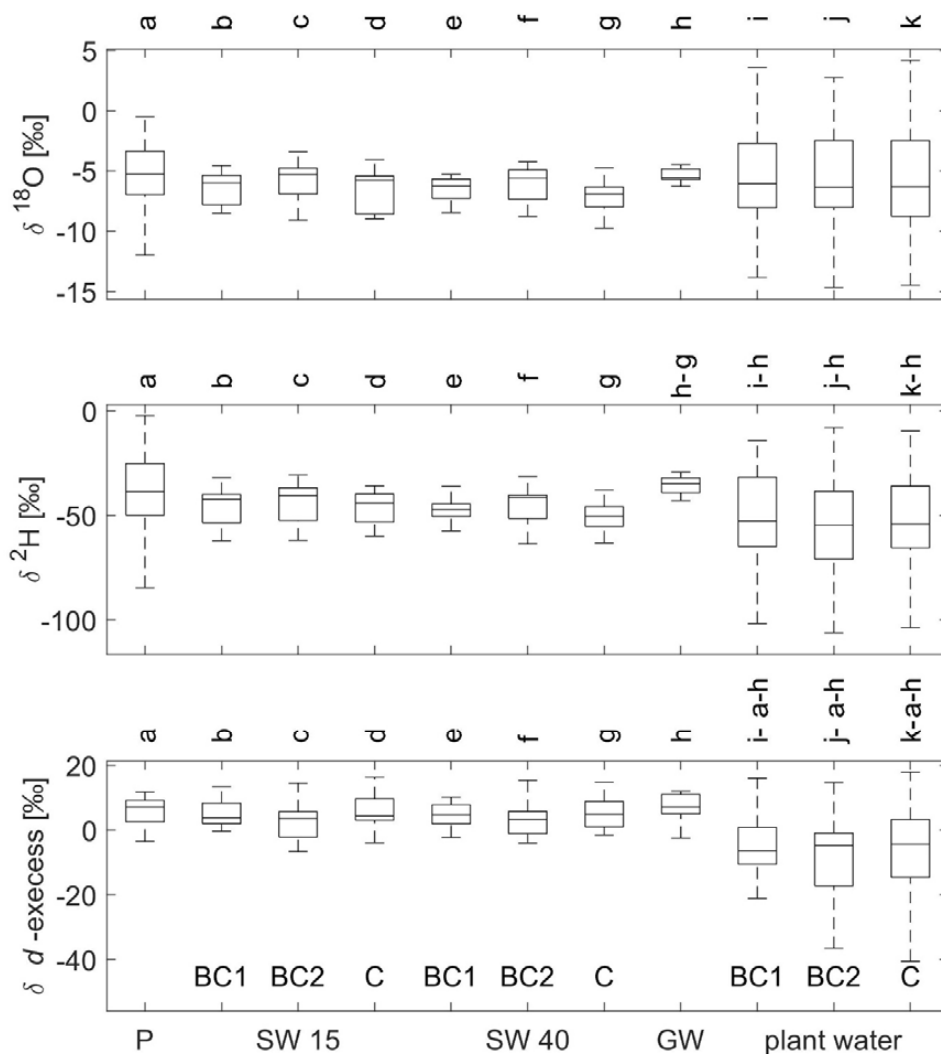
971

972 **Figure A1** Time series of (a)  $\delta^2\text{H}$  in rainfall, irrigation water, ground water and (b-d) soil water sampled at 15 cm (SW15)  
 973 and 40 cm (SW40), ranges of  $\delta^2\text{H}$  of SW (red line). The  $\delta^2\text{H}$  of plant water (grey circle) and its average (black  
 974 circle) are shown for the BC1 (b), BC2 (c) and control treatment (d), for sampling days 1-11 (indicated in each  
 975 panel as vertical dashed lines and numbered on top of panel a). Periods I, II and III are indicated on the top of  
 976 panel a. Italic numbers in panels b-d indicate the numbers of plants samples. Significant differences among the  
 977 average plant water values (per treatment  $n > 3$ ) of each sampling day are on the vertical dashed lines as letter  
 978 of the treatment e.g. BC1, BC2 or C (Tukey's honestly significant difference criterion  $\alpha = 0.05$ ).



979

980 **Figure A2** Time series of (a) *d*-excess in rainfall, irrigation water, ground water and (b-d) soil water sampled at 15 cm  
 981 (*SW*<sub>15</sub>) and 40 cm (*SW*<sub>40</sub>), ranges of *d*-excess of *SW* (red line). The *d*-excess of plant water (grey circle) and its  
 982 average (black circle) are shown for the BC1 (b), BC2 (c) and control treatment (d), for sampling days 1-11  
 983 (indicated in each panel as vertical dashed lines and numbered on top of panel a). Periods I, II and III are  
 984 indicated on the top of panel a. Italic numbers in panels b-d indicate the numbers of plants samples. Significant  
 985 differences among the average plant water values (per treatment *n*>3) of each sampling day are on the vertical  
 986 dashed lines as letter of the treatment e.g. BC1, BC2 or C (Tukey's honestly significant difference criterion  $\alpha =$   
 987 0.05). The *d*-excess was defined as  $d\text{-excess} = \delta^2\text{H} - 8 \cdot \delta^{18}\text{O}$  (Dansgaard, 1964) using data from Figure 5 and  
 988 A1.



989

990 **Figure A3** The variability in stable isotope composition  $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$  and  $d$ -excess. The x-axis indicates the sampled  
 991 precipitation, soil water collected at 15 cm ( $SW_{15}$ ) and 40 cm ( $SW_{40}$ ) below surface, groundwater and plant  
 992 water where BC1, BC2 and C indicate the three different treatments. The boxes show the range of values for  
 993 different sample groups (showing the median and the interquartile range, with whiskers indicating 10th and  
 994 90th percentiles). Letters on top of each box indicate significant differences among the average values of the  
 995 different groups (Tukey's honestly significant difference criterion  $\alpha = 0.05$ ).