

1           **Ground cover rice production systems increase soil carbon**  
2                           **and nitrogen stocks at regional scale**

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21 **Abstract**

22 Rice production is increasingly limited by water scarcity. Covering paddy rice soils with films  
23 (so called ground cover rice production system: GCRPS) can significantly reduce water  
24 demand as well as overcome temperature limitations at the beginning of the growing season,  
25 which results in greater grain yields in relatively cold regions and also in those suffering from  
26 seasonal water shortages. However, it has been speculated that both increased soil aeration  
27 and temperature under GCRPS result in lower soil organic carbon and nitrogen stocks. Here  
28 we report on a regional-scale experiment conducted in Shiyan, a typical rice-producing  
29 mountainous area of China. We sampled paired adjacent Paddy and GCRPS fields at 49  
30 representative sites. Measured parameters included soil carbon (C) and nitrogen (N) stocks (to  
31 1m depth), soil physical and chemical properties,  $\delta^{15}\text{N}$  composition of plants and soils,  
32 potential C mineralization rates, and soil organic C (SOC) fractions at all sampling sites. Root  
33 biomass was also quantified at one intensively monitored site.

34 The study showed that: 1) GCRPS increased SOC and N stocks 5-20 years following  
35 conversion from traditional Paddy systems; 2) there were no differences between GCRPS and  
36 Paddy in soil physical and chemical properties for the various soil depths with the exception  
37 of soil bulk density; 3) GCRPS increased above-ground and root biomass in all soil layers  
38 down to a 40 cm depth; 4)  $\delta^{15}\text{N}$  values were lower in soils and plant leaves indicating lower  
39  $\text{NH}_3$  volatilization losses from GCRPS than in Paddy systems; and 5) GCRPS had lower C  
40 mineralization potential than that observed in Paddy systems over a 200 days incubation  
41 period. Our results suggest that GCRPS is an innovative production technique that not only  
42 increases rice yields using less irrigation water, but that it also increases SOC and N stocks.

43

44 Key words: soil organic carbon and nitrogen stocks, region scale evaluation, water-saving rice,  
45 above- and below-ground biomass,  $\delta^{15}\text{N}$ , potential carbon mineralization rates.

## 46 **1 Introduction**

47 Globally more than 3 billion people depend on rice as a staple food (FAOSTAT,  
48 2011). Water used for irrigation is becoming increasingly scarce due to growing water  
49 demands from increasing populations and economies across Asia and from projected  
50 climatic changes. It is expected that by 2025 about 15 million ha of irrigated rice, 27  
51 million ha of rainfed rice, and nearly 20 million ha of rainfed upland rice will suffer  
52 from water scarcity worldwide (Bouman, 2007). An annual increase of about 8-10  
53 million tons will be required to meet the global forecasted needs over the next 20  
54 years (IRRI, 2011). In this scenario, water-saving technologies are urgently needed to  
55 cope with such rice production demands.

56

57 China is the world's largest rice producer with an average rice production rate of 197  
58 million tons yr<sup>-1</sup>, which in 2009 was grown on c.30 million hectares and accounted for  
59 43.7% of the total national cereal grain production (Fan et al., 2010). Water shortages  
60 already affect more than 4 million ha devoted to rice production in China, and a  
61 significant proportion of this area also show comparatively low yields resultant from  
62 low-temperature limitations. One of the most promising techniques to overcome these  
63 limitations is the Ground Cover Rice Production System (GCRPS). Here, the soil is  
64 covered - typically with plastic film - to reduce evaporation, seepage losses and  
65 increase springtime soil temperatures. The soil is kept moist between irrigation  
66 periods thanks to the covering material, which reduces irrigation water demand by 50-  
67 90%. The actual reduction in irrigation water demand is dependent on soil type,  
68 precipitation and cultivation duration (Tao et al., 2006; Liu et al., 2003). Furthermore,  
69 high-yielding lowland rice varieties (middle-duration cultivar, about 140 days) can  
70 still be grown in upland locations using GCRPS, which results in similar or even

71 greater yields than Paddy systems (Qu et al., 2012; Liu et al., 2013, 2014, Tao et al  
72 2015). Thus, GCRPS is consistent with China's 12<sup>th</sup> Five Year Plan that requires  
73 development of technologies to reduce the water demand and greenhouse gas  
74 emissions (GHG) in agricultural production (Yao et al., 2014; Tao et al., 2015).

75

76 Improving rice production systems should not be solely focused on increasing  
77 productivity, but should also consider other aspects affecting sustainability, such as  
78 preservation of optimal levels of SOC and total N. Soil organic matter (SOM) helps  
79 maintain soil structure and fertility, decreases the risk of soil erosion and degradation  
80 (Watts et al., 2006; Powlson et al., 2011), provides nutrients to plants and soil  
81 microbes (Tiessen et al., 1994), and increases soil water holding capacity, thereby  
82 improving the systems' ability to resist drought stress (Rawls et al., 2003). The  
83 sustainability of a production system tends to be correlated with the maintenance or  
84 increase of SOM stocks, which tends to lead to increased yield potentials worldwide  
85 (Lehmann, 2007). The amount of organic C stored in a soil is a fine balance between  
86 organic C inputs, mineralization and lateral exports (Jenny, 1941; Amundson, 2001).  
87 These processes are strongly affected by temperature, plant available water, soil  
88 mineral composition, and the chemical properties of the precursor biomass (Swift,  
89 2001; Saiz et al., 2012).

90

91 Compared to upland cereals production systems, submerged paddy rice cultivation is  
92 considered to be a sustainable cropping system because the permanent presence of  
93 water results in anoxic conditions that drive soil redox potential to the lowest natural  
94 levels (Gao et al., 2004; Pan et al., 2010). It is widely acknowledged that  
95 decomposition of SOM is slower in submerged than in aerated soils (Sahrawat, 2004),

96 and previous studies have shown that continuous rice cropping on submerged soils  
97 may favour the maintenance, and even the increase of SOM stocks (Cassman et al.,  
98 1995; Bronson et al., 1997; Witt et al., 2000). While some studies have shown that  
99 GCRPS accelerated SOM decomposition and resulted in a decline in soil SOM stocks  
100 in the topsoil above the hardpan (between 20-40 cm) (Li et al., 2007; Fan et al., 2012;  
101 Qu et al., 2012), a thorough regional-scale evaluation of GCRPS effects on SOC and  
102 total N stocks has not yet been reported. The shift from flooded soils to higher  
103 aeration and soil temperatures at the start of the growing season may result in reduced  
104 CH<sub>4</sub> emissions, while N<sub>2</sub>O emissions (Kreye et al., 2007; Yao et al., 2014) and C  
105 mineralization rates may increase (Koch et al., 2007). On the other hand, high  
106 ammonia volatilization in Paddy systems tends to result in low N use efficiency  
107 (approx. 30%) (Ju et al., 2009) and covering the soil surface might reduce ammonia  
108 volatilization rates.

109

110 To evaluate the impact of GCRPS on soil C and N stocks as well as identifying the  
111 primary N loss pathways from GCRPS and Paddy using the natural abundances of <sup>15</sup>N,  
112 we conducted a field study sampling 49 pairs of neighbouring GCRPS and Paddy  
113 fields in the Shiyan region, Central China, where the GCRPS technique was first  
114 introduced approximately 20 years ago. We hypothesized that improved soil moisture  
115 conditions and increased soil temperature and redox potential in GCRPS would  
116 stimulate soil C and N mineralization, leading to a reduction of soil C and N stocks  
117 under GCRPS at a regional scale.

118

119

120

## 121 **2 Materials and methods**

### 122 **2.1 Sampling region characteristics**

123 The study was situated in Shiyan region, Hubei province, Central China (32°02' to  
124 33°10'N, 109°44'to 111°04'E, 169 m to 661 m a.s.l., see Table S1), where GCRPS  
125 was introduced at the end of the last century (Shen et al., 1997; Liang et al., 1999).  
126 Shiyan is located in the QinBaShan Mountains with peaks reaching a maximum  
127 altitude of 2740 m a.s.l.. The area is in the northern subtropical agro-climatic zone of  
128 China's eastern monsoon region (Smit and Cai, 1996). Low temperatures at the start  
129 of the growing season together with severe seasonal water scarcity often limit rice  
130 production in these mountainous regions (Shen et al., 1997). The mean annual  
131 temperature and rainfall (calculated for the 1961-2009 period from seven  
132 meteorological stations located in the respective counties of Shiyan) are 15.3 °C and  
133 829 mm respectively (Zhu et al., 2010). There is little interannual variation in  
134 temperature and rainfall (coefficient of variations of 0.01 and 0.05). Annual rainfall  
135 patterns show pronounced seasonality, with approximately 45% (375 mm) of the  
136 rainfall occurring during the summer period (June to August). The mean total  
137 sunshine hours per year are 1835 h (Zhu et al., 2010). Given that GCRPS was  
138 introduced only two decades ago and the implications for farming activities, labour  
139 demand and associated costs, has resulted in GCRPS and traditional lowland rice  
140 cultivation (Paddy) often being spatially interwoven (Zhou et al., 2008). In most cases  
141 the adoption of GCRPS by individual farmers was documented by the local  
142 administration so it was possible to trace specific land management records for the  
143 selected sites and fields.

144

### 145 **2.2 Site and field selection**

146 Site selection was performed by experienced staff members from the Department of  
147 Agriculture in Shiyao and extension personnel who have been working closely with  
148 farmers at the individual local villages. Specific attention was paid to ensure proper  
149 representativeness of the different rice growing areas (i.e. varying altitudes,  
150 contrasting soil types and proper coverage of the range of time since adoption of  
151 GCRPS). Information on fertilizer use, and soil and crop management was obtained  
152 through farmer interviews (Table S2). Topdressing is not used in GCRPS since the  
153 plastic film covers the soil surface; rather the farmers usually broadcast all the  
154 fertilizer before transplanting (Liu et al., 2013). The day before transplanting, a  
155 compound NPK fertilizer and urea were applied to the soil surface in a single dose  
156 and incorporated into the soil by ploughing. The total N input was about 150 kg N ha<sup>-1</sup>  
157 for GCRPS. The soil surface was then levelled and covered with a transparent film 5  
158 µm thick (Liu et al., 2013). For Paddy systems, an average of 100 kg N ha<sup>-1</sup> was  
159 applied as a compound NPK fertilizer to the soil surface and incorporated to a depth  
160 of 20 cm before transplanting. At tillering and grain filling stages, additional doses of  
161 40 kg N ha<sup>-1</sup> were given as urea in order to increase rice milling quality, protein  
162 content (Wopereis-Pura et al., 2002; Leesawatwong et al., 2005) and yield. This  
163 resulted in a total N application rate of approximately 180 kg N ha<sup>-1</sup> for the paddy rice  
164 system.

165

166 We compared, across a region of 5 000 km<sup>2</sup>, 49 pairs of neighbouring fields that were  
167 managed either as traditional paddy rice fields or where GCRPS had been applied  
168 continuously for 5-20 years. A total of 49 sites with paired treatments consisting of  
169 GCRPS vs permanent flooding paddy fields (hereafter referred to as GCRPS and  
170 Paddy) were selected for soil and plant sampling. Regardless of the current production

171 system, all sites had been growing rice for more than 40 years. The distance between  
172 the paired plots were in most cases less than 100 m, with only 9 out of 49 paired plots  
173 being more than 250 m apart (Table S1). Geographical coordinates of the sites and  
174 fields were recorded by GPS (Garmin Colorado 300) and altitudes were obtained  
175 using the Global Digital Elevation Model (GDEM) provided by NASA and METI  
176 (2008).

177

### 178 **2.3 Sampling methodology and analytical procedure**

179 Soil samples from the 49 paired sites were collected before field preparation during  
180 March and April 2011. These sites represented a wide range of different soil types  
181 (Table S1). At each of the 98 fields, six to nine spatial replicates were taken with the  
182 aid of a soil corer (3.5 cm diameter) at four depths intervals (0-20, 20-40, 40-60, 70-  
183 90 cm). Additionally, three replicate samples were collected from each soil profile  
184 excavated in each field for each depth and analysed for bulk density (Blake and  
185 Hartge, 1986) and soil texture (Gee, 1986).

186

187 Soil samples for each depth interval were air dried for 5 days and sieved to 2 mm.  
188 Identifiable plant material (>2 mm) was removed during sieving. Soil pH (Mc Lean,  
189 1982) was measured in 1:2.5 soil-water solution using a combined electrode pH meter  
190 (HI 98121, Hanna Instruments, Kehl am Rhein, Germany). Extractable soil  $\text{NO}_3^-$ -N  
191 and  $\text{NH}_4^+$ -N (Keeney and Nelson, 1982) was estimated from 1:10 soil- $\text{CaCl}_2$  (0.01M)  
192 extracts using an autoanalyser (AA3, Bran & Luebbe, Nordstadt Germany). Sub-  
193 samples for determination of soil C and N concentration and  $^{15}\text{N}$  isotope natural  
194 abundance were powdered in a ball mill (MM200, Retsch, Haan Germany) with the  
195 soil carbonates removed prior to C analyses (Harris et al., 2001; Walthert et al., 2010).



196 Analyses were conducted using a Costech Elemental Analyzer (Costech International  
197 S.p.A., Milano, Italy) fitted with a zero-blank auto-sampler coupled via a ConFloIII to  
198 a Thermo Finnigan Delta V Plus isotope ratio mass spectrometer (Thermo Scientific,  
199 Waltham, MA, USA). Soil C and N stocks were calculated using element  
200 concentrations and bulk density data for all sites.

201

202 Leaves at maximum tillering stage and aboveground plant biomass at maturity stage  
203 were sampled from 36 paired sites (at some sites rice was not planted as foreseen due  
204 to a severe drought) with three replicates from each site used for analysis of  $^{15}\text{N}$   
205 natural abundance using a CN analyser coupled to a mass spectrometer (see above).  
206 Carbon and N concentrations were then determined by an elemental analyzer  
207 (EA1108). Carbon and N assimilated in aboveground biomass were calculated as the  
208 sum of grain and straw dry matter multiplied by grain and straw C or N concentration  
209 at harvest.

210

211 Root biomass was quantified at a long-term experimental site in Fang County  
212 ( $32^{\circ}07'\text{N}$ ,  $110^{\circ}43'\text{E}$ ; Fig. S1; Tao et al., 2015) where 22 paired GCRPS and Paddy  
213 sites were located (Table S1). The site consists of the two production systems (Paddy  
214 and GCRPS) and two N fertilizer application rates ( $0$ ,  $150 \text{ kg N ha}^{-1}$ ) in three-fold  
215 replication. All 12 subplots ( $8.5 \text{ m} \times 9.5 \text{ m}$ ) were arranged in a complete randomized  
216 block design. Root biomass was quantified for three replicate cores in each of the  
217 subplots. For this purpose, soil columns ( $40 \text{ cm}$  height and  $15 \text{ cm}$  diameter) were  
218 collected at the maximum tillering stage using stainless steel cylinders. The soil  
219 column was separated into depth intervals of  $0$ - $10$ ,  $10$ - $20$  and  $20$ - $40 \text{ cm}$ . Soil samples  
220 were placed in mesh bags and set in a water stream to remove soil particles and then

221 cleaned by tap water on a 0.2 mm mesh. Cleaned root samples in different soil depths  
222 were transferred into small envelopes and oven-dried at 75 °C for 24 h.

223

224 Potential soil C mineralization rates from all 49 paired Paddy and GCRPS sites were  
225 determined using a laboratory incubation assay. Three soil samples with a volume of  
226 20 cm × 10 cm × 20 cm (depth) were sampled at each site using a spade. Samples  
227 were composited and air dried. Three replicates with 30 g of soils were incubated for  
228 200 days at 25 °C at 60% soil water-holding capacity in 150 ml bottles. CO<sub>2</sub> fluxes  
229 were measured daily for the first 10 days, then every three days for the following  
230 three weeks and then every 1-2 weeks afterwards. The gas measurement period was  
231 from 5 min to 4 hours depending on CO<sub>2</sub> flux rates. For flux measurements, the jars  
232 were closed gas-tight and CO<sub>2</sub> headspace concentrations were measured with a non-  
233 dispersive infrared sensor (Premier, Dynamet, United Kingdom) at 10-second  
234 intervals. CO<sub>2</sub> fluxes were calculated from concentration changes with time,  
235 considering headspace volume, temperature and air pressure. Total cumulative  
236 emissions were obtained by summing the measured daily fluxes using trapezoidal  
237 integration assuming a linear change in flux between measurements.

238

239 Organic matter (OM) fractions were physically separated before and after incubation  
240 using a slightly modified procedure to that described in Zimmermann et al. (2007).  
241 Briefly, 30 grams of dried soil (<2 mm) were added to 161 mL water and dispersed by  
242 means of a calibrated ultrasonic probe (Labsonic 2000, B Braun, Melsungen,  
243 Germany) using a light output energy (22 J ml<sup>-1</sup>). The dispersed suspension was then  
244 wet sieved over a 53 µm mesh size until achievement of clear rinsing water. The  
245 fraction > 53 µm was dried at 40 °C and weighed. This fraction contained sand-size

246 particles and aggregates (Heavy fraction, HF), as well as particulate organic matter  
247 (Light fraction, LF). These two fractions were separated using the procedure for  
248 recovery of organic matter from soils using static dense media as described in Wurster  
249 et al. (2010). The dried fraction  $>53 \mu\text{m}$  was stirred in a water:sodium polytungstate  
250 solution with a density of  $1.87 \text{ g cm}^{-3}$ . The mixture was centrifuged at 1000 g for 15  
251 min, and allowed to settle overnight prior to freezing. The LF was subsequently  
252 decanted and both fractions were then washed with deionized water, dried at  $40 \text{ }^\circ\text{C}$  and  
253 weighed. The solution  $<53 \mu\text{m}$  (silt and clay) was filtered through a  $0.45 \mu\text{m}$   
254 membrane filter and the material retained in the membrane (s+c) was then dried at  
255  $40 \text{ }^\circ\text{C}$  and weighed. An aliquot of the filtrate was frozen to determine the amount of  
256 dissolved organic carbon (DOC) using a C/N liquid analyser (Multi N/C 3100  
257 Anaytik Jena, Jena, Germany).

258

## 259 **2.4 Statistical Analyses**

260 All statistical analysis and calculations were performed in the Statistics Analysis  
261 System (SAS, version 8.2). Shapiro-Wilk tests were applied to check for normal  
262 distribution. Non-parametric tests were applied if the data was not normally  
263 distributed. Before any statistical test was performed, we tested for significant  
264 differences between GCRPS and Paddy according to a model that included soil type,  
265 years since conversion, soil type and elevation as potential variables influencing the  
266 percentage change of SOC/N stocks between both systems. However, we found that  
267 the percentage change of SOC/N stocks was not significantly affected by soil type,  
268 years since conversion, elevation nor by any of the interactions. Therefore, we pooled  
269 over different soil types, years since conversion and elevation in the subsequent  
270 statistical analysis (Table S3). A paired t-test was used to test for differences in soil

271 texture (clay, silt and sand content), bulk density, pH and mineral N concentrations  
272 (Nmin) between GCRPS and Paddy. All statistical analyses and calculations were  
273 performed using parametric (paired and two-tailed t-test, Pearson chi-square) and non-  
274 parametric (Wilcoxon matched pairs rank sum test; two-tailed) tests. Differences in  
275 root biomass between the two systems were tested using the general linear model  
276 (GLM) procedure. Results are expressed as arithmetic means  $\pm$  standard error of the  
277 means, levels of significance for all tests of \*=0.05, \*\*= 0.01, \*\*\*=0.001%  
278 probability level respectively and ns=not significant were used.  
279

### 280 **3 Results**

281 Average SOC concentrations and stocks were higher in GCRPS than in Paddy for  
282 each soil depth interval except for the top layer (0-20 cm; Fig. 1a, c; see Table S4 for  
283 details). Similarly, total N concentrations and stocks over the 1m profile also tended  
284 to be larger in GCRPS than in Paddy, although significant differences were only  
285 observed in the 20-40 cm depth interval (Fig. 1b, d; Table S4). There were no  
286 detectable differences in soil texture (Fig. 2a, b, c; Table S4), pH or mineral N content  
287 (Fig. 2e, f; Table S4) between GCRPS and Paddy for any soil depth interval. Soil bulk  
288 density (Fig. 2d; Table S4) tended to be lower in GCRPS than in Paddy over the 1m  
289 soil profile, although significant differences were only found in the 20-40 cm depth  
290 interval ( $P < 0.0001$ ).

291

292 Mean C and N assimilation rates in aboveground biomass at maturity were higher in  
293 GCRPS than in Paddy (Fig. 3;  $P < 0.0001$ ,  $\approx 0.0002$  for C and N). Root biomass from  
294 the one selected site was significantly affected by production system, but not by N  
295 fertilizer rates or by the interaction of production system and N fertilization (Fig. 4;  
296 Table S4). Pooled over the two N fertilizer rates, the root biomass at maximum  
297 tillering stage was significantly greater in GCRPS than in Paddy for all depth intervals  
298 down to 40 cm depth (Fig. 4).

299

300 Potential C mineralization rates did not differ between GCRPS and Paddy (data not  
301 shown), although Paddy soils showed a tendency towards higher cumulative C loss  
302 compared to GCRPS over the 200-day incubation period (Fig. 5). For the GCRPS, the  
303 SOC contents of the various fractions were similar before and after the incubation  
304 experiment (Fig. 6). However for the Paddy treatment, the amount of SOC in the

305 heavy fraction was significantly lower after incubation compared to before the  
306 incubation ( $P < 0.05$ ). No differences were found in the s+c, LF and DOC fractions  
307 before and after the incubation (Fig. 6).

308

309 Mean soil  $\delta^{15}\text{N}$  signatures were lower in GCRPS than in Paddy at each depth interval  
310 (Fig. 7a; Table S4). The average  $\delta^{15}\text{N}$  signature in plant leaves was also lower ( $P <$   
311  $0.0001$ ) in GCRPS compared to Paddy at maximum tillering stage (Fig. 7b). Ln-  
312 transformed soil N concentrations were inversely correlated with corresponding  $\delta^{15}\text{N}$   
313 values in either GCRPS or Paddy (Fig. 8).

314

315

## 316 **4 Discussion**

317 It has been hypothesized that the absence of permanently anaerobic conditions and  
318 increased soil temperatures under GCRPS may result in either no change or even  
319 increased SOC losses as a result of potentially enhanced microbial decomposition  
320 (Pan et al., 2003, 2010; Qu et al., 2012). Earlier studies showed trends towards lower  
321 SOC and total N stocks in fields using the plastic film-based GCRPS technique.  
322 However, these studies have only investigated the topsoil (0-20 cm) above the  
323 hardpan at a single experimental site (Li et al., 2007; Fan et al., 2012; Qu et al., 2012).  
324 By contrast, we sampled cultivated fields at 49 paired sites (i.e. adjacent sites  
325 experiencing comparable soil and environmental conditions, Figs. 2 and S1 and  
326 Tables S1 and S4) down to 1 m depth across an entire geographical region. Our  
327 results show that within the sampling region, conversion of Paddy to GCRPS  
328 increased SOC concentrations (Fig. 1a; Table S4) and storage (Fig. 1c; Table S4) after  
329 5 years since the time of conversion. We were able to identify two main processes that  
330 contributed to the positive effect of GCRPS on SOC stocks.

331 *a) Increased above- and belowground carbon inputs* Plant residues and organic  
332 fertilizers directly affect the amount and quality of organic matter above the hardpan  
333 (between 20 - 40 cm), while the accumulation and stabilisation of subsoil OM in these  
334 agricultural systems derives mainly from dissolved OM leached from the plough layer  
335 (Tanji et al., 2003). In our study we observed larger aboveground biomass and grain  
336 yields for GCRPS compared to traditional Paddy (Fig. 3; Liu et al., 2013).  
337 Furthermore, root biomass was also found to be greater under GCRPS cultivation in  
338 all soil layers down to 40 cm depth (Fig. 4; Table S4).

339 Recent literature has confirmed that rice cultivation under variable soil water regimes  
340 such as GCRPS results both in higher root biomass (Thakur et al., 2011; Uga et al.,

341 2013), and more rhizodeposits (Tian et al., 2013) compared to traditional flooded  
342 Paddy, likely because the larger aboveground biomass and grain yields require a  
343 larger root system to absorb more nutrients from the soil (Liu et al., 2003). GCRPS  
344 also promotes increased soil  $\text{NO}_3^-$  concentrations that can lead to more balanced plant  
345 N nutrition ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ), which is beneficial for crop growth (Nacry et al., 2013).  
346 Moreover, the fluctuating soil water content inherent to GCRPS, which varies  
347 between 80-90% water holding capacity (WHC), can limit the accessibility to some  
348 micronutrients (e.g. Mn, Fe) in the topsoil if they are oxidised to forms that cannot be  
349 directly assimilated by the plant (Tao et al., 2007; Kreye et al., 2009). For example,  
350 the lack of standing water may cause increased soil aeration, and thus, higher redox  
351 potentials (Tao et al., 2007), resulting in the oxidized form of Mn that greatly lowers  
352 its availability to the plant (Norvell, 1988). Therefore, rice plants in GCRPS need to  
353 develop stronger root systems capable of accessing deeper soil layers to obtain a  
354 balanced micro-nutrient supply. Even if just a few fine roots penetrate the hardpan  
355 they may represent a large difference in deep SOC storage as root channels may  
356 further promote percolation of organic compounds into the subsoil.

357 *b) Greater physical protection of soil organic matter against microbial degradation*

358 We conducted soil incubations under controlled environmental conditions using soils  
359 from all field sites to test whether GCRPS would enhance SOM stabilisation or  
360 increase C mineralization, promoting net losses of SOM (Xiong et al., 2014). Our  
361 results showed no significant differences in mineralization rates between soils from  
362 the GCRPS and Paddy systems for all measuring dates over a 200-day incubation,  
363 although cumulative C losses over the entire incubation period were consistently  
364 greater for Paddy soils (Fig. 5). This could suggest that SOM in fields managed under  
365 GCRPS may be more effectively preserved than SOM in traditional Paddy systems.



366 Besides the physicochemical protection offered by clay minerals (Koegel-Knabner et  
367 al., 2010; Saiz et al., 2012) other stabilizing mechanisms could be conferred through  
368 higher OM inputs resultant from enhanced above and belowground biomass  
369 production, as higher OM input rates are known to promote stable micro and  
370 mesoaggregates (Six et al., 2004). However, we did not observe significant  
371 differences between both systems in the physically protected fractions for the topmost  
372 soil layer (Fig. 6). It is likely though, that aggregation and/or stabilisation might  
373 become more relevant at deeper locations where the differences in SOC  
374 concentrations were greater. Indeed, the strong anaerobiosis and stabilisation  
375 conditions prevailing at depth would likely promote OM accumulation below the  
376 hardpan, as we found in our study (Fig. 1; Koegel-Knabner et al., 2010). Also relevant  
377 within this context is the contrasting soil redox conditions observed between the two  
378 systems (Liu et al., 2013). The more frequent oscillation in redox conditions (aerobic  
379 to anaerobic and back) in GCRPS may have a strong positive influence on the  
380 generation of organo-mineral complexes, which are of paramount importance for  
381 stabilisation of OM in Paddy soils (Koegel-Knabner et al., 2010).

382

383 Similar to SOC concentrations and stocks, soil organic N concentrations and stocks  
384 were larger in GCRPS than in paddy fields over the 1m soil profile. However,  
385 significant differences were only observed in the 20-40 cm depth interval (Fig. 1b, 1d).  
386 In addition, we observed  $\delta^{15}\text{N}$  enrichment in paddy soils for all soil depths (Fig. 7a),  
387 which was also reflected in the plant biomass (Fig. 7b). Bulk soil  $\delta^{15}\text{N}$  is a combined  
388 signal for organic and mineral N compounds and may be affected by (1) the amount  
389 and isotopic signature of applied fertilizer (Yun et al., 2011), (2) isotopic fractionation  
390 occurring during N cycle processes such as N mineralization, nitrification and

391 assimilation (Bedard-Haughn et al., 2003), and (3)  $^{15}\text{N}$  depletion of gaseous N  
392 compounds produced during denitrification and ammonia volatilization with  
393 subsequent  $^{15}\text{N}$  enrichment of the remaining soil N (Bedard-Haughn et al., 2003).  
394 Based on farmers' interviews, the dominant fertilizer used was a compound NPK  
395 fertilizer with urea as the N form ( $\delta^{15}\text{N}$  of ca. 0.5‰) (Yun et al., 2011). As well as  
396 urea-N, 11 of the 98 sites received manure ( $\delta^{15}\text{N} > 10\text{‰}$ ). Most crucially, N  
397 fertilization rates were comparable for both management systems (GCRPS: approx.  
398  $150 \text{ kg N ha}^{-1}$ ; Paddy: approx.  $180 \text{ kg N ha}^{-1}$ ). Therefore, kinetic isotope fractionation  
399 processes in the soil rather than mixing of different N sources with distinct  $\delta^{15}\text{N}$   
400 signatures likely account for the observed differences in soil  $\delta^{15}\text{N}$ . This is confirmed  
401 by the observation that Ln-transformed soil N concentrations were inversely  
402 correlated with the  $\delta^{15}\text{N}$  values (Fig. 8).

403

404 The largest fractionation factors are consistently reported for gaseous N losses  
405 (Bedard-Haughn et al., 2003; Robinson, 2001) so it is likely that changes in  $\text{N}_2$ ,  $\text{N}_2\text{O}$ ,  
406  $\text{NO}$  and  $\text{NH}_3$  losses account for the  $^{15}\text{N}$  enrichment in Paddy soils. Nitrification- and  
407 denitrification - induced losses of  $\text{N}_2$ ,  $\text{N}_2\text{O}$  and  $\text{NO}$  were expected to increase under  
408 unsaturated soils typical for GCRPS cultivation as compared to continuous flooding  
409 of Paddy soils that has also been documented in earlier studies (Kreye et al., 2007;  
410 Yao et al., 2014). Therefore, we can rule out both fertilizer effects and changes in  
411 denitrification losses as significant factors explaining lower  $\delta^{15}\text{N}$  in GCRPS soils. The  
412  $^{15}\text{N}$  enrichment in Paddy soils and increased soil N stocks under GCRPS are therefore  
413 more likely related to ammonia volatilization following fertilizer application.  
414 Ammonia loss from urea fertilization in Paddy rice fields can be very high with  
415 emission factors ranging from 9-40% of applied N (Xu et al., 2013). Covering the soil

416 with a plastic film immediately after fertilizer application (Zhuang and Wang. 2010)  
417 or manure deposits (Webb et al., 2013) greatly reduces NH<sub>3</sub> volatilization losses.  
418 Therefore, we expect that the greater soil N stocks in GCRPS fields were associated  
419 with decreased NH<sub>3</sub> volatilization.

420

## 421 **5 Conclusion**

422 We demonstrate for the first time, across a wide range of spatially representative  
423 paired sites under real farming conditions, that GCRPS significantly increased soil  
424 organic C and N stocks under varying edaphic conditions. GCRPS also increased  
425 above - and belowground root biomass in all soil layers down to 40 cm depth. These  
426 indicate that GCRPS is a stable and sustainable technique that maintains key soil  
427 functions while increasing rice yields and expanding the cultivation of a valuable crop  
428 into regions where it has been hampered by low seasonal temperatures and/or a lack  
429 of irrigation water. However, the use of plastic sheets as cover material remains an  
430 obstacle because plastic residues often remain in the field and pollute the environment.  
431 Biologically degradable films may be a suitable solution to overcome this problem,  
432 and supplying such films with micronutrients may allow a more effective and  
433 integrated nutrient management that could further boost grain yields.

434

435 **Author contributions.** M. Liu and M. Dannenmann contributed equally to this work.  
436 S. Lin and K. Butterbach-Bahl designed the experiments. M. Liu, S. Lin, M.  
437 Dannenmann, S. Sippel, Z. Yao and K. Butterbach-Bahl conducted the regional field  
438 sampling. M. Liu performed the lab analysis and statistical analysis. G. Yan and G.  
439 Saiz performed the incubation and fractionation experiment. Y. Tao and Y. Zhang  
440 carried out the field experiment and were in charge of the root biomass. M. Liu, S. Lin,  
441 M. Dannenmann, G. Saiz, K. Butterbach-Bahl and D.E. Pelster wrote the manuscript.  
442 All authors commented and revised the manuscript.

443

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450

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672 **Figure captions**

673

674 **Figure 1 Concentrations and stocks of soil organic carbon and total nitrogen in**  
675 **traditional Paddy and GCRPS at different soil depths.** Data presented are the  
676 mean values pooled over 49 paired sites (for 0-20 & 20-40 cm, n=147; 40-60 cm,  
677 n=108; 70-90 cm, n=63). Errors bars indicate the standard error of the means. \*\*\*, \*\*, \*  
678 \* Significant at 0.001, 0.01, 0.05 probability level respectively; ns-not significant.

679

680 **Figure 2 Average soil clay, silt and sand contents (for 0-20 and 20-40 cm, n=49;**  
681 **40-60 cm, n=36; 70-90 cm, n=21), soil bulk density, pH and mineral nitrogen**  
682 **concentrations ( $N_{min}$ ; for 0-20 and 20-40 cm, n=147; 40-60 cm, n=108; 70-90 cm,**  
683 **n=63) at different soil depths from 49 paired sites cultivated either under**  
684 **traditional Paddy or GCRPS.** Errors bars indicate s.e.m. \*\*\* Significant at 0.001  
685 probability level respectively; ns-not significant.

686

687 **Figure 3 Carbon and nitrogen assimilated in aboveground biomass at maturity**  
688 **(n=108).** Data presented are the means pooled over 36 paired sites (these represent all  
689 the sites where rice was grown in 2011) with three replicates at each site. Errors bars  
690 indicate s.e.m. Bars labeled with different lowercase letters indicate statistically  
691 significant differences ( $P < 0.05$ ) between Paddy and GCRPS.

692

693 **Figure 4 Root dry matter at maximum tillering stage for different soil depths in**  
694 **traditional Paddy and GCRPS.** n = 18. Error bars denote s.e.m. Bars labelled with  
695 different lowercase letters indicate differences ( $P < 0.05$ ) between Paddy and GCRPS.

696

697 **Figure 5 Differences in cumulative organic carbon mineralization during a 200 d**  
698 **incubation period of top soils (0 - 20 cm) collected from either Paddy or GCRPS.**

699 Data presented are the mean values pooled over 49 paired sites. Error bars indicate  
700 s.e.m.

701

702 **Figure 6 Relative SOC fractionation (% of total) of topsoils (0 - 20 cm) from**  
703 **either Paddy or GCRPS grown rice fields for the different physically separated**  
704 **fractions before and after a 200 d incubation period.** s+c = fraction < 53  $\mu\text{m}$ ,

705 HF/LF = heavy/light fraction > 53  $\mu\text{m}$ , DOC = dissolved organic carbon < 0.45  $\mu\text{m}$ .

706 GCRPS (n=18) and Paddy (n=18) (random selection of 18 out of 49 paired sites).

707 Error bars denote s.e.m. The asterisk indicates significant differences between pre and  
708 post incubation (P<0.05).

709

710 **Figure 7 (a) Soil  $\delta^{15}\text{N}$  isotopic signature in traditional Paddy and GCRPS at**  
711 **different soil depths.** Data presented are the mean values pooled over 49 paired sites  
712 (for 0-20 & 20-40 cm, n=147; 40-60 cm, n=108; 70-90 cm, n=63). **(b)  $\delta^{15}\text{N}$  signature**

713 **in plant leaves at maximum tillering stage.** Data presented are the means pooled  
714 over 36 paired sites (these represent all the sites where rice was grown in 2011) with  
715 three replicates at each site, n=108. Errors bars indicate the s.e.m. \*\*\*, \*\*, \*

716 Significant at 0.001, 0.01, 0.05 probability level respectively; ns-not significant. Bars

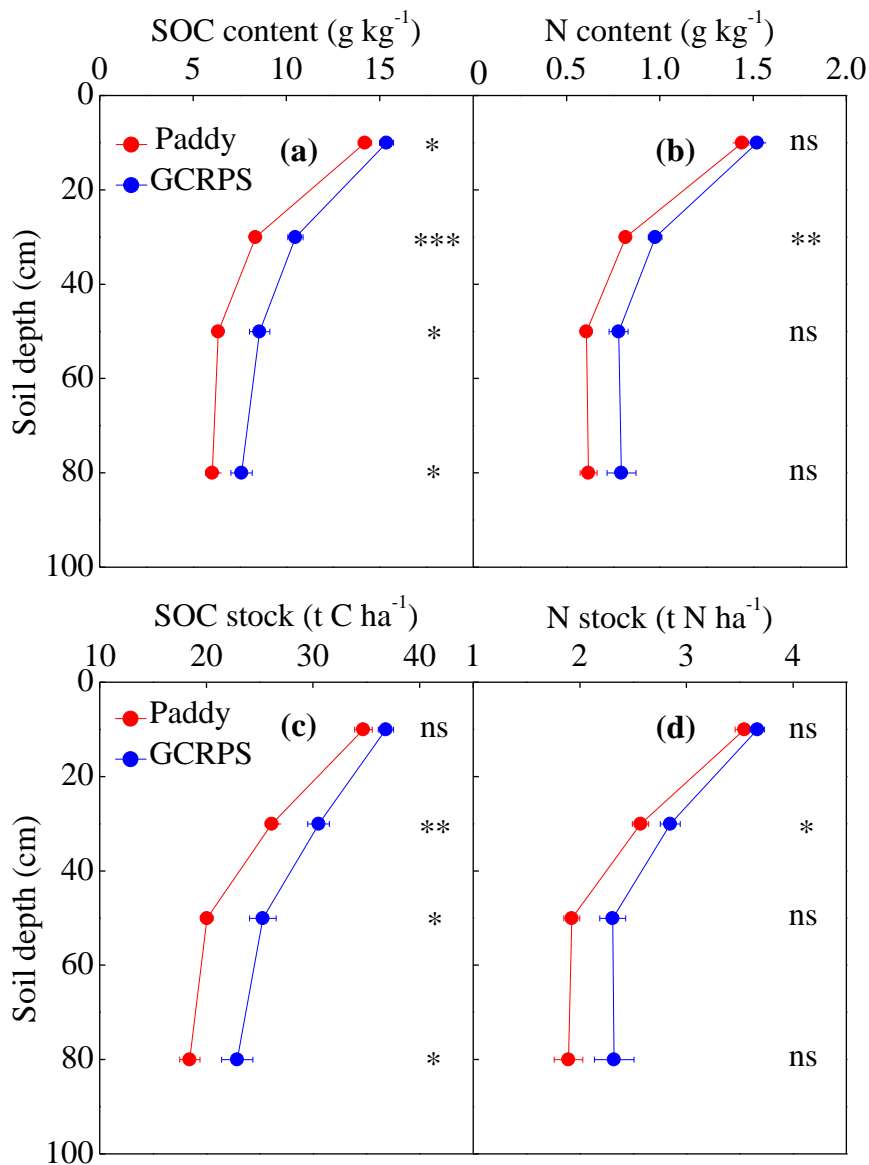
717 labelled with different lowercase letters indicate differences (P < 0.05) between Paddy  
718 and GCRPS.

719

720 **Figure 8 Correlation of  $\delta^{15}\text{N}$  with Ln transformed soil total nitrogen content up**  
721 **to 1 m depth.** Data presented are all the individual samples measured across the 49  
722 paired sites, which consist of three replicates for each site (n=465).  
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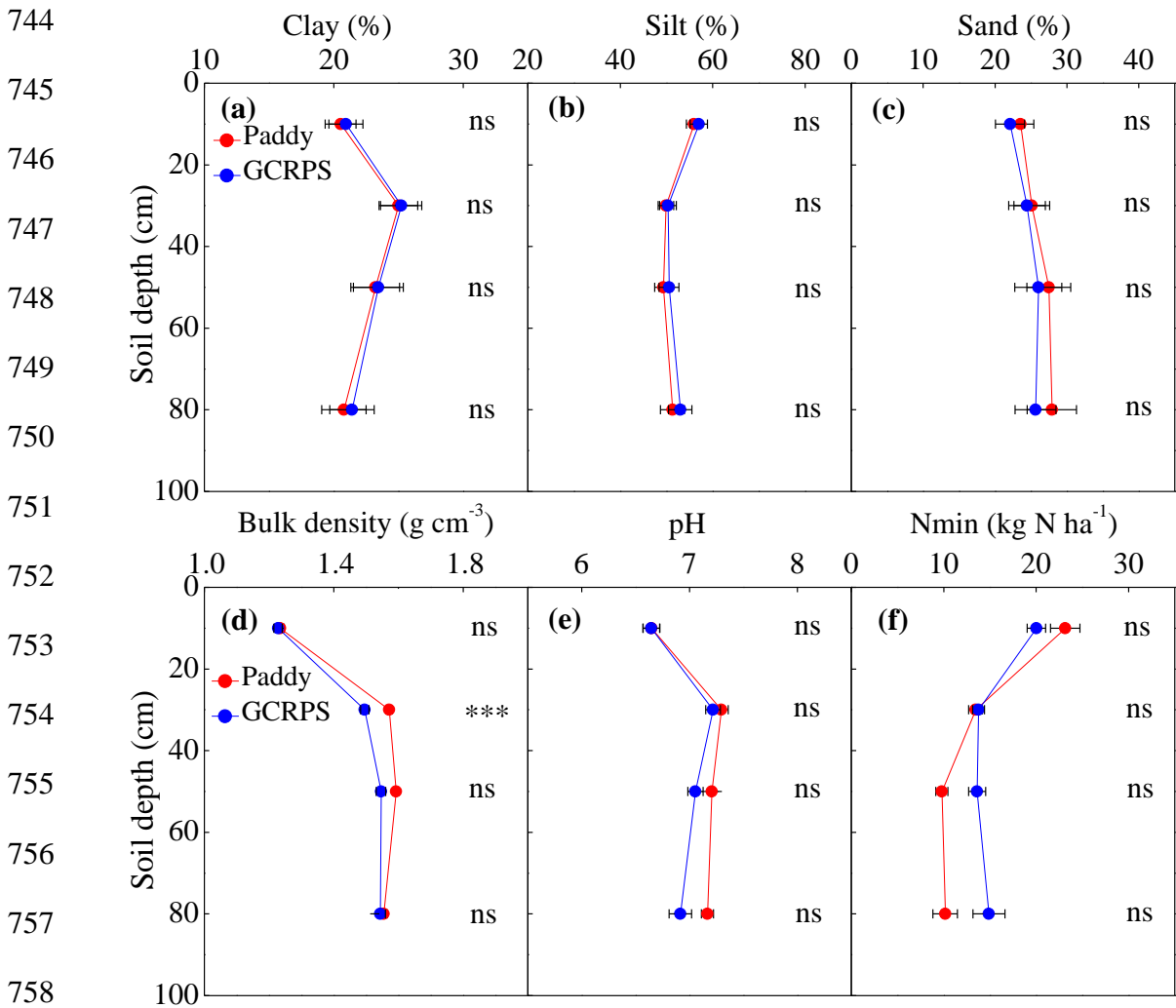


724 **Figure 1**



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743 **Figure 2**



761 **Figure 3**

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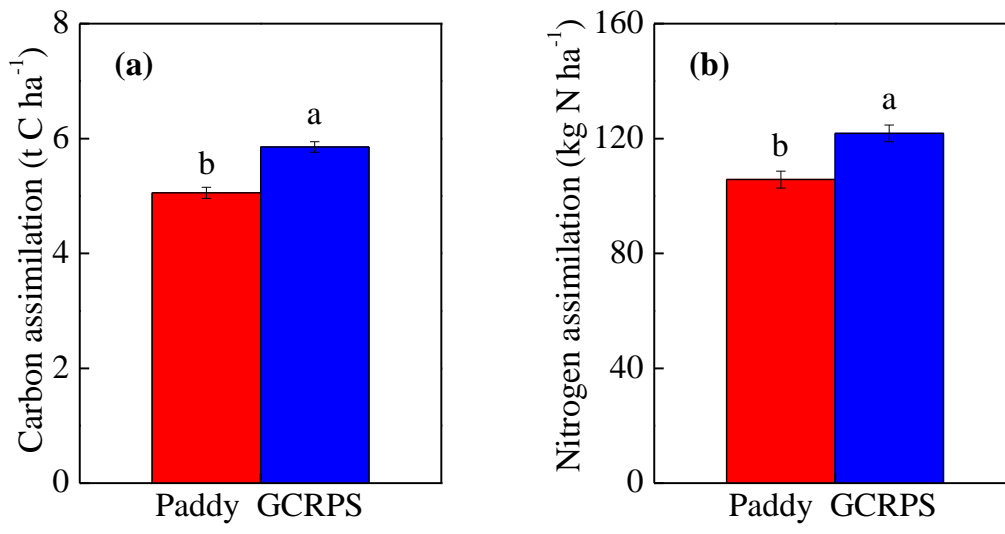
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771 **Figure 4**

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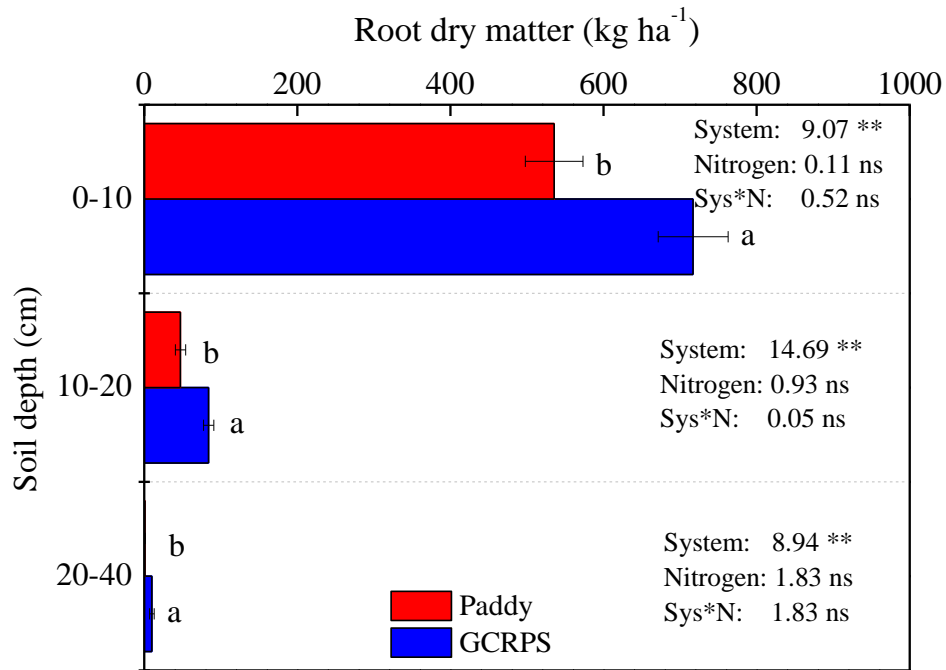
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784 **Figure 5**

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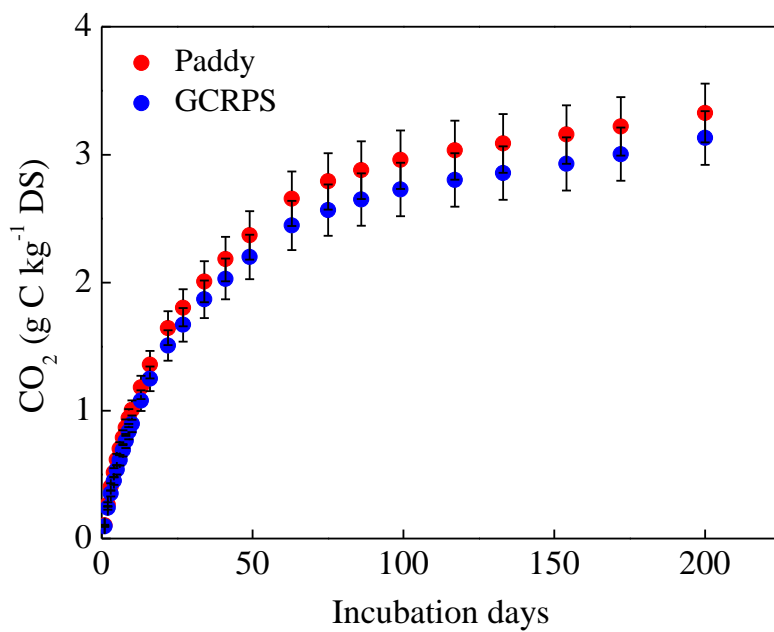
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795 **Figure 6**

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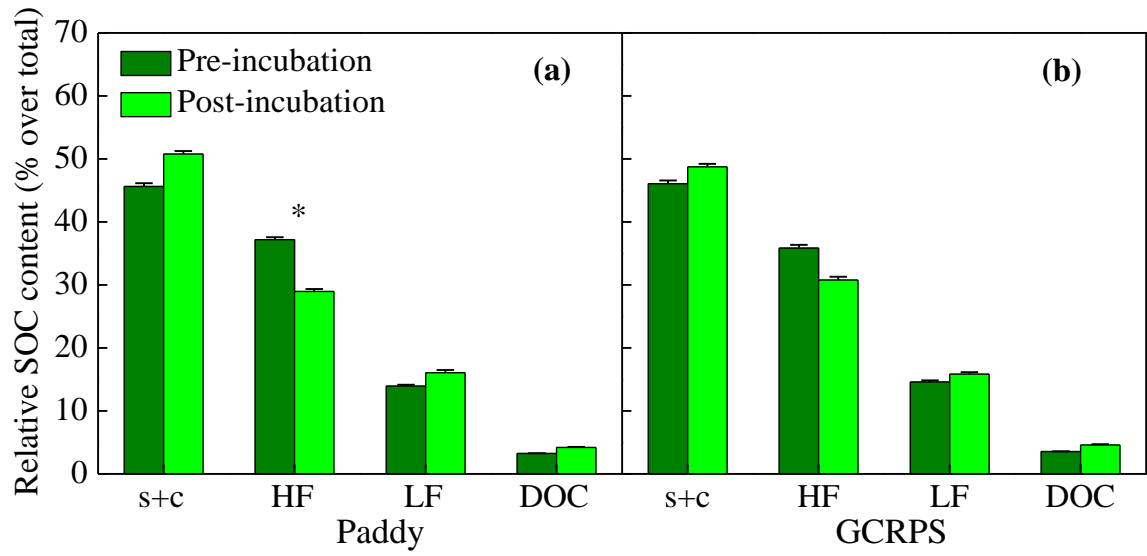
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806 **Figure 7**

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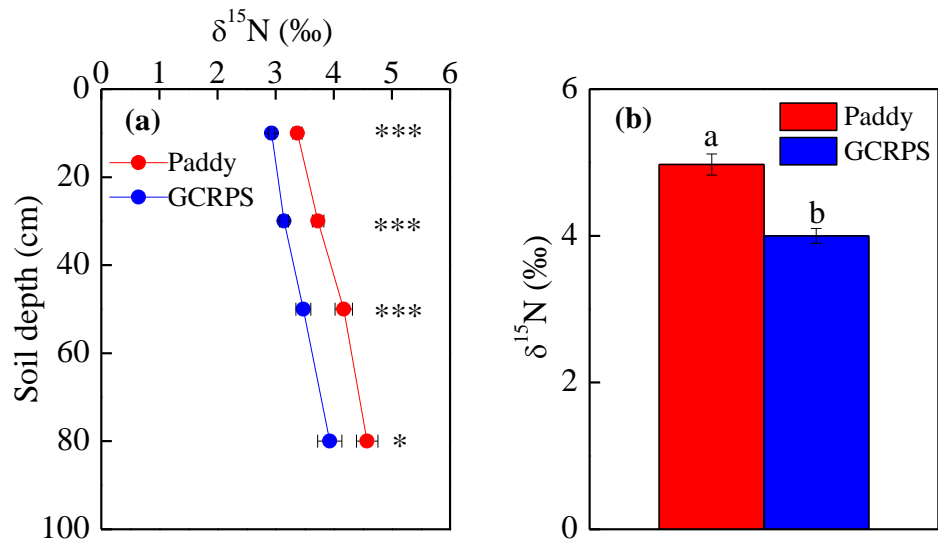
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816 **Figure 8**

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