1	<u>_Ground cover rice production system may facilitates soil</u>
2	carbon and nitrogen stocks at regional scale
3	Ground cover rice production systems increase soil carbon
4	and nitrogen stocks at regional scale
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23 Abstract

24 Rice production is increasingly challengedlimited by irrigation water scarcity., Hhowever 25 Ceovering paddy rice soils with films (so called ground cover rice production system: GCRPS) 26 can significantly reduce water demand as well as overcome temperature limitations at the beginning of the vegetation period growing season, which resultings in increased greater grain 27 28 yields in relatively colder regions and also in those of rice production with suffering from 29 seasonal water shortages. However, lit has been speculated that btheoth increased soil 30 aeration and temperature under GCRPS may results in losses of lower soil organic carbon and 31 nitrogen stocks. Here we report on a regional-regional-scale experiment, conducted in Shiyan, a typical rice-producing mountainous area of China. Weby samplinged paired adjacent Paddy 32 33 and GCRPS fields at 49 representative sites. in the Shiyan region;, which is a typical riceproducing, offor many mountainous areas for rice production acrossin China. We 34 35 MmMeasured pParameters evaluated included soil carbon (C) and nitrogen (N) stocks (to 1m depth), soil physical and chemical properties, δ^{15} -stable nitrogenN isotopic composition of 36 37 plants and -soils, , root biomass was quantified at maximum tillering stage at one of our 38 paired sites.potential carbon C mineralization rates and, fractions of soil organic carbon C 39 (SOC) fractions and stable carbon isotopic composition of plant leaves. Furthermore, stable 40 carbon isotopic composition of plant leaves, potential carbon mineralization rates and 41 fractions of soil organic carbon at all sampling sites, while rRoot biomass was onlyalso quantified at one intensively monitored site.and, root biomass was quantified at maximum 42 43 tillering stage at one of our paired sites. 44 TAgainst expectations the study showed that: 1) GCRPS significantly increased soil organic

45 <u>SO</u>C and N stocks 5-20 years following conversion of production from traditional Paddy 46 systems; 2) there were no differences between GCRPS and Paddy in soil physical and 47 chemical properties for the various soil depths with the exception of soil bulk density; 3)

48	GCRPS increased above-ground biomass yields and root biomass (n=18) in all soil layers
49	down to a 40 cm depth; 4) GCRPS showed lLower $\delta^{15}N$ values were lower in the soils and
50	plant leafves indicatinginged lessower NH3 volatilization losses fromin GCRPS than-in in
51	Paddy systems; and 5) soil organic C in GCRPS had lower C mineralization potential for soil
52	organic C compared withthan that observed in from Paddy systems over the a 200 days
53	incubation period; 4) GCRPS showed lower δ^{15} N in the soils and plant leafs indicating less
54	NH ₃ -volatilization in GCRPS than in Paddy; and 5) GCRPS increased yields and root biomass
55	in all soil layers down to 40 cm depth. Our results suggest that GCRPS is an innovative rice
56	production technique that not only increases rice yields using less irrigation water, but that
57	itthat itis also is system sustainabley and stably system environmentally beneficial due to its
58	increased soil-SOC and N stocks at a regional scale.
59	
60	Key words: soil organic carbon and nitrogen stocks, region scale evaluation, water-saving rice,
61	<u>rice yields above- and below- ground root-biomass, δ^{15}Nstable isotopes ¹⁵N, potential carbon</u>

62 mineralization rates., stable isotopes ¹⁵N, rice yields and root biomass

64 **1 Introduction**

65 Globally more than 3 billion people depend on rice as a staple food (FAOSTAT, 66 2011). Water used for China is the world's largest rice producer, with an average rice production rate of 197 million tons yr⁻¹, which in 2009 was grown on c.approximately 67 29.930 million hectares in 2009, and accounteds for 43.7% of the total national cereal 68 69 grain production (Fan et al., 2010). Iirrigation water is becoming increasingly scarce 70 due to the With growing water demands from increasing populations and economies in 71 across Asia and in view of ongoing from projected expected climatic changes, 72 irrigation water is becoming increasingly scarce. It is expected that by 2025 about 15 73 million ha of irrigated rice, 27 million ha of rainfed rice, and nearly 20 million ha of 74 rainfed upland rice will suffer from water scarcity worldwide (Bouman, 2007). 75 However, Alin order to meet the global forecasted needs globally over the next 20 76 years though, ann annual increase of about 8-10 million tons will be production 77 increaseing must be produced is required to meet the global forecasted needs over the 78 next 20 years (IRRI, 2011). In the scenario-Therefore, water-saving technologies are urgently proposed needed to cope with for the futuresuch worldwide rice production 79 80 demands-worldwide.

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82 China is the world's largest rice producer with an average rice production rate of 197 83 million tons yr⁻¹, which in 2009 was grown on c.30 million hectares and accounted for 84 43.7% of the total national cereal grain production (Fan et al., 2010). Within China, 85 wWater shortages and temperature limitations already affect more than 4 million ha 86 devoted to of rice production in China, and a significant proportion of this area also 87 show comparatively low yields resultant from low-temperature limitations. oOne of 88 the most promising techniques to overcome these limitations is the Ground Cover

89 Rice Production System (GCRPS). Here, the soil is covered - typically with plastic 90 film - to reduce evaporation, seepage losses and increase springtime soil temperatures. 91 The soil is kept moist between irrigation periods thanks to by the covering material, 92 reducing which reduces irrigation water demand by 50-90%. The actual reduction in 93 irrigation water demand which is dependented on soil types, precipitation and 94 cultivation duration (Tao et al., 2006; Liu et al., 2003). As with conventional paddy 95 rice systems (Paddy), hFurthermore, high-yielding lowland rice varieties (middle-96 duration cultivar, about 140 days) can still be grown in upland locations using GCRPS, 97 which resultsing in similar or even greater yields as compared tothan Paddy systems 98 (Qu et al., 2012; Liu et al., 2013, 2014, Tao et al., 2015). Thus, GCRPS is well in lineconsistent with China's 12th Five Year Plan that requires development of and 99 100 technologies to reduce the water demand and greenhouse gas emissions 101 (GHG)environmental footprint about to increase SOC/N stocks ofin agricultural 102 production (Yao et al., 2014; Tao et al., 2015).

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104 Improving rice production systems should not be solely focused on increasing 105 productivity however, but should also consider be linked to other aspectsfactors 106 affecting that affecting production the stability and sustainability of production, such 107 as preservation of optimal levels of -soil organic-SOC and total N. On a global scale, 108 optimal sSoil organic matter (SOM)contents helps maintain and or improve soil 109 structure and fertility, decreases risks of soil erosion and soil-degradation (Watts et al., 110 2006; Powlson et al., 2011), provides nutrients to plants and soil microbesial populations (Tiessen et al., 1994), and increases soil the water holding capacity, 111 112 thereby improving the systemss' soils' ability of soils to resist drought stress (Rawls et 113 al., 2003).

115	The sustainability of a production system tends to be correlated with the
116	maintenanceaining or increasinge of SOM stockssoil organic matter content,
117	whichand also tends to lead to result in increased yield potentials worldwide
118	(Lehmann, 2007). The amount of organic C stored in a soil is a fine balance between
119	The Cchanges of soil organic carbonC inputs, mineralization and lateral exports
120	(Jenny, 1941; Amundson, 2001). These processes are strongly stocks depend on the
121	relative rates of input and loss of soil organic matter, which is not only affected by
122	temperature, plant available ground vegetation, soil mineral composition, water
123	content and temperature conditions, soil mineral composition, and the but also by the
124	chemical properties of the precursor biomass soil organic matter and its resistance to
125	microbial decomposition ability, depends on the relative rates of input and loss of soil
126	organic matter (Swift, 2001;). WhithIinAmong these factors, temperature and soil
127	water content determined were the most important in determining the formation and
128	decomposition (Saiz et al., 2012) and above and -below ground biomass under
129	different land uses patterns and the formation and decomposition of soil organic
130	matter (Saiz et al., 2012).
131	<u>Meanwhile, Bulk soil δ^{15}N is an index that could reveal the relation between N</u>
132	compounds produced during denitrification and ammonia volatilization (Bedard-
133	Haughn et al. 2003) and SON stock.
134	Compared to upland cereals production systems, Ssubmerged paddy rice cultivated
135	system is considered to be a stable and sustainable cropping system compared with
136	upland systems because the submergencepermanent presence of water results in
137	anoxic conditions, that the depletion of soil O22- by microorganisms drivinge the soil
138	redox potential to the lowest natural levels (Gao et al., 2004; Pan et al., 2010). It is

139	widely acknowledged that decomposition of <u>SOM</u> , plant residues and other organic
140	matter is slower in submerged than in aerated soils (Acharya, 1935Sahrawat, 2004),
141	and previous studies have shown that continuous rice cropping on submerged soils
142	may and prolonged soil submergence favours the maintenance, and even or the
143	increase of soil organic matter (SOM stocks) (Cassman et al., 1995; Bronson et al.,
144	1997; Witt et al., 2000). However, three earlier studies have already showned that
145	GCRPS could accelerated SOM decomposition and thus resultedresulting in declining
146	soil SOM stocks ion the topsoil above the hardpan (between 20-40 cm) at
147	experimental fields (Li et al., 2007; Fan et al., 2012; Qu et al., 2012).
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151	Previous research has already demonstrated that tThe water saving GCRPS technique
152	increased both grain yields and water use efficiency in areas where seasonal water
153	shortages and/or low temperatures during early growth stages were the main limiting
154	factors for rice production (Qu et al., 2012; Liu et al., 2013, 2014; Tao et al 2015).
155	The GCRPS also minimized the effects of varying edaphic conditions on yields at a
156	regional scale (Liu et al., 2013). While some studies have shown that GCRPS
157	accelerated SOM decomposition and resulted in a decline in soil SOM stocks in the
158	topsoil above the hardpan (between 20-40 cm) (Li et al., 2007; Fan et al., 2012; Qu et
159	al., 2012), However, a thorough regional-scale evaluation of GCRPS effects on soil
160	organic-SOC and total N stocks has not yet been reported. Although Also, tThe shift
161	from flooded, anaerobic paddy soils to higher aeration and soil temperatures at the
162	start of the growing season may result in reduced CH4 emissions, while N_2O
163	emissions (Kreye et al., 2007; Yao met al., 2014) and soil-organic-C mineralization
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164 rates of soil organic C (SOC) stocks may increase (Stanford et al., 1973Koch et al., 165 2007) and lower SOC and N stocks in fields using the plastic film-based GCRPS 166 technique on the topsoil above the hardpan (Li et al., 2007; Fan et al., 2012; Qu et al., 167 2012). We hypothesized that optimal soil moisture and increased soil temperature and redox potential would stimulate soil C and N mineralization, leading to a reduction in 168 169 soil C and N stocks under GCRPS at a regional scale. In the long-term this will affect soil fertility and nutrient retention and threaten the stability and sustainability of 170 171 GCRPS production systems. Meanwhile, On the other hand, high ammonia 172 volatilization in Paddy systems tends to result in low N nitrogen-use efficiency was 173 only(approx. 30%) in rice production system due to high ammonia volatilization lost 174 (Ju et al., 20xx; xxx, 20xx09) and. cCovering the soil surface, like in GCRPS, might reduce the ammonia volatilization rates. The natural abundance of stable isotope ¹⁵N 175 176 is an index which can indirectly indicate the main pathway of nitrogen lost through 177 nitrate leaching, denitrification and ammonia volatilization (Bedard-Haughn et al., 178 2003).

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180 To evaluate the environmental consequences impact of GCRPS on soil C and N 181 stocks as well as identifying the primary N loss pathways from GCRPS and Paddy 182 using the on the difference of natural abundances of ¹⁵N between GCRPS and Paddy, we conducted a field study with sampling 49 pairs of neighbouring GCRPS and Paddy 183 184 neighbouring farmer fields in the Shiyan region County, Central China, where the 185 GCRPS technique was first introduced approximately 20 years ago-due to water and temperature limitations of rice cultivation (Zhou et al., 2008). We hypothesized that 186 187 the improved soil moisture conditions and increased soil temperature and redox

188	potential in GCRPS would stimulate soil C and N mineralization, leading to a
189	reduction of soil C and N stocks under GCRPS at a regional scale.
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192	In our study we compared 49 pairs of neighbouring farmer fields across a cultivation
193	region of 5000 km ² that were managed either as traditional paddy rice fields or where

194 GCRPS has been introduced and applied continuously for 5-20 years.

196 **2 Materials and methods**

197 **2.1 Sampling region characteristics**

198 The study was situated in Shiyan region, Hubei province, Central China (32°02'to 199 33°10'N, 109°44'to 111°04'E, 169 m to 661 m a.s.l., see Table S1), where GCRPS 200 was introduced at the end of the last century (Shen et al., 1997; Liang et al., 1999). 201 Shiyan is located in the QinBaShan Mountains with peaks reaching a maximum 202 altitude of 2740 m a.s.l., According to Smit and Cai (1996) tThise area is in the 203 northern subtropical agro-climatic zone of China's eastern monsoon region (Smit and 204 Cai, 1996). Low temperatures at the start of the growing season and together with 205 severe seasonal and regional water scarcity often limit rice production in these 206 mountainous regions (Shen et al., 1997). The mean annual temperature and total 207 average annual rainfall (calculated for the 1961-2009 period from seven 208 meteorological stations located in the respective counties of Shiyan) are is 15.3 °C and 209 829 mm respectively (Zhou et al., 201008). There is little interannual variation in 210 rainfall and temperature and rainfall (coefficient of variations of 0.015% and 0.051%)-. 211 (Zhu et al., 2010) Annual rainfall patterns show pronounced seasonality, with 212 approximately 45% (375 mm) of the rainfall occurring during the summer period 213 (June to August) (Zhu et al., 2010). The mean total sunshine hours per year are 1835 h 214 (Zhu et al., 2010). Given that GCRPS has only been was introduced only two decades 215 ago and this growing technique has the implications for farming activities, labour 216 demand and associated costs, has resulted in GCRPS and traditional lowland rice 217 cultivation (Paddy) are often being spatially interwoven, i .e. some farmers have 218 adopted the technique while others have not (Zhou et al., 2008). However, Iin most 219 cases the adoption of GCRPS by individual farmers is was well-documented by the

local administration so that it was possible to trace specific land management records
for the selected sites and fields.

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223 **2.2 Site and field selection**

Site selection was performed by experienced staff members from the local 224 225 Department of Agriculturale Bureau in Shiyan and extension personnel persons who have been working closelyd with farmers at the individual local villages, with 226 227 Sspecific attention being was paid to ensure proper representativeness coverage of the 228 different rice growing areas at-(i.e. varying altitudes, on-contrasting soil types and 229 proper over acoverageing of the range of time spans since adoption of the GCRPS 230 technique). Information on fertilizer use, and soil and crop management and fertilizer 231 was obtained through farmer interviews (Table S2). TSince the plastic film covers the 232 soil surface, topdressing is not used in GCRPS since the plastic film covers the soil surface; rather, (i.e. the farmers usually broadcast all the fertilizer before transplanting 233 (Liu et al., 2013)) (Table S4). The day before transplanting, a compound NPK 234 fertilizer and urea containing about 150 kg N ha⁻¹-wasere applied to the soil surface in 235 a single dose and incorporated into the soil by plowingploughing., The total N input 236 was about 150 kg N ha⁻¹ for GCRPS. and then The soil surface which was then 237 followed by levellinged and covered with a⁵ µm thick, transparent film 5 µm thick 238 with the thick of 5 um (Liu et al., 2013). For Paddy systems, approximately an 239 240 average of 100 kg N ha⁻¹ was applied as a compound NPK fertilizer to the soil surface 241 and incorporated to a soil-depth of 20 cm before transplanting. At both-tillering and grain filling stages, additional doses of 40 kg N ha⁻¹ were given as urea in order to 242 243 increase rice milling quality, and protein content (Wopereis-Pura et al., 2002;

<u>Leesawatwong et al., 2005</u>) and yield., This all of which resultedresultinged in a total
 <u>N application rate of approximately 180 kg N ha⁻¹ for the paddy rice system.</u>

WGCRPS fields received compound fertilizer containing approx. 150 kg N ha⁻¹ and 247 Paddy fields received compound fertilizer containing approx. 180 kg fertilizer N ha⁻¹ 248 249 in applications. In our study we compared, across a region of 5000 km², 49 pairs of 250 neighbouring farmer fields across a cultivation region of 5000 km²-that were managed 251 either as traditional paddy rice fields or where GCRPS has beenwas had been 252 introduced and applied continuously for 5-20 years. A total of 49 sites with paired 253 treatments consisting of GCRPS vs permanent flooding paddy fields (hereafter 254 referred to as GCRPS and Paddy) were selected for soil and plant sampling. 255 Regardless of current production systems, aAll ofsites sampleshad haved been 256 growing paddy-rice for more than 240 years. The distance between the paired plots 257 were in most cases less than 100 m with only only 9 out of 49 paired plots being more 258 than 250 m apart (Table S1). Geographical coordinates of the sites and fields were 259 recorded by GPS (Garmin Colorado 300) and altitudes were obtained using the Global Digital Elevation Model (GDEM) provided by NASA and METI (2008). 260

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262 **2.3 Sampling methodology and analytical procedure**

Soil samples from <u>the 49 paired GCRPS vs Paddy</u> sites were collected before field preparation during March <u>and to April 2011 across Shiyan County</u>, <u>Hubei province</u> (32°02′ to 33°10′N, 109°44′ to 111°04′E) of Central China</u>. These sites represented a wide range of different soil types (Table S21). At each of the 98 fields, six to nine spatial replicates were taken with the aid of a soil corer (3.5 cm diameter) at four depths intervals (0-20, 20-40, 40-60, 70-90 cm). <u>FurthermoreAdditionally</u>, three replicate samples were collected from each soil profile excavated in each field for
each depth and analysed for bulk density (Blake and Hartge, 1986) and soil texture
(Gee, 1986).

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273 Soil samples for each depth interval were air dried for 5 days and sieved to grounded 274 by hand to pass a 2.0 mm sieve;. Iidentifiable plant material (>2.0 mm) was removed during sieving. These samples were used for analysis of physical and chemical soil 275 276 properties. Soil pH (Mc Lean, 1982) was measured in 1:2.5 soil-water solution using a 277 combined electrode pH meter (HI 98121, Hanna Instruments, Kehl am Rhein, 278 Germany). Extractable soil NO₃⁻-N and NH₄⁺-N (Keeney and Nelson, 1982) was 279 estimated from 1:10 soil-CaCl₂ (0.01M) extracts using an autoanalyser (AA3, Bran & 280 Luebbe, Nordstadt Germany). Sub-samples for determination of soil C and N 281 concentrationtent and ¹⁵N and ¹³C-isotope natural abundance were powdered in a ball 282 mill (MM200, Retsch, Haan Germany) and with had the soil had carbonates removed 283 prior to C analyses (Harris et al., 2001; Walthert et al., 2010). Analyses were 284 conducted using a Costech Elemental Analyzer (Costech International S.p.A., Milano, 285 Italy) fitted with a zero-blank auto-sampler coupled via a ConFloIII to a Thermo 286 Finnigan Delta V Plus isotope ratio mass spectrometer (Thermo Scientific, Waltham, 287 MA, USA). Soil C and N stocks were calculated using element concentrations and 288 bulk density data for all sites.

289

LThe latest expanded leaves at maximum tillering stage and aboveground plant biomass at maturity stage were sampled from 36 paired sites (at some sites rice was not planted as foreseen due to a severe drought) with three replicates from each site used for analysis of the content and ¹⁵N natural abundance using a CN analyser coupled to a mass spectrometer (see above).; carbonSample_Carbon and N
concentrationtents of the samples_were then analyzeddetermined by an elemental
analyzer (EA1108). Carbon-(CAGB) and nitrogen (NAGB)N assimilated in aboveground
biomass were calculated as the sum of grain and straw dry matter multiplied by grain
and straw C or N concentration at harvest. For further details see Liu et al. (2013).

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301 Root biomass was quantified at aone of our paired sites (32 °.07' N, 110 °.43' E) our 302 well-managed own-long-term experimental site in Fang County (32 07'N, 110 43'E; 303 Fig. S1; Tao et al., 2015) where 22 paired GCRPS and Paddy sites wereare located 304 (Table S21). The experimentsite consistsed of the two production systems (Paddy and 305 GCRPS) and two N fertilizer application rates (0, 150 kg N ha⁻¹) in three-fold 306 replication. All 12 subplots (8.5 m \times 9.5 m) were arranged in a complete randomized block design. Root biomass was quantified for three replicate cores in each of the 307 308 subplots. For this purpose, soil columns (with 40 cm height and 15 cm diameter) were 309 collected at the maximum tillering stage using stainless steel cylinders. The soil column was separated into depth intervals of 0-10-cm, 10-20 cm, and 20-40 cm. SThe 310 311 soil samples from the different soil depths wereas placed in mesh bags and set in a 312 water stream to remove soil particles and then cleaned by tap water on a 0.2 mm mesh. 313 The cCleaned root samples in different soil depths wasere transferred into small 314 envelope and oven-dried at 75 °C for 24 h.

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Potential <u>soilcarbon C</u> mineralization rates for 0-20 cm <u>soil depths</u> samples from all 49 paired Paddy and GCRPS sites were determined using a laboratory incubation assay. Three soil samples with a volume of $0.20 \text{ cm} \times 0.10 \text{ cm} \times 0.20 \text{ cm}$ (depth) were 319 sampled at each site using a spade. Samples were composited and air dried. Three 320 replicates with 30 g of soils were incubated for 200 days at 25 °C and 60% soil water-321 holding capacity in 150 ml bottles. Carbon-CO2_dioxide_fluxes were measured daily 322 during for the first 10 days, at three-day intervals then every three days for the 323 following next-three weeks and in-then every 1-2 weeks intervals-afterwards. The gas 324 measurement period was from 5 min to 4 hours depending on the CO₂ fluxes rates. 325 For flux measurements, the jars were closed gas-tight and CO₂ headspace 326 concentrations were measured with a non-dispersive infrared sensor (Premier, 327 Dynament, United Kingdom) at 10-second intervals. CO₂ fluxes were calculated from 328 concentration changes with time, considering headspace volume, temperature and air 329 pressure. The <u>T</u>total cumulative emissions were obtained by integrating summing the 330 measured daily fluxes, with daily fluxes of the observational intervals being estimated 331 as the arithmetic means of neighbouring datausing trapezoidal integration assuming a 332 linear change in flux between measurements.

333

334 OSoil organic matter (OM) fractions before and after incubation were physically 335 separated before and after incubation using a slightly modified procedure to that 336 described in Zimmermann et al. (2007). Briefly, 30 grams of dried soil (<2 mm) were 337 added to 161 mL water and dispersed by means of a calibrated ultrasonic probe 338 (Labsonic 2000, B Braun, Melsungen, Germany) using a light output energy (22 J ml⁻ 339 ¹). The dispersed suspension was then wet sieved over a 53 μ m mesh size until 340 achievement of clear rinsing water. The fraction > 53 μ m was dried at 40 °C and 341 weighed. This large fraction contained sand-size particles and stable aggregates 342 (Heavy fraction, HF), as well as particulate organic matter (Light fraction, LF). Both 343 These two fractions were separated using the procedure for recovery of organic matter

344 from soils using static dense media as described in Wurster et al. (2010). The dried fraction >53 µm was stirred in a water:sodium polytungstate solution with a density of 345 1.87 g cm⁻³. The mixture was centrifuged at 1000 g for 15 min, and allowed settling to 346 347 settle overnight prior to freezing. The light fraction (LF) was subsequently decanted 348 and both fractions were then washed with deionized water, dried at 40 $^{\circ}$ C and weighed. 349 The solution $<53 \mu m$ (silt and clay) was filtered through a 0.45 μm membrane filter 350 and the material retained in the membrane (S+Cs+c) was then dried at 40 °C and 351 weighed. An aliquot of the filtrate was frozen to determine the amount of dissolved 352 organic carbon (DOC) using a C/N liquid analyser (Multi N/C 3100 Anaytik Jena, 353 Jena, Germany).

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355 Root biomass was quantified at one of our paired sites (32.07 N, 110.43 E). The 356 experiment consisted of two production systems (Paddy and GCRPS) and two N 357 fertilizer application rates (0, 150 kg N ha⁻¹) in three fold replication. All 12 subplots 358 $(8.5 \text{ m} \times 9.5 \text{ m})$ were arranged in a complete randomized block design. Root biomass 359 was quantified for three replicate cores in each of the subplots. For this purpose, soil 360 columns 40 cm height and 15 cm diameter were collected at the maximum tillering 361 stage using stainless steel cylinders. The soil column was separated into depth 362 intervals of 0-10 cm, 10-20 cm, and 20-40 cm. The soil from the different soil depths was placed in mesh bags and set in a water stream to remove soil particles and then 363 364 cleaned by tap water on a 0.2 mm mesh. The cleaned root sample in different soil 365 depth was transferred into small envelope and oven-dried at 75 °C for 24 h.

366

367 2.4 Statistical Analyses

368 All statistical analysis and calculations were performed in the Statistics Analysis 369 System (SAS, version 8.2). Pairs were used as statistical units to test for significant 370 differences between both treatments (GCRPS vs. Paddy). Shapiro-Wilk tests were 371 applied to check for normal distribution. Non-parametric tests were applied if the data 372 was not normally distributed. Before any statistical test was performed, using 373 parametric or non-parametric tests to investigate differences between GCRPS and 374 Paddy at the regional scale, we tested for significantee differences betweenof GCRPS 375 and Paddy treatments according to athe-model that included sources of variation due 376 to system, altitude, year, village, interaction of system × altitude, system × year and 377 system \times villagesoil type, and covering years (years since conversion, soil type and 378 elevation) as potential variables influencing on the percentage change of soil organic carbonSOC/N stocks between both systemsGCRPS and Paddyconcentration and 379 380 stocks. However, we found that the percentage change of SOC/N stocks was not 381 significantly affected neither by soil type, covering years since conversion, elevation 382 nor by any the interaction of the interactionssoil type and covering years soil organic 383 carbon/N concentration and stocks were not significantly affected by soil type at 384 regional scalethe interactions of system and altitude, system and year, system and 385 village, which means soil organic carbon/N concentration and stock were not only 386 affected by simple two factors and their interactions. Thereforenso, we pooled over 387 different soil types, years since conversion and elevationaltitude, year and village in 388 the subsequent following statistical analysis (Table S23). Non parametric tests were 389 applied if the data was not normally distributed. First, A paired t-test was used to test 390 for significant differences in soil texture (clay, silt and sand content), bulk density, pH 391 and mineral N concentrations (Nmin) between GCRPS and Paddy in soil texture (clay, 392 silt and sand content), bulk density, pH and mineral nitrogen concentrations (Nmin) at

393	the <u>a</u> regional scale. All statistical analyses and calculations were performed using
394	parametric (paired and two-tailed t-test, Pearson chi-square) and non-parametric
395	(Wilcoxon matched pairs rank sum test; two-tailed) tests to investigate differences
396	between GCRPS and Paddy at the regional scale. Statistical analyses of Differences in
397	root biomass between the two systems were tested was performed using the general
398	linear model (GLM) procedure of the Statistics Analysis System (SAS, version 8.2).
399	Results are expressed as arithmetic means ± standard error of the means, levels of
400	significance for all tests of *=0.05, **= 0.01, ***=0.001% probability level
401	respectively and ns=not significant were used.
402	

404 **3 Results**

405 Average SOsoil organic C concentrations and stocks and concentrations were 406 significantly higher in GCRPS than in Paddy for each soil depth interval except for 407 the top layer (0-20 cm; depth of soil organic C stock (Fig. 1a, c; statistic results in 408 detail see Table S²⁴ for details) P=0.0244, 0.001, 0.0176 and 0.0459 for 0-20, 20-40, 409 40 60 and 70 90 cm for SOC concentrations; P= 0.0557, 0.0059, 0.0108 and 0.0415 410 for 0-20, 20-40, 40-60 and 70-90 cm for SOC stocks; P=0.0244, 0.001, 0.0176 and 411 0.0459 for 0-20, 20-40, 40-60 and 70-90 cm for SOC concentrations). Similarly, total 412 to SOC concentrations and stocks and concentrations, soil organic N concentrations 413 and stocks over the 1m profile and concentrations also tended to be larger in GCRPS 414 than infor pPaddy, fields over the 1m soil profile although. However, significant 415 differences were only observed in the 20-40 cm depth interval (Fig. 1b, d; -Table 416 S432P= 0.0978, 0.0053, 0.1307, 0.0829 for 0-20, 20-40, 40-60 and 70-90 cm for N 417 concentrations; P= 0.2809, 0.0392, 0.12, 0.0562 for 0-20, 20-40, 40-60 and 70-90 cm 418 for N stocks; P= 0.0978, 0.0053, 0.1307, 0.0829 for 0-20, 20-40, 40-60 and 70-90 cm 419 for N concentrations). There were no detectable differences in soil texture (Fig. 2a, b, 420 c;- Table S432P= 0.8165, 0.9231, 0.9297, 0.8002, 0.6713, 0.8537, 0.6738, 0.6496, 421 0.5946, 0.8512, 0.7459, 0.6117 for 0-20, 20-40, 40-60 and 70-90 cm and for clay, silt 422 and sand content respectively), pH or mineral N content (Fig. 2e, f; -Table S432P= 423 0.9104, 0.5442, 0.58, 0.2819, 0.9797, 0.3634, 0.0977, 0.1152 for 0-20, 20-40, 40-60 424 and 70-90 cm and for pH and mineral content respectively) between GCRPS and 425 Paddy for each-any soil depth interval. Soil bulk density (Fig. 2d; -Table S432P= 426 0.7293, 0.0759, 0.4236 for 0-20, 40-60 and 70-90 cm) tended to be lower in GCRPS 427 than in Paddy over the 1m soil profile, although significant differences were only 428 found in the 20-40 cm (P<0.0001) depth interval (P<0.0001).

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430 431

432 The average Mean C and N assimilation rates of in above ground biomass at maturity 433 for GCRPS were significantly higher in GCRPS than forin Paddy at maturity stage 434 (Fig. 3; P < 0.0001, ==0.0002 for C_{AGB} and N_{AGB}). Root biomass from the one selected 435 site was significantly affected by production system, but was not affected by N 436 fertilizer rates or by the interaction of production system and N nitrogen-fertilization 437 from one selected experimental site (Fig. 4; Table S234). Pooled over the two N 438 fertilizer rates, the root biomass at the maximum tillering stage was significantly 439 greater in GCRPS than in Paddy for all depth intervals soil layers down to 40 cm depth (Fig. 4; P=0.0041, 0.0004, 0.0062 for 0-1-, 10-20 and 20-40 cm depth). 440 441 442 Over the 200 day incubation period, t Potential C mineralization rates did not differ

- 442 Over the 200-day incubation period, t Potential C mineralization rates did not differ
- 443 <u>There were no differences between GCRPS and Paddy systems in average potential C</u>
- 444 mineralization rates (data not shown), although. In contrast, Paddy soils systems

445 <u>showed aim tendency towards higher cumulative C loss rates</u> compared withto in

446 <u>GCRPS over the 200-day incubation period for the entire dataset (Fig. 5).</u>

- 447
- For the GCRPS, the SOC contents of the various fractions were similar before and after the incubation experiment (Fig. 6). However for the Paddy treatment, the amount of SOC in the heavy fraction was significantly significantly-lower after incubation compared to before the incubation (P<0.05); However, although nNo differences were found in the fractions of s+c, LF and DOC fractions before and after the incubation (Fig. 6).</p>

The average Mean soil δ^{15} N signatures were significantly-lower in GCRPS than in Paddy at for each depth interval (Fig. <u>75a; Table S42 P< 0.0001, < 0.0001, = 0.0002</u>, = 0.0289 for 0.20, 20.40, 40.60 and 70.90 cm). <u>TMeanwhile</u>, the average δ^{15} N signature in plant leaves was also lower (P< 0.0001) in GCRPS compared <u>to with in</u> Paddy at the maximum tillering stage (Fig. <u>75b</u>). Ln-transformed soil N concentrations were inversely correlated with corresponding δ^{15} N values for either GCRPS or Paddy (Fig. <u>68</u>).

462

463 Over the 200 day incubation period, there were no differences between GCRPS and
464 Paddy systems in average potential C mineralization rates. In contrast, Paddy systems
465 showed higher cumulative C loss rates compared with in GCRPS over the incubation
466 period for the entire dataset (Fig. 7).

467

For the GCRPS, the SOC contents of the various fractions were similar before and after the incubation experiment (Fig. 8). However for the Paddy treatment, the amount of SOC in the heavy fraction was significantly lower after incubation compared to before incubation; although no differences were found in fractions of s+c, LF and DOC before and after the incubation (Fig. 8).

473

475 **4 Discussion**

476 The amount of organic C stored in soil is a finerelated to the balance between C inputs 477 and decomposition processes (Jenny, 1941; Amundson, 2001; Saiz et al., 2012). It has 478 been hypothesized that an-the absence of permanently anaerobic conditions and 479 increased soil temperatures for-under GCRPS maywould result in either no change or 480 even increased SOC losses as a result of potentially due to enhanced microbial 481 decomposition (Pan et al., 2003, 2010; Qu et al., 2012). Therewo-Eearlier studies 482 showed trends towards lower SOC and total N stocks in fields using the plastic film-483 based GCRPS technique., HhHowever, these previous studies have only investigated 484 the topsoil (0-20 cm) above the hardpan (normally 12 – 20 cm below the ground level 485 in the paddy field) at a single experimentstudy site (Li et al., 2007; Fan et al., 2012; 486 Qu et al., 2012). By contrast, in our study we the large regional dataset presented here 487 has been obtained by samplingsampled cultivated fields at 49 paired sites-(i.e. 488 adjacent-pairedboth sites experiencinge comparable soil and environmental conditions, 489 Figs. 2 and S1 and Tables S1 and S4 and 2) down to 1 m depth across an entire 490 geographical region. Our results show the indication that adoption of within the 491 sampling region, conversion of Paddy to GCRPS has a positive trendeffect 492 onincreasedd SOC concentrations storage (Fig. 1a) and storage concentrations (Fig. 493 1c) after SOC concentrations (Fig. 1a; Table S4) and storage (Fig. 1c; Table S4) at 494 after least 5 years since conversion from compared to the time of conversion the 495 traditional Paddy cultivation system in context of the sampling region. We were able 496 to identify two main processes that contributed to the positive effect of GCRPS on 497 SOC stocks.

498 <u>a) Increased above- and belowground carbon inputs</u> Plant residues and organic
 499 fertilizers directly impact-affect the amount and quality of organic matter above the

500 hardpan_at the depths(-between 20 toand- 40 cm-depth), while the accumulation and 501 stabilisation of subsoil OM organic matter in these agricultural systems derives 502 mainly from dissolved OM organic matter leached from the plough layer- (Tanji et al., 503 2003). In our study we observed significantly larger aboveground biomass and grain 504 yields for GCRPS compared to traditional Paddy systems (Fig. 3; Liu et al., 2013, 505 2014) (Fig. 3). Furthermore, root biomass was also found to be significantly largergreater under GCRPS cultivation in all soil layers down to 40 cm depth (Fig. 4; 506 507 Table S4).

508 Accordingly to mutual feedback mechanisms located both above and below ground, 509 significantly larger aboveground biomass and grain yields need more and better root 510 system to absorb more nutrients from the soil (Liu et al., 2003), which meansThis 511 shows that plants growing in GCRPS have a more dynamic root system capable of 512 acquiring soil nutrients in soil layers down to 40 cm depth. Recent literature has 513 confirmed that rice cultivation under variable soil water regimes such as GCRPS 514 result both in higher root biomass (Thakur et al., 2011; Uga et al., 2013), and in-more 515 rhizodeposits (Tian et al., 2013) compared to traditional the flooded Paddy-system, 516 likely because the larger aboveground biomass and grain yields require a larger root 517 system to absorb more nutrients from the soil (Liu et al., 2003). The GCRPS also promotes also-increased soil NO3⁻ concentrations, that which can lead to more 518 519 balanced plant N nutrition (NO3⁻ and NH4⁺), which is that is beneficial for crop 520 growth (Nacry et al., 2013). Moreover-Also, the fluctuating soil water content 521 inherent to GCRPS, which varies between 80-90% water holding capacity (WHC), limits can limit the accessibility toof some micronutrients (e.gi.e. Mn, Fe) in the 522 523 topsoil ifas they become are oxidised to to forms that cannot be directly assimilated 524 by the plant (Tao et al., 2007; Kreye et al., 2009). For example, the lack of standing

525 water may cause increased soil aeration, Θ_2 -content and thus, a higher redox potential 526 (Tao et al., 2007), resulting in the oxidized form of Mn, that greatly lowerings its 527 availability to the plant-availability (Norvell, 1988). Such as Mn, at critical growth 528 stages, shoot Mn concentrations were below the critical deficiency concentration 529 (Dobermann and Fairhurst, 2000) due to there is no standing water and the soil 530 oxygen content may increase leading to higher soil redox potential (Tao et al., 2007). 531 Therefore, Mn is oxidized, and its plant availability is lowered (Norvell, 1988). On the 532 other hand, GCRPS promotes increased soil NO3⁻ concentrations thus leading to a 533 more balanced plant N nutrition (NO₃⁻ and NH₄⁺), which is beneficial for the growth 534 of the crop (Nacry et al., 2013). Therefore, the rice plants in GCRPS need to develop 535 stronger root systems capable of accessing deeper soil layers to obtain a balanced 536 micro-nutrient supply, while avoiding iron toxicity effects (Benejiser et al., 1984). 537 Even if just a few fine roots penetrate the hardpan they may represent a large difference in deep_SOC storage below the hardpan_as root channels may further 538 539 promote percolation of organic compounds into the subsoil. The strong anaerobiosis 540 and stabilisation conditions prevailing at depth will likely promote OM accumulation 541 below the hardpan;; as was we found in our study (Fig. 1).

542 b) Greater physical protection of soil organic matter against microbial degradation 543 We conducted soil incubations under controlled environmental conditions across 544 using soils from all field sites to test the hypothesis that, in contrast to Paddy 545 systems, whether the consistently high soil moisture conditions and high soil 546 temperatures characteristic of GCRPS would enhance SOM stabilisation or might 547 increase C mineralization, and drivepromoting net losses of SOM (Farooq et al., 548 2009Xiong et al., 2014). Our results showed no significant differences in 549 mineralization rates-potentials between soils from the GCRPS and Paddy systems for

550 all measuring dates over a 200-day incubation, although cumulative C losses over the 551 entire incubation period weretended to bewere consistently greater forrom Paddy 552 soils . On the contrary, soils from paddy fields showed higher cumulative C loss rates 553 over the incubation period (Fig. -57). This could suggest indicated that SOM in fields 554 managed under GCRPS is may be more effectively preserved than SOM in traditional 555 Paddy systems. Besides the physicochemical protection offered by clay minerals (Koegel-Knabner et al., 2010; Saiz et al., 2012)Such other stabilizing mechanisms 556 557 could may be conferred through because of the higher OM inputs due to resultant from 558 enhancedimproved above and belowground biomass production, as with higher OM 559 input rates are known to promote stable macro and mesoaggregates (Six et al., 2004). 560 However, we did not observe significant differences between both systems in the 561 physically protected fractions for at-the topmost soil layer ($\frac{1}{2}$ (Fig. -68);. It is likely 562 though, that aggregation and/or stabilisation might become more relevant at deeper 563 locations where the differences in SOC concentrations were greater. in addition to the 564 physicochemical protection offered by clay minerals (Koegel-Knabner et al., 2010; 565 Saiz et al., 2012). Also Indeed, the strong anaerobiosis and stabilisation conditions prevailing at depth would likely promote OM accumulation below the hardpan, as we 566 567 found in our study (Fig. 1; Koegel-Knabner et al., 2010). Also relevant within this 568 context is the contrasting soil redox conditions observed between the two systems 569 (Liu et al., 2013). The more frequent oscillation in redox conditions (aerobic to 570 anaerobic and back) in GCRPS may have a strong positive influence on the generation of organo-mineral complexes, which are of paramount importance for stabilisation of 571 572 OM organic matter in Paddy soils (Koegel-Knabner et al., 2010).

574 Similar to SOC concentrations and stocks and contents, soil organic N concentrations 575 and stocks and contents were larger in GCRPS than for in paddy fields over the 1m 576 soil profile. However, significant differences were only observed in the 20-40 cm depth interval (Fig. 1b, Fig. 1d). In addition, we observed $\delta^{15}N$ enrichment in paddy 577 578 soils for all soil depths (Fig. 75a), which was also reflected in the plant biomass (Fig. 75b). Bulk soil δ^{15} N is a combined signal for organic and mineral N compounds and 579 580 may be affected by (1) the amount and isotopic signature of applied fertilizer (Yun et 581 al., 2011), (2) isotopic fractionation occurring during N cycle processes such as N 582 mineralization, nitrification and assimilation (Bedard-Haughn et al., 2003), as well asand (3) ¹⁵N depletion of gaseous N compounds produced during denitrification and 583 ammonia volatilization with subsequent ¹⁵N enrichment of the remaining soil N 584 (Bedard-Haughn et al., 2003). Based on farmers' interviews, the dominant fertilizer 585 586 used was a compound NPK fertilizer with urea as the N form (δ^{15} N of ca. 0.5-‰) 587 (Yun et al., 2011). As well as urea-N, only-11 out of of the 98 sites received manure, 588 $(\delta^{15}N \rightarrow 10 - \infty)$. Most crucially, N fertilization rates were comparable for both management systems (GCRPS: approx. 150 kg N ha⁻¹; Paddy: approx. 180 kg N ha⁻¹). 589 590 Therefore, kinetic isotope fractionation processes in the soil rather than mixing of different N sources with distinct δ^{15} N signatures likely account for the observed 591 differences in soil δ^{15} N. This is confirmed by the observation that L4n-transformed 592 soil N concentrations were inversely correlated with the δ^{15} N values (Fig. 86). 593

595 The largest fractionation factors are consistently reported for gaseous N losses 596 (Bedard-Haughn et al., 2003; Robinson, 2001) so <u>it is likely</u> that changes in N₂, N₂O, 597 NO and NH₃ losses may account for the ¹⁵N enrichment in Paddy soils. Nitrification-598 and denitrification-induced losses of N₂, N₂O and NO were expected to increase under

599 unsaturated soils typical for GCRPS cultivation as compared to continuous flooding of Paddy soils that has also been documented in earlier studies (Kreve et al., 2007; 600 Yao et al., 2014). Therefore, we can rule out both fertilizer effects and changes in 601 mineral N cycling and associated denitrification losses as significant factors 602 explaining lower δ^{15} N in GCRPS soils. The ¹⁵N enrichment in Paddy soils and 603 604 increased soil N stocks under GCRPS are therefore more likely related to ammonia volatilization following fertilizer application. Ammonia loss from urea fertilization in 605 606 Paddy rice fields can be very high with emission factors ranging from 9-40% of 607 applied N (Xu et al., 2013). Covering the soil with a plastic film immediately after 608 fertilizer application (Zhuang and Wang. 2010) or manure deposits (Webb et al., 2013) 609 greatly reduces surface water NH4⁺ concentrations. Meanwhile, NH3 volatilization 610 losses.s were significantly positively correlated with NH4⁺ concentrations of the 611 surface water. Low surface water NH4⁺ concentrations could be greatly reduced N 612 loss through NH₃ volatilization in GCRPS than in Paddy (Xu et al., 2013). NH₃ loss. 613 Therefore, we expect that the observed greater soil N stocks in GCRPS fields were 614 associated with decreased NH₃ volatilization.

615

616 **5 Conclusion**

We demonstrate for the first time, that across a wide range of spatially representative paired sites under real farming conditions, that GCRPS significantly increased soil organic C and N <u>concentrations and</u> stocks and concentrations at a regional scale under varying edaphic conditions., which no differences in soil physical and chemical properties for the various soil depths with the exception of soil bulk density. <u>Meanwhile, GCRPS also increased above- yields and belowground root biomass in all</u> soil layers down to 40 cm depth. These indicate that GCRPS is a stable, and 624 sustainable_<u>and environmentally sound</u> technique that <u>can</u> maintains key soil 625 functions while increasing rice yields and expanding the cultivation of a valuable crop 626 into regions where it has been hampered by low seasonal_temperatures at the 627 beginning of the growing season and/or a lack of irrigation water. However, the use of 628 plastic sheets as cover material remains an obstacle, since because plastic residues 629 often remain in the field and pollute the environment. Biologically degradable films 630 maybe a suitable solution to overcome this problem, and supplying such films with 631 micronutrients may allow a more effective and integrated nutrient management that 632 could further boost grain yields.

633

635 Author contributions. M. Liu and M. Dannenmann contributed equally to this work. 636 S. Lin and K. Butterbach-Bahl designed the experiments. M. Liu, S. Lin, M. 637 Dannenmann, S. Sippel, Z. Yao and K. Butterbach-Bahl conducted the regional field 638 sampling. M. Liu performed the lab analysis and statistical analysis. G. Yan and G. 639 Saiz performed the incubation and fractionation experiment. Y. Tao and Y. Zhang 640 carried out the field experiment and were in charged of on the root biomass. M. Liu, S. 641 Lin, M. Dannenmann, G. Saiz, K. Butterbach-Bahl and D.E. Pelster wrote the 642 manuscript. All authors commented and revised the manuscript.

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882 Figure captions

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Figure 1 <u>Concentrations and Ss</u>tocks and concentrations of soil organic carbon and total nitrogen in traditional Paddy and GCRPS at different soil depths. Data
presented are the mean values pooled over 49 paired sites (for 0-20 & 20-40 cm, n=147; 40-60 cm, n=108; 70-90 cm, n=63). <u>Errors Bb</u>ars indicate the standard error of the means. ***, **, * Significant at 0.001, 0.01, 0.05 probability level respectively; ns-not significant.

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Figure 2 Average soil clay, silt and sand content (for 0-20 and 20-40 cm, n=49; 4060 cm, n=36; 70-90 cm, n=21), soil bulk density, pH and mineral nitrogen
concentrations (N_{min}; for 0-20 and 20-40 cm, n=147; 40-60 cm, n=108; 70-90 cm,
n=63) at different soil depths from 49 paired sites cultivated either under with
either-traditional Paddy or GCRPS. Errors bars indicate s.e.m. *** Significant at
0.001 probability level respectively; ns-not significant.

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Figure 3 Carbon (CAGB) and nitrogen (NAGB) assimilated in aboveground biomass at the maturity stage (n=108). Data presented are the means pooled over 36 paired sites (these represent all the sites where rice was grown in 2011) with three replicates at each site. Errors bars indicate s.e.m. Bars labeled with different lowercase letters indicate statistically significant differences (P < 0.05) between Paddy and GCRPS. For further details see Liu et al. (2013).

905Figure 4 Root dry matter at maximum tillering stage for different soil depths in906 $\underline{traditional}$ Paddy and GCRPS. n = 18. Error bars denote s.e.m. Bars labelled with907different lowercase letters indicate differences (P < 0.05) between Paddy and GCRPS.</td>908

- Figure 5-(a) <u>Differences in cumulative organic carbon mineralization during a</u>
 200 d incubation period of top soils (0 20 cm) collected from either Paddy or
 GCRPS-grown rice fields. Data presented are the mean values pooled over 49 paired
 sites. Error bars indicate s.e.m. GCRPS and Paddy showed no significant differences
 for individual incubation times.
 Soil δ¹⁵N isotopic signature in traditional Paddy and GCRPS at different soil
- **depths.** Data presented are the mean values pooled over 49 paired sites (for 0 20 & 20-40 cm, n=147; 40-60 cm, n=108; 70-90 cm, n=63). (b) δ^{15} N signature in plant **leaves at the maximum tillering stage.** Data presented are the means pooled over 36 paired sites (these represent all the sites where rice was grown in 2011) with three replicates at each site, n=108. Errors bars indicate the s.e.m. ***, **, * Significant at 0.001, 0.01, 0.05 probability level respectively; ns not significant. Bars labelled with different lowercase letters indicate differences (P < 0.05) between Paddy and GCRPS. 922
- Figure 6 <u>Relative SOC distributionfractionation (% of total) of topsoils (0 20</u> <u>cm-depth) from either Paddy or GCRPS grown rice fields for the different</u> <u>physically separated fractions before and after a 200 d incubation period</u>. s+c =fraction < 53 µm, HF/LF = heavy/light fraction > 53 µm, DOC = dissolved organic carbon < 0.45 µm. GCRPS (n=18) and Paddy (n=18) (random selection of 18 out of 49 paired sites). Error bars denote s.e.m. The asterisk indicates significant differences between pre and post incubation (P<0.05). <u>Correlation of δ^{15} N with Ln transformed</u>

soil total nitrogen content up to 1 m. Data presented are all the individual samples
 measured across the 49 paired sites, which consist of three replicates for each site
 (n=465).

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934 Figure 7 (a) Soil $\delta^{15}N$ isotopic signature in traditional Paddy and GCRPS at different soil depths. Data presented are the mean values pooled over 49 paired sites 935 (for 0-20 & 20-40 cm, n=147; 40-60 cm, n=108; 70-90 cm, n=63). (b) δ^{15} N signature 936 in plant leaves at the maximum tillering stage. Data presented are the means pooled 937 938 over 36 paired sites (these represent all the sites where rice was grown in 2011) with 939 three replicates at each site, n=108. Errors bars indicate the s.e.m. ***, **, * 940 Significant at 0.001, 0.01, 0.05 probability level respectively; ns-not significant. Bars 941 labelled with different lowercase letters indicate differences (P < 0.05) between Paddy 942 and GCRPS.Differences in cumulative organic carbon mineralization during a 200 d incubation period of top soils (0-20 cm) collected from either Paddy or 943 944 GCRPS grown rice fields. Data presented are the mean values pooled over 49 paired 945 sites. Error bars indicate s.e.m. GCRPS and Paddy showed no significant differences 946 for individual incubation times.

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948Figure 8 Correlation of δ^{15} N with Ln transformed soil total nitrogen content up949to 1 m depth. Data presented are all the individual samples measured across the 49950paired sites, which consist of three replicates for each site (n=465). Relative SOC951distribution (% of total) of topsoils (0 - 20 cm depth) from either Paddy or952GCRPS grown rice fields for the different physically separated fractions before953and after a 200 d incubation. s+c = fraction < 53 µm, HF/LF = heavy/light</td>954fraction > 53 µm, DOC = dissolved organic carbon < 0.45 µm. GCRPS (n=18) and</td>

- 955 Paddy (n=18) (random selection of 18 out of 49 paired sites). Error bars denote s.e.m.
- 956 The asterisk indicates significant differences between pre and post incubation
- 957 (P<0.05).



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Figure 2













Dear editor and reviewers:

Thank you for the numerous and constructive suggestions, questions and comments. We addressed all of those in our revision. In the following, we respond to all the comments. Where applicable, we describe the associated changes in the manuscript, and where we disagree with the reviewers' suggestions, we clearly describe the reasons.

>> To reviewer 399

The manuscript discussed an interesting issue that whether ground cover rice production system depletes soil carbon and nitrogen compared to traditional paddy rice system. The authors did a valuable job using a paired sampling method to examine the difference of soil carbon and nitrogen between these two systems at 49 sites on a regional scale. The results showed that the ground cover rice production system benefits soil C and N sequestration. The results are interesting and have been fully discussed in the manuscript. I think this study is worthy of publishing, but still needs improve huge.

Specific comments

1. The sampling was selected from 49 paired sites. As mentioned in the manuscript, these sites represented a wide range of different soil types. The differences in soil C and N between the contrasting systems could vary considerably across soil types or sites. Therefore, spatial heterogeneity should be considered in the manuscript.

We have included the factors "soil type" and "elevation" in our statistical model and found no significant effect of these factors on the relative change of SOC and TN stocks due to GCRPS cultivation. In the revised manuscript, we present the associated details of the statistical model.

Furthermore, the outlined mechanisms for the observed GCRPS effects on C and N storage in soils (e.g. deeper penetration of roots, decreased NH₃ losses due to coverage) are generally valid irrespective of soil type. That is precisely what we demonstrate through the sampling of the 49 paired sites.

2. The root biomass samples were sampled from two N-treatment subplots. However, in Fig. 4, root dry matter of different soil depth in two farm systems was present. What the effect of N fertilizer on root biomass? Please check the values and analysis process.

>> Many thanks for your concern and suggestion. Root biomass was significantly affected by the production system, but not by the N fertilizer rates or the interaction of production system and nitrogen fertilization rates. Therefore we have pooled data over the two N fertilizer rates. This was clarified both in the Result section and in the Figure caption in the revised version.

3. A laboratory incubation experiment was conducted to test the hypothesis that GCRPS releases more soil carbon than paddy systems. However, the same controlled incubation conditions were dissimilar to the field conditions of the two systems. It

seems better to conduct a field monitoring for test the hypothesis.

>> We are fully aware of the limitations of the chosen laboratory approach under controlled conditions. The approach of choosing identical incubation conditions was followed to address changes of C mineralization due to different C substrates, different microbial communities or because of differences in the physical protection of C between the Paddy and GCRPS systems. Hence, it was our purpose to eliminate any confounding temperature or soil moisture effects. The information derived from such an experiment is an important piece of supporting information to interpret the observed effects on SOC storage in the field. Nonetheless we agree that the results should be interpreted in the light of the limitations of such an approach under controlled conditions. This is what we do in the revised version, together with a clearer outline of the rationale behind this experiment.

4. Greater stability of soil organic matter partly contribute to higher soil C under GCRPS systems. In addition, "the more frequent oscillation in redox conditions in GCRPS may have a strong positive influence on the generation of organo-mineral complex", which implies that GCRPS may hold higher mineral-associated organic matter (s+c). However, SOC contents in various fractions were similar between the two systems before incubation experiments, as seen in Fig. 8, indicating the difference in SOM stability between the two systems were not large. The difference in potential SOM mineralization may attribute to other factors, such like microbial composition.

>> We have better clarified our argumentation in the revised version. First of all, it is important to note that s+c and HF are both organo-mineral fractions, which provide physical protection against microbial decomposition through aggregation and adsorption of SOM on mineral surfaces. It is true that the relative SOC content for the different fractions (% over the total) before incubation were similar between the two systems. However, these fractions may differ with respect to their stability. The significant change of the heavy fraction within paddy soils during the incubation may indicate lower aggregate stability compared to GCRPS (we develop this point further in our response to Reviewer 495). Furthermore, it should be noted that information on physical soil fractions is only available for the topsoil, whereas the most pronounced effects on SOC stocks were observed in the deeper horizons. This aspect is also highlighted in the discussion of the revised version. Information on physical soil fractions from deeper soil layers is unfortunately not available.

We have also added the argument of different microbial communities to the revised version. In fact we have started experiments to analyze the microbial community in Paddy and GCRPS soils using molecular tools. And indeed the first available information shows that the microbial community differs between GCRPS and Paddy soils and that microbial activity is lower in GCRPS soils than in Paddy soils, which is in line with the observed mineralization dynamics in the laboratory and with field observations on the SOC stocks. We have added this information as "personal communication" to the revised version, as the related manuscript is under preparation.

5. The manuscript requires significant language editing. Many of the paragraphs need

to be tightened up and at times the sentence structure is confusing and needs to be simplified or edited carefully.

>> Dr. David Pelster, who is also a co-author, is a native English speaker who edited and polished the manuscript.

6. Sections describing the statistical analyses are poorly described.

>> This section was rewritten to improve clarity. In particular, we tested significance of treatments according to the model that included effects of soil type on soil organic carbon/N stocks. However, we found that soil organic carbon/N stocks were not significantly affected by soil type at regional scale. So we pooled these over the different soil types in the statistical analysis.

>> To reviewer 464

1. Page 3652, lines 10-16: The table containing the site information is well-done, but within the manuscript it would be good to include the elevation range of the sampling sites.

>> Revised as suggested - the elevation of all sampling sites was added to the table. The elevation ranges from 169 m to 661m above sea level.

2. P. 3652, l. 19: Is there any idea of the inter-annual variation in rainfall or temperature in this region? Perhaps error of some type here. Also, are there any present temperature/rainfall trends seen during this time period?

>> Yes, we have added the inter-annual variation and presented rainfall and air temperature in this region based on seven meteorological stations located in the respective counties in this region (Shiyan City, Danjiangkou City, Yun County, Yunxi County, Fang County, Zhuxi County and Zhushan County) from 1961 to 2009. This information is given in the publication of Zhu et al., 2010. The data show that there is little interannual variation in rainfall and temperature for these sites (coefficient of variations of 5% and 1%). Present temperature / rainfall trends were (not) observed in the experiment year. All this information was added to the M+M section of the revised version.

3. P. 3652, l. 22: Where did the measure of sunshine hours come from? >> It comes from the reference of Zhu et al., 2010. We added this citation to the reference list.

4. P. 3653, l. 4-8: The description of the site selection process is lacking. How did "experienced staff members" select this sites? Where the selections random? Soil type and elevation have the potential to greatly influence the outcomes of these findings, the manner in which these site characteristics were consider in selecting study sites is crucial and thus this area of the manuscript needs further explication. Is the information from interviews with farmers available?

>> The mentioned "experienced staff members" have been working in the Department of Agriculture in Shiyan with close interaction with the farmers in the individual villages since more than 20 years, also overseeing the introduction of GCRPS in the region. The site selection process was as follows: Information on topography, geology, soil type, and land use was collected from Shiyan Agricultural Bureau to identify a large set of potential villages and sites. Then, villages and potentially suitable paired sites were visited and information on agronomic parameters (e. g., transplanting data) and the time since conversion from Paddy to GCRPS cultivation as provided by the local extension staff was compared with the related information collected from farmers interviews. In case of sites were selected that provided unambiguous information on site history. Otherwise, we continued the site search until a representative set of paired sites with respect to elevation and geology was gained for the target region (i. e., 49 paired sites). Farmer interviews are available in form of Table S2. We have added this information to the revised version.

5. P. 3653, l. 20-21: What are the soil types? Maybe an additional table could be provided or perhaps table S1 could be expanded to include more information about each site.

>> Revised as suggested - we have added the soil types in Table S1 of the revised version for each single sample site. The soil types are: Dystric Cambisols, Haplic Luvisols, Dystric Regosols, Calcaric Regosols and Eutric Gleysols.

6. P. 3656, l. 5-13: Where all analyses conducted in SAS? Are data/code/output posted anywhere for review and reproducibility? This section is lacking on specifics and details and requires clarification.

>> Yes, all analyses have been conducted in SAS 8.2. The section on statistical analyses in the revised version has been rephrased and extended to improve clarity. We have also added Tables S3, S4.

7. P.3656-3657: The results section could be expanded to include more specific numbers. As is, the results section mostly identifies differences and points the reader to the plots without including specific numbers, significance levels, or error. Lines 5-10 on p. 3657 represent a more thorough representation of the findings. Given the thorough and well-detailed methods section, I was expecting more explicit results.

well-detailed results of statistical analyses were listed. In order to comply with these suggestions, we have rephrased the results section describing in more detail the differences observed between GCRPS and Paddy systems. This explicitly includes the addition of numbers, error ranges and significance levels.

8. P. 3658, I. "Our results show that adoption of GCPRS has a positive effect . . ."

This sentence in the manuscript may be overstating the findings of the results. While there is an indication of a positive trend, the findings should be placed in context of the region and the relatively scant time scale. Overstatements should be avoided.

>> We do not agree with the term "trend" proposed by the reviewer, because increased SOC concentrations were statistically significant over the entire soil profile and increased SOC stocks were significant in 3 out of 4 sampled soil depths. While the term "trend" suggests statistical insignificance, we found significant results based on an extremely robust dataset with 49 replicated sites. However, in order to avoid overstatement, we limit the statement that GCRPS has a positive effect on SOC to the investigated region in Central China in the revised manuscript. We had included the factor "time since conversion to GCRPS" in our statistical analyses; however we found that this factor was insignificant (Table S3 in the revised version). This may be due to an insufficient number of sites in some of the investigated age classes due to the short timespan since the introduction of this technique and the generally slow changes in SOC and TN stocks. A significant time effect, and thus the calculation of a change rate per year may indeed be possible in another 10-20 years from now. 9. P. 3658, l. 15-19: "... root biomass was found to be significantly larger under GCPRS ..." on p. 3655, l. 22 in the methods, it is noted that root biomass was examined at only one of the paired sites. While the identified method of the increased dynamism of root systems under GCPRS influencing soil nutrient acquisition may be what is going on, the predictive ability of the outlined method does not seem to have the power to confirm this. I would reexamine this analysis and consider this a possible further area of exploration as the findings are interesting, but overarching proclamations regarding this mechanism are not necessarily supported by one site.

>> It is actually true that, unfortunately, and due to logistic reasons it is just not possible to sample root biomass at all investigated sites. However, the observed effects of GCRPS cultivation on the root system at one of the sites was consistent with earlier independent publications (e.g. Li et al., 2007; Thakur et al., 2011; Uga et al., 2013). Furthermore,, we intensively sampled 22 plots at a well-managed site with a well-known land management history that can be considered as representative for the rest of sites.

Overall, the positive effect of GCRPS cultivation on root biomass and rooting depth is well acknowledged from previous studies, and the following reasons may explain this effect:

1) higher translocation of photosynthetic product into the root; 2) reduced anaerobiosis favoring root development and 3) relocation of nutrients such as NO_3 in deeper soil depth, requiring more vigorous root development.

10. The figures for each graph/plot should mention the statistical test which the significance levels are referring too. Visually, the plots are quite nice and are nicely suited to presenting the data.

A major concern here is the confounding of findings stemming from the lack of explicit consideration for independent variables. Without considering variance in soil type and elevation among the sites, and looking for relationships among and within treatments, the findings here are constrained considerably depending on the range of soil types.

And also what about time? A time range of 5-20 years is mentioned multiple times in the manuscript, but never tested explicitly to see how much of an impact time from conversion has on any variable.

It would be preferential if the data and analysis were posted publicly so that results could be verified and reproducibility could be considered.

This study is worth of publication, but does also require significant editing for language and grammar.

>> In the revised, we have added information on the statistical test used to the caption of each figure in the Table S4.

We have included the variables "soil type" and "time since conversion" in our statistical model, but neither these factors nor their interaction were significant. We have added this information to the revised version. For potential explanations of the insignificance of the factors time and soil type, see responses above.

We are willing to add our database on soil C and N content (and further data if desired such as soil texture) at the level of single fields and soil layers as supplementary data

to the revised version (although this will be a very large table).

In the context of the comments on potential biases of different elevation or soil type (this information was also added for each site), we feel that it is important to state that it is exactly the consideration of 49 replicated paired sites with different soil types and elevation, including a good spatial replication at each single field and the sampling down to 90 cm depth which makes our findings on GCRPS effects on soil C and N stocks extremely robust. We are not aware of any study with a comparable site replication.

Language quality was checked by a native speaker.

>> To review 495

Dear Authors and Editor,

The article "Ground cover rice production system facilitates soil carbon and nitrogen stocks at regional scale" by Liu et al. is based on sophisticatedly designed soil sampling from geographically representative field sites in Central China. I found it of good value to understand local soil responses to film coverage. Its novelty in regional scale may also provide supportive information to local policy makers. However, the data obtained in this article was largely devalued by its weak argument in Introduction, lack of rationalization in Method, as well by the far-fetched interpretation in Discussion.

>> Many thanks for your comments. In the revised version we have extended the introduction as suggested below. Also the chosen methodology is better justified. As we take from the reviewer comments, the rationale behind our argumentation in the discussion section was partly misunderstood. We have addressed the issues raised in the responses to the specific questions below, and have further clarified our argumentation. Also we have extended the introduction section of the revised version to provide a better framework for the later discussion of our results. However, in this context some suggestions of the reviewer, e. g., to move the biogeochemical framework we provide in the discussion for the interpretation of the results to earlier sections, appear contradictory to us. This would weaken the readability of the paper in our view (please also see our specific responses below). Finally, we believe that the interpretation is not far-fetched and provide the reasons for it in the answers to the specific comments.

Here are my general comments:

1) The authors very often cite a great length of literature in Discussion, which should have been reviewed and argued in Introduction to build up your own argument, clarify knowledge gap and rationalize your own research question.

>> In order to comply with this comment, we have extended the introduction section with more literature, thus guiding the reader more straightforward to the rationale behind the hypothesis we had developed to be tested in our experiment.

2) Why and how could you make a hypothesis (C, N stocks would reduce under GCRPS), but then observed completely opposite results? Do you indicate that you did sufficient literature review to guide you to such hypothesis? If yes, then how could you reject it later on with your own results? If no, then please take full use of literature review to thoroughly debate which factors could be relevant to increase or decrease C and N stocks under GCRPS.

>> Our hypothesis was based on general findings in the literature that aerobic soils have a higher organic carbon turnover and lower C content as compared to soils being predominantly anaerobic. Of course one can adapt their hypothesis to suit the findings, but we just report our initial hypothesis based on what is in the literature. What is wrong here, what would have been your starting hypothesis? For example, in the recent review article of Kögel-Knabner et al (2010, Geoderma), it is stated that the high soil organic matter content of Paddy soils may

be associated with retarded decomposition under anaerobic conditions. Consequently, it appeared straightforward to hypothesize that the more aerobic conditions under GCRPS cultivation would increase the C loss rates and thus overall reduce the SOC stocks under GCRPS cultivation. Based on our extremely robust dataset as gained from regional sampling at 49 paired sites we found the opposite and thus rejected the hypothesis. All this illustrates that soil organic matter dynamics of rice soils is still not well understood, as was also pointed out in this review article by K ögel-Knabner and colleagues.

Science is based on testing hypotheses and then either rejecting them or not.

3) Besides, if you decide to stay on the hypothesis of reducing C, N stocks under GCRPS, then it would be contradictory to use positive word such as "facilitate" in your article title. >> Thank you for this comment, we agree that the title is ambiguous. Consequently we changed it to "Ground cover rice production systems increase soil organic carbon and nitrogen stocks at a regional scale"

4) The relevance of 13C, 15N, and respiration rates should have been clarified in Introduction, i.e. why these properties are relevant, what additional information can they provide than the total C and N, what they can tell you to support your argument? Otherwise, it would be lack of ground to just bring it up in Method and Results.

>> We added the necessary information based on your suggestion to the introduction section of the revised version.

5) Why did you air-dry all the soil samples before incubation? How much do you think such drying treatment will affect the mineralization potential? The community of microbes could change, I assume?

>> Soil sampling and final soil analysis (in this case C decomposition potentials) were done at different locations, with weeks between the sampling and the analysis. We only ensured a standardized treatment of sampling, as hundreds of studies before. This equal treatment of all samples allowed us to reduce storage bias resulting in a similar effect for all samples, as well as allowing us to adjust soil water content to make it consistent for all samples. The results are then referred to as "mineralization potential", which makes it clear that they are the derived CO_2 fluxes from a standardized experiment and should not be confused with field measurements. Our procedure will likely increase mineralization rates, which is why it was termed "mineralization potential". Nonetheless, this data can still provide qualitative information on differences in mineralization dynamics soil processes between Paddy and GCRPS soils.

6) The Results are better reorganized to first deliver the most primary results, link them with logics, and then the secondary results. For instance, information such as soil texture, pH and bulk density could be moved below, unless you can reasonably link them to your primary results C and N stocks. On the other hand, the average C and N assimilation of aboveground biomass could be considered to be moved up directly following the C and N stocks. This may make a better reading flow.

>> We have reorganized the results section according to your suggestion. However we would

like to keep soil physical and chemical properties after the SOC and N stocks. Our logic is to firstly verify that the significant difference on SOC and N stocks does not come from soil differences between paired GCRPS and Paddy.

7) In the Discussion part, authors tended to use a lot of observations from other reports to interpret the results observed in this study. This makes the Discussion less convincing. Peer reports should be used to compare and discuss, not to explain your own results.

>> We disagree that using the literature to back up and explain our results makes the discussion less convincing. We believe that it is common practice to use information from and existing theories developed in other studies to unravel our results explain observed results.

8) The authors attributed the greater total C and N in GCRPS to more residues returns. Have those newly returned C and N been converted to stable form? Or they are just less decomposed litter buried or simply mixed into topsoils?

>> Plant biomass production (aboveground and belowground) was higher for GCRPS. The fraction of aboveground residues left on the field and finally being ploughed is the same for Paddy and GCRPS. This results in higher total amounts (in kg) of residue returns. Figure 6 in the revised version shows comparatively more particulate organic matter (LF) in GCRPS than in Paddy systems, which supports the above statement.

9) With respect to C stabilization/liability, what does the 13C and 15N show? What could be captured from the 13C, 15N and mineralization rates? For instance, Figure 5 shows that Paddy soils are less depleted in 15N than GCRPS. This indicates that soils from GCRPS are less decomposed than that from Paddy, suggesting greater mineralization potential in GCRPS soils (I am not expert in stable isotope. Excuse me, if I am not correct here.). Then, why did Paddy soils show greater cumulative mineralization? What could be the factors? Local aeration, temperature, community or accessibility of microbes?

>> As outlined in the discussion, $\delta^{15}N$ of bulk soil N is a proxy for N loss pathways. These pathways are characterized by clearly pronounced discrimination of ¹⁵N vs ¹⁴N. Consequently, NH₃ volatilization or denitrification result in relative enrichment of ¹⁵N in the remaining N compounds. Therefore, the higher $\delta^{15}N$ values in Paddy soils indicate a higher N loss. Hence, our observation of lower ¹⁵N in the soils and plant leaves indicates less N volatilization along gaseous pathways (mainly NH₃ volatilization) for GCRPS than for Paddy fields. We did not show ¹³C data in this manuscript and we cannot unequivocally answer the question why mineralization potential was higher in Paddy soils (this was a statistically insignificant trend only). As you already mentioned, the microbial community may have been changed after conversion from Paddy to GCRPS.

10) Why heavy fractions have significant differences before and after incubation, but other fractions do not. Does it have anything to do with the stabilization mechanism of SOC? And how? How does this then affect the mineralization, and SOC stocks?

>> HF is the fraction containing micro- and meso-aggregates, which together with the s+c fraction confer physical protection to SOM. However, the HF can potentially be very sensitive to changes in land use and management (Baldock and Skjemstad, 2000).

During the incubation experiment, all soils were exposed to non-saturated conditions (60% WHC). It is therefore plausible that these large changes in soil redox conditions may affect Paddy systems more significantly that GCRPS (particularly in this sensitive fraction) as the latter ones generally occur under higher redox potentials (Liu et al. 2013; Tao et al., 2015). The relative decrease of SOC in the HF fraction is the result of the disruption of micro- and meso-aggregates. This unprotected OM may get mineralized, comminuted, and thus incorporated in the s+c fraction, which after incubation invariably showed an increase in their relative contribution to total SOM. Refs:

Baldock, J.A., Skjemstad, J.O.: Role of the soil matrix and minerals in protecting natural organic materials against biological attack, Org. Geochem., 31, 697-710, 2000.

11) When choosing the sampling sites, you also considered the time spans since adoption of the GCRPS technique. Then, did you do any analysis against the time variable? Any patterns of total C and N stocks over adoption time? Are the increase C and N stocks consistently observed in different adoption years? Are the increasing rates constant over different years? Could it be possible that the benefits of C and N increase only occur for the first several years and then cease when soil C and N stocks approach their maximum capacities?

>> These are interesting questions that we have also asked ourselves. We had included the factor "time since conversion to GCRPS" in our statistical analysis, but no significant effect was found despite a trend towards increasing differences between GCRPS and Paddy with time since conversion. Statistical insignificance in this case may be a result of the still relatively short times since conversion from Paddy to GCRPS (5-20 years since conversion) in the light of the slow rates of change for SOC stocks. Furthermore, we may not have had a sufficient sample size for all years of the 5-15 years period, preventing proper testing of the time factor. This also may have prevented clear responses to the question "Are the increasing rates constant over different years?" We did such an analysis, but no significant difference was observed. The last question remains our research goal in the future, however a thorough addressing of this question requires the availability of longer time series of Paddy-GCRPS conversion.

12) If out of practical reasons, it is just not feasible to investigate root biomass for all field sites. Then, why did you choose this particular site? How well this site could represent all other sites of different soil types, and varying altitudes?

>> Yes, it is just not feasible to investigate root biomass for all field sites. This particular site was chosen because it is a well-managed long-term monitoring site with well-documented agronomic history (e.g., Tao et al., 2015 European Journal of Agronomy). Hence, the risk for unrepresentative effects at this intensively studied site was very low.

13) In Conclusion part, it is better to summarize the key results first before relating to implications. The ideal case would be that the readers can get the most valuable information from just reading your conclusions.

>> This is what we did in the Conclusion part, first to summarize the key results and then

relating to our research goal in the future. Nonetheless, we have slightly changed the conclusion section of the revised version to guide the reader more straightforward to the most important conclusion from our experiment.

Specific comments: Page 3650 L13-18: lack of literature reference. >> We added additional literature references in the revised version.

L18-20: "As with conventional paddy rice systems: : :as compared to Paddy systems: : :": Either grammatically incorrect, or convoluted expressed. >> This was changed in the revised version.

Page 3651

L5 to 30: There should be less description on general effects of SOM on soil properties, but more related to rice system and what could possibly be the effects of GCRPS to SOM. >> Revised as suggested – we have added more rice-specific information, and describe potential pathways how GCRPS could alter SOM.

Page 3652

L5-7: Such detailed description should be moved to Method.

>> This sentence was moved to the Methods section in the revised version.

L20-22: Lack of literature reference or data source.

>> We added references in the revised version.

L23: What does "implications" mean here?

>> Due to additional field work, labor demand and costs (e.g., the need for buying the PE foliage), not all farmers have adopted this technique even though GCRPS has clear advantages.

Page 3653

L10: "180kgfertilizerNha-1": improper way to express measurement unit. At least, there should be space between numbers and measurement unit. And, is it different from the above "150kgNha-1"?

>> We corrected this. Furthermore, we provide detailed information on field management and fertilization in the revised version in order to clarify the management regimes for GCRPS and Paddy. This includes a better clarification of the differences in fertilizer application rates between Paddy (180 kg N ha⁻¹) and GCRPS (150 kg N ha⁻¹).

Page 3654

L1-13: It would be much more convinced if you could provide some literature references for all the methods you used here.

>> Revised as suggested, we have added references to the revised version.

Page 3657

L12: ": : :no differences in average potential C mineralization rates: : :": how did you calculate the average? You mean, averaging the mineralization rates over 200 days? Then, it seems meaningless to me. And why there are no differences in average but a higher value in cumulative mineralization rates?

>> We apologize for unclear writing of this section. The section was rephrased in order to clarify that mean cumulative C mineralization rates were not significantly different between soils of the Paddy and GCRPS systems. Paddy showed an insignificant trend towards higher C mineralization rates. We only used cumulative rates calculated for the 200 days period.

L21-25: These sentences should belong to Introduction.

>> We do not agree here – we believe that this sentence improves readability because it puts the subsequent discussion of our findings in a biogeochemical context. Page 3658

L2-5: These sentences should belong to Method.

>> Again, we disagree. We provide this information here to explain why our findings may differ from results of earlier studies. This clearly belongs to the discussion in our view.

L10-14, L19-30: They should be used in Introduction.

>> Also here we do not agree. This sentence establishes an important link between our results and other research. We feel that it would be inappropriate to discuss all these details in the Introduction.

L15-19: These sentences are just repeating your description in Results.

>> This sentence contains results, yes, but this appears hard to avoid in order to establish the context between our results and earlier publications and thus serves to improve readability.

Page 3659

L1-11: If these sentences are moved to Introduction, then it could be a good literature review. >> Also here we do not agree. As mentioned above, this sentence describes essential information required to explain our results. We feel that it would be inappropriate to discuss all of these details in the Introduction. We have however, made some changes to this in the revised version.

L14-19: Just from "higher cumulative C loss rates", you cannot directly get the conclusion that SOM under GCRPS is more effectively persevered. Besides, you did not do aggregate fractionation, you could not simply relate your interpretation to the conceptual model of Six et al., 2004.

>> We have eliminated such a conclusion, as we agree that the data provided does not justify that GCRPS provides greater SOM stability than Paddy systems. The section has been reorganized.

L20-25: Too much observations from other reports rather than your own observations. Such interpretations are far-fetched.

>> This needs to be seen in the light of literature. We are not in agreement with this statement. Here we refer to Figure 6 in the revised version, which shows that the C content of the heavy fraction significantly declines throughout the lab incubation experiment for Paddy soils only but not for GCRPS soils. This provides hints on the physicochemical protection mechanisms we discuss – and the cited literature explicitly deals with Paddy soils.

Page 3660

L2-15: Such discussion or information should have been either discussed in Introduction, or clarified in Method.

>> We disagree. We feel that it would be inappropriate to discuss details of the interpretation of natural abundance of ¹⁵N in bulk soil N in the introduction of this paper. Again, having this in the discussion seems to us to be essential to the rationale behind our discussion. Omitting this here would allow only experts in the field of isotopic fractionation to follow our line of thought for the nitrogen turnover processes. From the previous comment above (comment 9) we conclude that it is essential to clarify why and how we interpret our d¹⁵N data.

L17-25: Most of these sentences should be mentioned earlier in Introduction or Method.

>> We do not necessarily agree with this. The introduction is a section that introduces the problem and place it in a general research context, but is not a section where one would go into every fine specific detail.

L29: It is not readily convinced to simply attribute "less loss of ammonia" to "the covering of soil immediately after fertilizing". More in-depth interpretation may be needed.

>> We have added a sentence with a more detailed outlined rationale on the mechanism of how covering the soil reduces NH_3 emissions. But this is well accepted in the current literature.

Technical comments:

Figure 1: I would suggest to place SOC content above and SOC stock below, as, logically, SOC stock is calculated from SOC content.

>> We have changed this in the revised version.

Figure 3: What does CAGB represent here? You did not explain it in your text body. The text body and figures should be consistent.

>> Carbon (CAGB) and nitrogen (NAGB) assimilated in aboveground biomass were calculated as the sum of grain and straw dry matter multiplied by grain and straw C or N concentration at harvest. We omitted these abbreviations in the revised version

Figure 4: Y-axis is missing. >> Y-axis was added in the revised version.

>> To reviewer 486

This paper investigates a novel technique namely Ground Cover Rice Production System (GCPRS) and its effects on Soil Organic Carbon (SOC) and nitrogen stocks since this techniques can increase rice yields in areas with lower temperature and water supply. The article is of significance given the critical issue of increasing rice yields in future without compromising its sustainability (with the its negative environmental impact only briefly touched upon in the conclusion). This paper has several scientific issues before it can be published.

Scientific issues

The main issue is in the interpretation of the results and 'direct' conclusion that this technique 'facilitates' SOC and N stocks as stated in the title. The results are not so clear cut for both and more importantly there is a high chance that independent variables may have confounded the results. This is because there is a lack of information on the soil samples:

what soil types are we looking at (a range?), what elevation?

>> Information on the elevation of the sites was already provided in the Table S1 of the submitted version. We have added an additional information on soil type for each site in Table S1. As mentioned earlier, we have also tested the influence of the variable "soil type" on the GCRPS effect (not significant, Table S3) and we provide the related information in the revised version. The elevation range was too small to have an influence in this study, with most sites concentrated at medium elevation.

What past land use if only recently turned into rice production?

>> This is obviously a misunderstanding – rice was grown on all sampled sites for more than 40 years. This information was already provided in the submitted version and is better highlighted in the revised version.

Who and why were they chosen?

>> The previously mentioned "experienced staff members" who assisted in the site selection have been working in the Department of Agriculture in Shiyan with close interaction with the farmers in the individual villages for more than 20 years. The site selection process was as follows: Information on topography, geology, soil type, and land use was collected from Shiyan Agricultural Bureau to identify a large set of potential villages and sites. Then, villages and potentially suitable paired sites were visited and information on agronomic parameters (e. g., transplanting data) and the time since conversion from Paddy to GCRPS cultivation as provided by the local extension staff was compared with the related information collected from farmer interviews. When information on the site history was unambiguous, the sites were selected. We continued the site search until a representative set of paired sites with respect to elevation and geology was gained for the target region, resulting in the 49 paired sites. An extended summary of the farmer's interviews is available in Table S2 in the revised version. We have added all this information to the revised version.

Have they been irrigated the same way?

>> We are not sure if this question refers to differences between Paddy and GCRPS cultivation or differences across sites within a cultivation system. Paddy and GCRPS have system-inherent differences in irrigation as outlined in the manuscript. For example, for the Paddy system, the field is maintained flooded with about 3-5 cm water layer until two weeks before harvest. For GCRPS cultivation, the soil remained almost water-saturated but without standing water during the first week after transplanting. After this initial stage, the soil was kept between 80-90% of its maximum water holding capacity for the remaining growing period.

Potential site differences within a cultivation system: Generally there is extension staff in the villages ensuring that the guidelines for irrigation are followed. However we cannot exclude that there were deviations, also in previous years. But this was also the reason why we chose 49 pair sites.

Some results in Fig 2 would suggest some Clay content variation for example. Surprisingly (or not) bulk density doesn't seem to show much variation at all. Information on fertilisation is confusing. Was there application of manure and the application between the two system is not comparable (150 vs 180 kg N ha-1) the latter being for Paddy system which most likely received manure as well. Such general information is critical to permit a sound discussion and proper conclusions.

>> We conducted farmers' interviews to learn about general field management practices and there were no major differences in manure or synthetic fertilizer applications across treatments. One exception was that GCRPS fields receive fertilizer in one dose at the beginning of the vegetation period, while Paddy received split application of fertilizer. We provided the fertilization information in more detail in the revised version. The absence of significant variations in soil bulk density is a common observation for such soils.

Refs:

Li, Y. S., Wu, L. H., Zhao, L. M., Lu, X. H., Fan, Q. L., and Zhang, F. S.: Influence of continuous plastic film mulching on yield, water use efficiency and soil properties of rice fields under non-flooding condition, Soil Till. Res., 93, 370–378, 2007.

The second main issue relates also to the proclamation of a conclusion, namely root biomass increase due to GCPRS influencing soil nutrient acquisition) from a method which is only tested at one site. Again confounding factors could be at play (as well as weather during that particular year!). Overall, as well as additional information in the M&M section and re-writing of the discussion, the manuscript would also benefit from additional details in the statistical section as well as editing for ease of reading and grammar.

>> It is actually true that, unfortunately, and due to logistic reasons it is just not possible to sample root biomass at all investigated sites. However, the observed effects of GCRPS cultivation on the root system at one of the sites was consistent with earlier independent publications (e.g. Li et al., 2007; Thakur et al., 2011; Uga et al., 2013). Nonetheless, we have further outlined the limitations of this single site sampling approach for root biomass in the

revised version. Large parts of the discussion were rephrased for the revised version, and the statistics section was extended, now providing the details requested by all reviewers. Furthermore, the manuscript was again checked in detail by a native speaker and co-author of our paper, Dr. David Pelster.

Other general comments:

3650 L16: 'reducing water demand by 50-90%'. This is a very wide range with no reference to back it up?

>> Yes, it is a very wide range of reported reduced water demand, which was found to depend on the precipitation, soil type and cultivation duration. We added two references in the revised version.

3650 L23: how is making and using more plastic and leaving it in nature reducing the environmental footprint. Be more specific here what kind of benefits is gained. Also how about its atmospheric impact?

>> This topic is already discussed and mentioned in the manuscript, specifically in the conclusion section. We are sensitive to this issue and we state more clearly in the revised version that the GCRPS technique may be environmentally suitable for further expansion only if biodegradable films are used. We hope that publication of this manuscript further increases the awareness of the pollution of landscapes with plastic films.

3651 L 11: 1935 reference? Anything newer?

>> New literature reference was added in the revised version.

3651 L14 how about CH4 emissions?

>> CH₄ emissions were found to be significantly reduced under GCRPS cultivation compared to Paddy cultivation, however we found increased N_2O emissions (Kreye et al., 2007; Yao et al., 2013) that did not outbalance the gain of reduced CH₄ emissions (Yao et al. 2013).

3651 L24 the impact of higher aeration and soil temp on SOC mineralization has been widely looked at recently (update reference Stanford 1973)

>> This is correct. However, there is a lack of information on it for the innovative water-saving GCRPS. We have revised this part in the new version.

3652 first paragraph belong to M&M

>> Revised as suggested – we have moved this paragraph to the M&M section.

3653 L 20 How wide is the range of soil type? All sub-tropical kind of soil? Information on what kind of soils are being sampled is totally omitted. More information on depth to hardpan would be required as discussed further.

>> We now provide information on soil types for each sampling site (see revised Table S1 in the revised version). The soil types are: Dystric Cambisols, Haplic

Luvisols, Dystric Regosols, Calcaric Regosols and Eutric Gleysols. The depth of the hard pan is located in 20-40 cm and was not influenced by the cultivation technique.

3653 L8: how is the fertiliser applied to the GCPS and for Paddy, how many applications per year? Manure is mentioned in the discussion but not in the M&M. >> Because the plastic film covers the soil surface, topdressing is not used for GCRPS, i.e., farmers apply all the fertilizer before transplanting. The day before transplanting, compound fertilizer containing about 150 kg N ha⁻¹ was applied to the soil surface in a single dose and incorporated into the soil by ploughing. The soil surface was then levelled and covered with a 5 μ m transparent film (Liu et al., 2013). For Paddy, an average of approximately 100 kg N ha⁻¹ was applied as compound NPK fertilizer to the soil surface and incorporated to a depth of 20 cm before transplanting. At both tillering and grain filling stages, additional doses of 40 kg N ha⁻¹ were given as urea in order to increase rice milling quality and protein content (Wopereis-Pura et al., 2002; Leesawatwong et al., 2005) and yield. Thus the total N application for Paddy systems was approximately 180 kg N ha⁻¹. In the revised version we have extended this section to provide all this information.

3653 Is it a short-duration or long-duration variety?

>> It is a middle-duration (about 140 days) cultivar that is used for both GCRPS and Paddy. We have added this information to the Introduction section of the revised version.

3655 L22 which site is this?

>> It is a site in Fang County where we took 22 paired samples for regional evaluation and our well-managed long-term experiment located (Tao et al., 2015). In the revised version, we are specifically marking this site on the location map.

3656 L16. 'except for C stocks at 0-20 cm depth' as at that depth, concentrations are significantly different according to Fig 1c >> Revised as suggested in the revised version.

3656 L25 Mention that the root biomass is from the one experimental site. >> Thanks for the good editorial comment that helps to clarify this – we revised as suggested.

3657 L8 and Fig 6: is this correlation real, very low R2? >>Yes, this is correct. The very significant P value at comparably low R^2 values is explained by the large sample size (N=465).

3657 L18 explain here what are s+c and LF as not explained in M&M. Also in Fig 8. Need to be introduced in M&M

>> We have added sentences to explain physical fractions to the M+M section of the revised version, as well as to the Figure caption of Fig. 6 in the revised version.

3658 L11 Hardpan is mentioned here in the context of the study for the first time. It would be beneficial to give some information on its depth in such soil.

>> In the revised version, we provide information on the depth of the hardpan in the Introduction section.

3658 L14. In our study: : : then followed by 2 references. Do you mean these studies or do they match these studies?

>> We mean these studies, as they were conducted in the same experimental framework. The sentence has been rephrased in the revised version to clarify this.

3658 L29 - 3659 L2. The arguments don't follow up congruently. Separate micro-nutrient and need to go deeper (this is not to avoid toxicity effects as Fe is oxidised) and explain separately the N nutritional balance.

>> In line with your comments, the sentence was split in the revised version to clearly separate these topics.

3659 L6. again what depth is the hardpan at?

>> Hardpan is located in 20 - 40 cm. This information was added in the revised version.

2659 L15-25. "This indicated: : :" not significantly different so how do you conclude this? Where is the higher OM in put coming from and while we have no information on the soil types samples, why do you assume clay minerals as a factor in both system? Also there is no higher SOM stability according to the fraction s + c so argument not valid.

>> We have eliminated such a conclusion, as we agree that these data do not justify the conclusion that GCRPS provides greater SOM stability than Paddy systems. The section has been reorganized.

2661 L8 suggest remove 'environmentally sound' as the sentence below explain this technique does pollute the environment!

>> For clarification, we have changed this sentence to "environmentally sound....., given that biodegradable films are used in order to prevent soil and landscape pollution"

Fig 3. Why refer to previous publication for further details? Why CAGB, no need for such abbreviation?

>> The abbreviation will be omitted in the revised version, as well as the reference to a previous publication.

Fig 4. This figure doesn't show the N-fertiliser treatment. Is it amalgamated. Please inform both in the M&M and in the graph.

>> Yes, data are amalgamated across fertilizer treatments. This is because root
biomass was neither affected by the N fertilizer rates nor by the interaction of N fertilizer rates and the cultivation system. We have added this information to the figure caption in the revised version.

Technical corrections Abstract L1: Full stop after 'scarcity' and start new sentence with 'However,: : :

Abstract L10: 'typical of' instead of 'for'

3650 L4 'grown on c. 29.9 million ha'

3650 L10 'production increase' instead of 'increasing'

3657 L8 'compared with Paddy'. Remove 'in'

Supplement material: Table heading should read 'Township' instead of 'Towship'

>> Many thanks for the thorough reading. All this was revised as suggested in the new version.