Dear Prof. Dr. Yakov Kuzyakov and dear Dr. Natascha Töpfer

Thank you very much for providing the opportunity to further revise the manuscript. Find our responses to the reviewers' comments below. We have revised the manuscript in line with the suggestions by the reviewers. However, reviewer #6 underwent some errors in the evaluation of our work, as we clarify below.
We believe that this revision has further resulted in a stronger manuscript and hope that it now can be accepted for publication in Biogeosciences.
Sincerely,

Prof. Dr. Shan Lin, on behalf of all co-authors.
15.07.2015

Report #1 (Referee #1)

L111: Paddy 'field system'?
>> Yes, to clarify, we changed this to “Paddy system”.

L137 to 140: sentence does not make sense. The authors wish to explain why GCRPS and traditional Paddy are found side by side spatially but this is not conveyed clearly. >> To clarify, sentence was rephrased as follows: “Traditional lowland rice cultivation (Paddy) and GCRPS are spatially interwoven, because only some farmer adopted GCRPS after its invention two decades ago. This limited adoption is due to the implications GCRPS cultivation has on farming activities, labour demand and associated costs”

L169 remove (hereafter referred to...) as this has been used already before in the manuscript.
>> Revised as suggested.

L325 'Figs 2 and S1': where is Fig S1 (if indeed a figure?)
>> Fig S1 is in the Supporting Information, which is accompanying the paper. Besides this figure, there are four tables in the Supporting information.

Report #2 (Referee #5)

Dear Editor and Authors,

This manuscript has been improved after the revision. I find the Methods and Results quite reasonable as it is in this revised version. The data is of value to publish. However, the Introduction needs more state-of-the-art, rather than just a general introduction. Discussion part still reads a bit over-stated, and lack of real crispy in-depth interpretation. It still needs revision to meet the standard of this journal.

Basically, the Discussion part is structured into two sections: SOC and SON. Yet, a large part of each of the two sections is actually literature review, not really in the sense of interpretation or peer reports comparison. If the literature could have been discussed and argued much earlier in the Introduction, it would spare a great amount of writing space to build up a much more in-depth interpretation of the observed results, and comparison with peer reports in the Discussion. In addition, if the literature cited in the Discussion is to support to reject the initial hypothesis that more C and N loss should be the consequence of GCRPS, then the literature might be also used in the Introduction to discuss and argue the potential knowledge gaps between those two conflicting consequences (increase or decrease SOC stock). The argument and structure of this manuscript then might be much stronger.

>> As we wrote in our earlier responses to the reviewers' comments in the last revision round, we feel that details provided in the introduction and discussion sections were already balanced. However, in order to comply with these comments, we moved several introducing sentences from the discussion section to the introduction section in our latest revision. Furthermore, we have added arguments and literature on potential positive effects of GCRPS on SOC and TN stocks to the introduction section, as requested by the reviewer.

At last, I am still not convinced about the sustainability of using plastic sheets as cover materials. It perhaps would also help to rectify the net benefits of this technique, if the authors could discuss and show more the limitation of such plastic sheets, and their potential impacts to the environment. Otherwise, over-stating the positive benefits of the plastic sheets in increasing SOC stock and mitigating climate change is very likely to mislead local policy decision to prompt large-scale use of the plastic sheets.

>> We fully agree with the reviewer that the use of plastic films remain a very critical issue and therefore we have mentioned this already in the last version at a very prominent position in the conclusion section. In the last sentences of our paper we then clearly outline the problem by stating that plastic films need to be replaced, and we provide solutions with potential further benefits (biodegradable films with micronutrients). So we feel that we have comprehensively addressed this important topic.

Report#3 (Referee #6)

General comments:

I liked this study for having 59 paired sites and examined soil profile down to 1 m, and the questions related to the organic C and N as affected by the new rice management technique. However, this study failed to provide convincing evidence to test the hypothesis "that improved soil moisture conditions and increased soil temperature and redox potential in GCRPS would stimulate soil C and N mineralization…"

First, the authors even did not even measure variables in the hypothesis such as redox potentials; Second, they did not perform experiment to control temperature; Third, they failed to control two soil moisture conditions in the field by incubating soils in laboratory conditions with the same soil moisture level.

Thus, their data on decomposition and C fractions are invalid to accept/reject their hypothesis. Although their field survey data may provide limited evidences.

>> Redox potential and soil temperature were actually measured in situ on the fields and published earlier in the study of Liu et al. 2013 (Field Crops Research), we frequently refer to this paper. Soil temperature as well as redox potential increased under GCRPS cultivation. Furthermore, plant leaf $\delta^{13}C$ showed that soil moisture decreased in the GCRPS fields (Liu et al., 2013).

As we had clarified already in our earlier responses, the mineralization potential experiment under controlled conditions such as also standardized soil moisture and temperature aimed to unravel altered mineralization potential due to different physical protection of organic matter. It is not possible to quantify mineralization potential under saturated (Paddy) or almost saturated (GCRPS) soil water conditions as is observed under field conditions, and it is not useful to have other confounding factors (different temperature, moisture e.g.) in such mechanistic experiments under controlled conditions.

Overall, we provide a comprehensive assessment how GCRPS cultivation affects SOC and TN stocks via its combined effects on soil temperature, soil moisture and soil aeration, aboveground and belowground plant biomass, fertilizer use efficiency and N loss, and physical protection of organic matter. Most of the presented evidence is field-based with a hitherto unavailable spatial coverage. We do not understand why this field survey should provide "limited evidence" and are convinced that this actually is the strongest possible evidence for the observed GCRPS effects. Definitely this is an excellent justification to reject our initial hypothesis that GCRPS would lead "to an overall reduction of soil C and N stocks ….at a regional scale".

Technical corrections

Line

61 resulted from

>> Revised as suggested.

79 to maintain soil…

>> Revised as suggested.

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

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Abstract

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

The study showed that: 1) GCRPS increased SOC and N stocks 5-20 years following conversion from traditional Paddy systems; 2) there were no differences between GCRPS and Paddy in soil physical and chemical properties for the various soil depths with the exception of soil bulk density; 3) GCRPS increased above-ground and root biomass in all soil layers down to a 40 cm depth; 4) $\delta^{15}N$ values were lower in soils and plant leaves indicating lower NH_3 volatilization losses from GCRPS than in Paddy systems; and 5) GCRPS had lower C mineralization potential than that observed in Paddy systems over a 200 days incubation period. Our results suggest that GCRPS is an innovative production technique that not only increases rice yields using less irrigation water, but that it also increases SOC and N stocks.

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

1 Introduction

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

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.30 million hectares and accounted for 43.7% of the total national cereal grain production (Fan et al., 2010). Water shortages already affect more than 4 million ha devoted to rice production in China, and a significant proportion of this area also show comparatively low yields result~~ant~~ed from low-temperature limitations. One of the most promising techniques to overcome these limitations is the Ground Cover Rice Production System (GCRPS). Here, the soil is covered - typically with plastic film - to reduce evaporation, seepage losses and increase springtime soil temperatures. The soil is kept moist between irrigation periods thanks to the covering material, which reduces irrigation water demand by 50-90% (Tao et al., 2015). The actual reduction in irrigation water demand is dependent on soil type, precipitation and cultivation duration (Tao et al., 2006; Liu et al., 2003). Furthermore, high-yielding lowland rice varieties (middle-duration cultivar, about 140 days) can still be grown in upland locations using GCRPS, which results in similar or

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

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

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

and previous studies have shown that continuous rice cropping on submerged soils may favour the maintenance, and even the increase of SOM stocks (Cassman et al., 1995; Bronson et al., 1997; Witt et al., 2000). Consequently, it has been hypothesized that the absence of permanently anaerobic conditions, in conjunction with increased soil temperatures under GCRPS cultivation may result in either unchanged or increased SOC losses as a result of potentially enhanced microbial decomposition (Pan et al., 2003, 2010; Qu et al., 2012). Indeed, earlier studies showed trends towards lower SOC and total N stocks in fields using the plastic film-based GCRPS technique. However, these studies have only investigated the topsoil (0-20 cm) above the hardpan at a single experimental site (Li et al., 2007; Fan et al., 2012; Qu et al., 2012). ~~While some studies have shown that GCRPS accelerated SOM decomposition and resulted in a decline in soil SOM stocks in the topsoil above the hardpan (between 20-40 cm) (Li et al., 2007; Fan et al., 2012; Qu et al., 2012), a thorough regional-scale evaluation of GCRPS effects on SOC and total N stocks has not yet been reported.~~ The GCRPS-induced shift from flooded soils to higher aeration and soil temperatures at the start of the growing season may result in reduced CH_4 emissions, while N_2O emissions (Kreye et al., 2007; Yao et al., 2014) and C mineralization rates may increase (Koch et al., 2007). On the other hand, high ammonia volatilization in Paddy systems tends to result in low N use efficiency (approx. 30%) (Ju et al., 2009) and covering the soil surface might reduce ammonia volatilization rates, increase fertilizer use efficiency, plant biomass and/or soil N stocks. Furthermore, variable soil water regimes such as observed under GCRPS cultivation can increase root biomass (Thakur et al., 2011; Uga et al., 2013), which in turn could promote C inputs into the soil. –A thorough regional-scale evaluation of GCRPS effects on SOC and total N stocks is needed to address these effects, but has not yet been reported.

To evaluate the impact of GCRPS on soil C and N stocks as well as identifying the primary N loss pathways from GCRPS and Paddy system using the natural abundances of ^{15}N, we conducted a field study sampling 49 pairs of neighbouring GCRPS and Paddy fields in the Shiyan region, Central China, where the GCRPS technique was first introduced approximately 20 years ago. We hypothesized that decreased ~~improved~~ soil moisture conditions and increased soil temperature and redox potential in GCRPS would stimulate soil C and N mineralization, leading to an overall reduction of soil C and N stocks under GCRPS at a regional scale.

2 Materials and methods

2.1 Sampling region characteristics

The study was situated in Shiyan region, Hubei province, Central China (32°02′ to 33°10′N, 109°44′to 111°04′E, 169 m to 661 m a.s.l., see Table S1), where GCRPS was introduced at the end of the last century (Shen et al., 1997; Liang et al., 1999). Shiyan is located in the QinBaShan Mountains with peaks reaching a maximum altitude of 2740 m a.s.l.. The area is in the northern subtropical agro-climatic zone of China's eastern monsoon region (Smit and Cai, 1996). Low temperatures at the start of the growing season together with severe seasonal water scarcity often limit rice production in these mountainous regions (Shen et al., 1997). The mean annual temperature and rainfall (calculated for the 1961-2009 period from seven meteorological stations located in the respective counties of Shiyan) are 15.3 ℃ and 829 mm respectively (Zhu et al., 2010). There is little inter-annual variation in temperature and rainfall (coefficient of variations of 0.01 and 0.05). Annual rainfall patterns show pronounced seasonality, with approximately 45% (375 mm) of the rainfall occurring during the summer period (June to August). The mean total sunshine hours per year are 1835 h (Zhu et al., 2010). Traditional lowland rice cultivation (Paddy) and GCRPS are spatially interwoven, because only some farmer adopted GCRPS after its invention two decades ago. This limited adoption is due to the implications GCRPS cultivation has on farming activities, labour demand and associated costs ~~Given that GCRPS was introduced only two decades ago and the implications for farming activities, labour demand and associated costs, has resulted~~

in GCRPS and traditional lowland rice cultivation (Paddy) often being spatially interwoven (Zhou et al., 2008). In most cases the adoption of GCRPS by individual farmers was documented by the local administration so it was possible to trace specific land management records for the selected sites and fields.

2.2 Site and field selection

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

We compared, across a region of 5 000 km^2, 49 pairs of neighbouring fields that were managed either as traditional paddy rice fields or where GCRPS had been applied continuously for 5-20 years. A total of 49 sites with paired treatments consisting of GCRPS vs permanent flooding paddy fields ~~(hereafter referred to as GCRPS and Paddy)~~ were selected for soil and plant sampling. Regardless of the current production system, all sites had been growing rice for more than 40 years. The distance between the paired plots were in most cases less than 100 m, with only 9 out of 49 paired plots being more than 250 m apart (Table S1). Geographical coordinates of the sites and fields were recorded by GPS (Garmin Colorado 300) and altitudes were obtained using the Global Digital Elevation Model (GDEM) provided by NASA and METI (2008).

2.3 Sampling methodology and analytical procedure

Soil samples from the 49 paired sites were collected before field preparation during March and April 2011. These sites represented a wide range of different soil types (Table S1). 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). Additionally, 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).

Soil samples for each depth interval were air dried for 5 days and sieved to 2 mm. Identifiable plant material (>2 mm) was removed during sieving. Soil pH (Mc Lean, 1982) was measured in 1:2.5 soil-water solution using a combined electrode pH meter

206 (HI 98121, Hanna Instruments, Kehl am Rhein, Germany). Extractable soil NO_3^--N
207 and NH_4^+-N (Keeney and Nelson, 1982) was estimated from 1:10 soil-$CaCl_2$ (0.01M)
208 extracts using an autoanalyser (AA3, Bran & Luebbe, Nordstadt Germany). Sub-
209 samples for determination of soil C and N concentration and ^{15}N isotope natural
210 abundance were powdered in a ball mill (MM200, Retsch, Haan Germany) with the
211 soil carbonates removed prior to C analyses (Harris et al., 2001; Walthert et al., 2010).
212 Analyses were conducted using a Costech Elemental Analyzer (Costech International
213 S.p.A., Milano, Italy) fitted with a zero-blank auto-sampler coupled via a ConFloIII to
214 a Thermo Finnigan Delta V Plus isotope ratio mass spectrometer (Thermo Scientific,
215 Waltham, MA, USA). Soil C and N stocks were calculated using element
216 concentrations and bulk density data for all sites.
217
218 Leaves at maximum tillering stage and aboveground plant biomass at maturity stage
219 were sampled from 36 paired sites (at some sites rice was not planted as foreseen due
220 to a severe drought) with three replicates from each site used for analysis of ^{15}N
221 natural abundance using a CN analyser coupled to a mass spectrometer (see above).
222 Carbon and N concentrations were then determined by an elemental analyzer
223 (EA1108). Carbon and N assimilated in aboveground biomass were calculated as the
224 sum of grain and straw dry matter multiplied by grain and straw C or N concentration
225 at harvest.
226
227 Root biomass was quantified at a long-term experimental site in Fang County
228 (32°07'N, 110°43'E; Fig. S1; Tao et al., 2015) where 22 paired GCRPS and Paddy
229 sites were located (Table S1). The site consists of the two production systems (Paddy
230 and GCRPS) and two N fertilizer application rates (0, 150 kg N ha^{-1}) in three-fold

replication. All 12 subplots (8.5 m × 9.5 m) were arranged in a complete randomized block design. Root biomass was quantified for three replicate cores in each of the subplots. For this purpose, soil columns (40 cm height and 15 cm diameter) were collected at the maximum tillering stage using stainless steel cylinders. The soil column was separated into depth intervals of 0-10, 10-20 and 20-40 cm. Soil samples were placed in mesh bags and set in a water stream to remove soil particles and then cleaned by tap water on a 0.2 mm mesh. Cleaned root samples in different soil depths were transferred into small envelopes and oven-dried at 75 ℃ for 24 h.

Potential soil C mineralization rates from all 49 paired Paddy and GCRPS sites were determined using a laboratory incubation assay. Three soil samples with a volume of 20 cm × 10 cm × 20 cm (depth) were sampled at each site using a spade. Samples were composited and air dried. Three replicates with 30 g of soils were incubated for 200 days at 25 ℃ at 60% soil water-holding capacity in 150 ml bottles. CO_2 fluxes were measured daily for the first 10 days, then every three days for the following three weeks and then every 1-2 weeks afterwards. The gas measurement period was from 5 min to 4 hours depending on CO_2 flux rates. For flux measurements, the jars were closed gas-tight and CO_2 headspace concentrations were measured with a non-dispersive infrared sensor (Premier, Dynament, United Kingdom) at 10-second intervals. CO_2 fluxes were calculated from concentration changes with time, considering headspace volume, temperature and air pressure. Total cumulative emissions were obtained by summing the measured daily fluxes using trapezoidal integration assuming a linear change in flux between measurements.

255 Organic matter (OM) fractions were physically separated before and after incubation
256 using a slightly modified procedure to that described in Zimmermann et al. (2007).
257 Briefly, 30 grams of dried soil (<2 mm) were added to 161 mL water and dispersed by
258 means of a calibrated ultrasonic probe (Labsonic 2000, B Braun, Melsungen,
259 Germany) using a light output energy (22 J ml^{-1}). The dispersed suspension was then
260 wet sieved over a 53 µm mesh size until achievement of clear rinsing water. The
261 fraction > 53 µm was dried at 40 ℃ and weighed. This fraction contained sand-size
262 particles and aggregates (Heavy fraction, HF), as well as particulate organic matter
263 (Light fraction, LF). These two fractions were separated using the procedure for
264 recovery of organic matter from soils using static dense media as described in Wurster
265 et al. (2010). The dried fraction >53 µm was stirred in a water:sodium polytungstate
266 solution with a density of 1.87 g cm^{-3}. The mixture was centrifuged at 1000 g for 15
267 min, and allowed to settle overnight prior to freezing. The LF was subsequently
268 decanted and both fractions were then washed with deionized water, dried at 40 ℃ and
269 weighed. The solution <53 µm (silt and clay) was filtered through a 0.45 µm
270 membrane filter and the material retained in the membrane (s+c) was then dried at
271 40 ℃ and weighed. An aliquot of the filtrate was frozen to determine the amount of
272 dissolved organic carbon (DOC) using a C/N liquid analyser (Multi N/C 3100
273 Anaytik Jena, Jena, Germany).
274
275 **2.4 Statistical Analyses**
276 All statistical analysis and calculations were performed in the Statistics Analysis
277 System (SAS, version 8.2). Shapiro-Wilk tests were applied to check for normal
278 distribution. Non-parametric tests were applied if the data was not normally
279 distributed. Before any statistical test was performed, we tested for significant

differences between GCRPS and Paddy according to a model that included soil type, years since conversion, soil type and elevation as potential variables influencing the percentage change of SOC/N stocks between both systems. However, we found that the percentage change of SOC/N stocks was not significantly affected by soil type, years since conversion, elevation nor by any of the interactions. Therefore, we pooled over different soil types, years since conversion and elevation in the subsequent statistical analysis (Table S3). A paired t-test was used to test for differences in soil texture (clay, silt and sand content), bulk density, pH and mineral N concentrations (Nmin) between GCRPS and Paddy. All statistical analyses and calculations were performed using parametric (paired and two-tailed t-test, Pearson chi-square) and non-parametric (Wilcoxon matched pairs rank sum test; two-tailed) tests. Differences in root biomass between the two systems were tested using the general linear model (GLM) procedure. Results are expressed as arithmetic means ± standard error of the means, levels of significance for all tests of *=0.05, **= 0.01, ***=0.001% probability level respectively and ns=not significant were used.

3 Results

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

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

Potential C mineralization rates did not differ between GCRPS and Paddy (data not shown), although Paddy soils showed a tendency towards higher cumulative C loss compared to GCRPS over the 200-day incubation period (Fig. 5). 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 lower after incubation compared to before the incubation ($P < 0.05$). No differences were found in the s+c, LF and DOC fractions before and after the incubation (Fig. 6).

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

4 Discussion

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

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

356 Recent literature has confirmed that rice cultivation under variable soil water regimes
357 such as GCRPS results both in higher root biomass (Thakur et al., 2011; Uga et al.,
358 2013), and more rhizodeposits (Tian et al., 2013) compared to traditional flooded
359 Paddy, likely because the larger aboveground biomass and grain yields require a
360 larger root system to absorb more nutrients from the soil (Liu et al., 2003). GCRPS
361 also promotes increased soil NO_3^- concentrations that can lead to more balanced plant
362 N nutrition (NO_3^- and NH_4^+), which is beneficial for crop growth (Nacry et al., 2013).
363 Moreover, the fluctuating soil water content inherent to GCRPS, which varies
364 between 80-90% water holding capacity (WHC), can limit the accessibility to some
365 micronutrients (e.g. Mn, Fe) in the topsoil if they are oxidised to forms that cannot be
366 directly assimilated by the plant (Tao et al., 2007; Kreye et al., 2009). For example,
367 the lack of standing water may cause increased soil aeration, and thus, higher redox
368 potentials (Tao et al., 2007), resulting in the oxidized form of Mn that greatly lowers
369 its availability to the plant (Norvell, 1988). Therefore, rice plants in GCRPS need to
370 develop stronger root systems capable of accessing deeper soil layers to obtain a
371 balanced micro-nutrient supply. Even if just a few fine roots penetrate the hardpan
372 they may represent a large difference in deep SOC storage as root channels may
373 further promote percolation of organic compounds into the subsoil.
374 *b) Greater physical protection of soil organic matter against microbial degradation*
375 We conducted soil incubations under controlled environmental conditions using soils
376 from all field sites to test whether GCRPS would enhance SOM stabilisation or
377 increase C mineralization, promoting net losses of SOM (Xiong et al., 2014). Our
378 results showed no significant differences in mineralization rates between soils from
379 the GCRPS and Paddy systems for all measuring dates over a 200-day incubation,
380 although cumulative C losses over the entire incubation period were consistently

greater for Paddy soils (Fig. 5). This could suggest that SOM in fields managed under GCRPS may be more effectively preserved than SOM in traditional Paddy systems. Besides the physicochemical protection offered by clay minerals (Koegel-Knabner et al., 2010; Saiz et al., 2012) other stabilizing mechanisms could be conferred through higher OM inputs resultant from enhanced above and belowground biomass production, as higher OM input rates are known to promote stable micro and mesoaggregates (Six et al., 2004). However, we did not observe significant differences between both systems in the physically protected fractions for the topmost soil layer (Fig. 6). It is likely though, that aggregation and/or stabilisation might become more relevant at deeper locations where the differences in SOC concentrations were greater. Indeed, the strong anaerobiosis and stabilisation conditions prevailing at depth would likely promote OM accumulation below the hardpan, as we found in our study (Fig. 1; Koegel-Knabner et al., 2010). Also relevant within this context is the contrasting soil redox conditions observed between the two systems (Liu et al., 2013). The more frequent oscillation in redox conditions (aerobic to 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 OM in Paddy soils (Koegel-Knabner et al., 2010).

Similar to SOC concentrations and stocks, soil organic N concentrations and stocks were larger in GCRPS than in paddy fields over the 1m soil profile. However, significant differences were only observed in the 20-40 cm depth interval (Fig. 1b, 1d). In addition, we observed $\delta^{15}N$ enrichment in paddy soils for all soil depths (Fig. 7a), which was also reflected in the plant biomass (Fig. 7b). Bulk soil $\delta^{15}N$ is a combined signal for organic and mineral N compounds and may be affected by (1) the amount

406 and isotopic signature of applied fertilizer (Yun et al., 2011), (2) isotopic fractionation
407 occurring during N cycle processes such as N mineralization, nitrification and
408 assimilation (Bedard-Haughn et al., 2003), and (3) ^{15}N depletion of gaseous N
409 compounds produced during denitrification and ammonia volatilization with
410 subsequent ^{15}N enrichment of the remaining soil N (Bedard-Haughn et al., 2003).
411 Based on farmers' interviews, the dominant fertilizer used was a compound NPK
412 fertilizer with urea as the N form ($\delta^{15}N$ of ca. 0.5‰) (Yun et al., 2011). As well as
413 urea-N, 11 of the 98 sites received manure ($\delta^{15}N$> 10‰). Most crucially, N
414 fertilization rates were comparable for both management systems (GCRPS: approx.
415 150 kg N ha^{-1}; Paddy: approx. 180 kg N ha^{-1}). Therefore, kinetic isotope fractionation
416 processes in the soil rather than mixing of different N sources with distinct $\delta^{15}N$
417 signatures likely account for the observed differences in soil $\delta^{15}N$. This is confirmed
418 by the observation that Ln-transformed soil N concentrations were inversely
419 correlated with the $\delta^{15}N$ values (Fig. 8).
420
421 The largest fractionation factors are consistently reported for gaseous N losses
422 (Bedard-Haughn et al., 2003; Robinson, 2001) so it is likely that changes in N_2, N_2O,
423 NO and NH_3 losses account for the ^{15}N enrichment in Paddy soils. Nitrification- and
424 denitrification - induced losses of N_2, N_2O and NO were expected to increase under
425 unsaturated soils typical for GCRPS cultivation as compared to continuous flooding
426 of Paddy soils that has also been documented in earlier studies (Kreye et al., 2007;
427 Yao et al., 2014). Therefore, we can rule out both fertilizer effects and changes in
428 denitrification losses as significant factors explaining lower $\delta^{15}N$ in GCRPS soils. The
429 ^{15}N enrichment in Paddy soils and increased soil N stocks under GCRPS are therefore
430 more likely related to ammonia volatilization following fertilizer application.

Ammonia loss from urea fertilization in Paddy rice fields can be very high with emission factors ranging from 9-40% of applied N (Xu et al., 2013). Covering the soil with a plastic film immediately after fertilizer application (Zhuang and Wang. 2010) or manure deposits (Webb et al., 2013) greatly reduces NH_3 volatilization losses. Therefore, we expect that the greater soil N stocks in GCRPS fields were associated with decreased NH_3 volatilization.

带格式的：正文，两端对齐，行距：单倍行距

5 Conclusion

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

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

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Figure captions

Figure 1 Concentrations and stocks 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). Errors bars indicate the standard error of the means. ***, **, * Significant at 0.001, 0.01, 0.05 probability level respectively; ns-not significant.

Figure 2 Average soil clay, silt and sand contents (for 0-20 and 20-40 cm, n=49; 40-60 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 traditional Paddy or GCRPS.** Errors bars indicate s.e.m. *** Significant at 0.001 probability level respectively; ns-not significant.

Figure 3 Carbon and nitrogen assimilated in aboveground biomass at maturity (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.

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

Figure 5 Differences in cumulative organic carbon mineralization during a 200 d incubation period of top soils (0 - 20 cm) collected from either Paddy or GCRPS. Data presented are the mean values pooled over 49 paired sites. Error bars indicate s.e.m.

Figure 6 Relative SOC fractionation (% of total) of topsoils (0 - 20 cm) from either Paddy or GCRPS grown rice fields for the different physically separated fractions before and after a 200 d incubation period. 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 ($P<0.05$).

Figure 7 (a) Soil δ^{15}N 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 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.

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

Figure 1

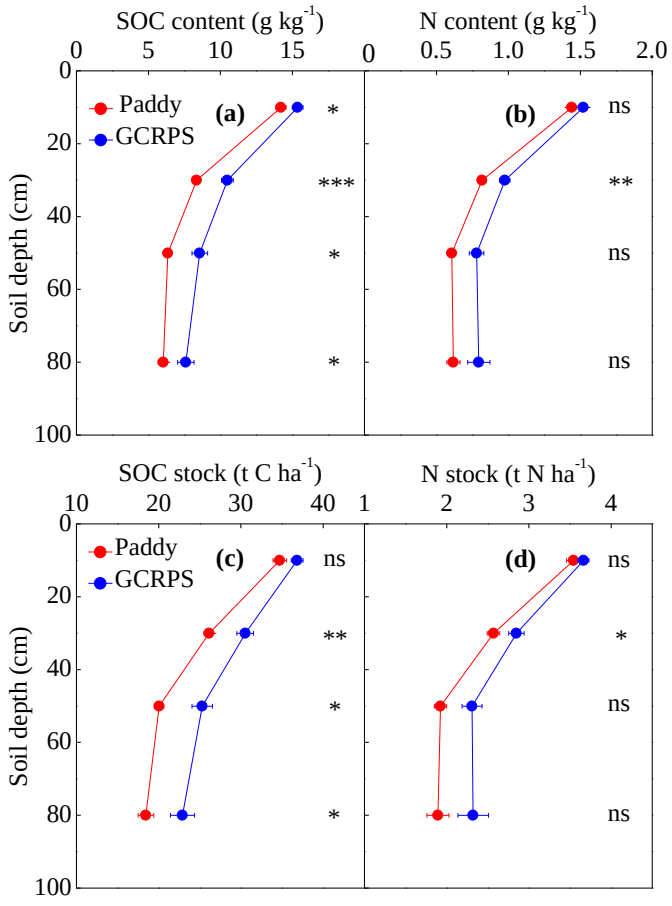

SOC content (g kg-1)
N content (g kg-1)
Soil depth (cm)
Paddy
GCRPS
(a)
(b)
(c)
(d)
SOC stock (t C ha-1)
N stock (t N ha-1)
*
***
ns
**

Figure 2

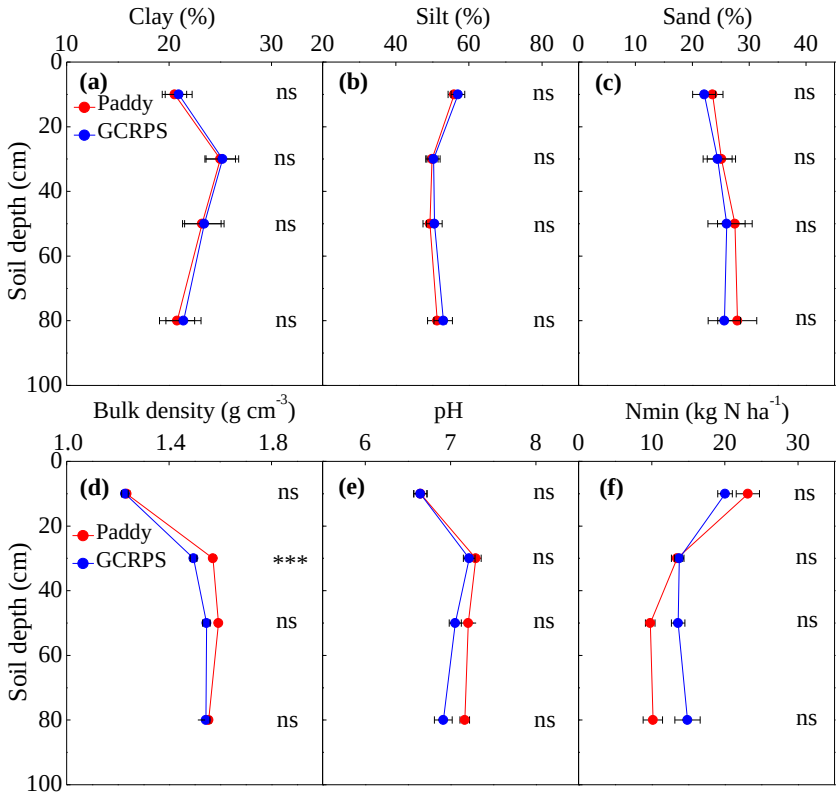

Clay (%)
Silt (%)
Sand (%)
(a)
(b)
(c)
Paddy
GCRPS
Soil depth (cm)
ns
Bulk density (g cm-3)
pH
Nmin (kg N ha-1)
(d)
(e)
(f)
***

Figure 3

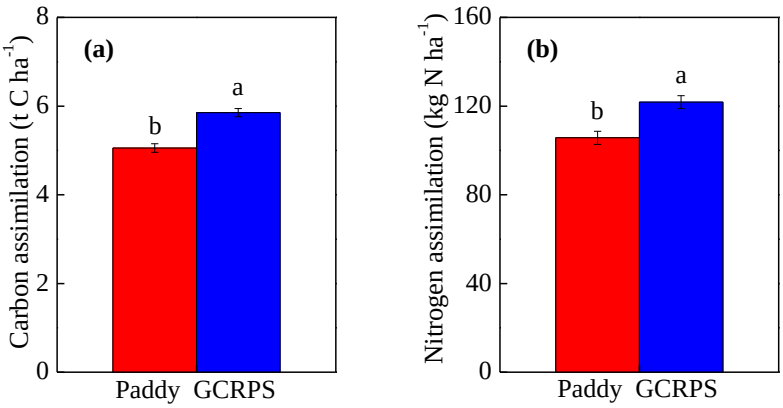

(a)
Carbon assimilation (t C ha-1)
8
6
4
2
0
b
a
Paddy
GCRPS
(b)
Nitrogen assimilation (kg N ha-1)
160
120
80
40
0
b
a
Paddy
GCRPS

Figure 4

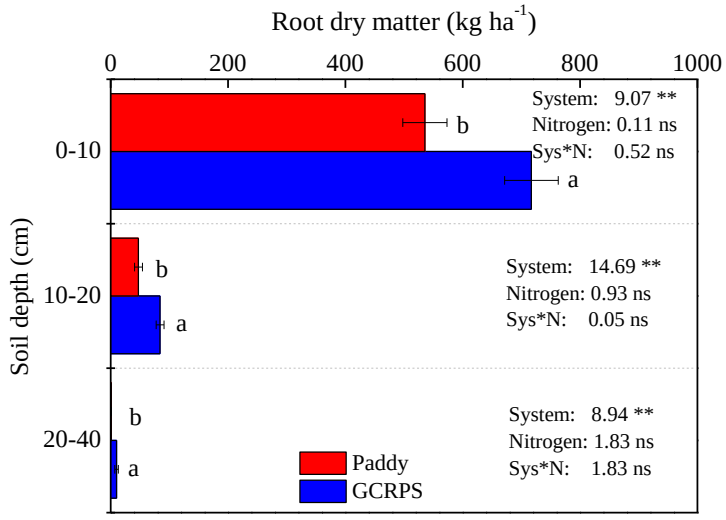

Root dry matter (kg ha-1)
0
200
400
600
800
1000
Soil depth (cm)
0-10
10-20
20-40
b
a
b
a
b
a
System: 9.07 **
Nitrogen: 0.11 ns
Sys*N: 0.52 ns
System: 14.69 **
Nitrogen: 0.93 ns
Sys*N: 0.05 ns
System: 8.94 **
Nitrogen: 1.83 ns
Sys*N: 1.83 ns
Paddy
GCRPS

Figure 5

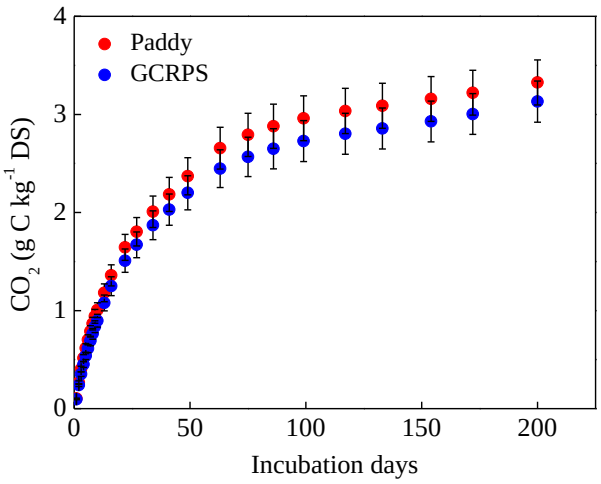

Paddy
GCRPS
CO_2 (g C kg^{-1} DS)
Incubation days
0
1
2
3
4
0
50
100
150
200

Figure 6

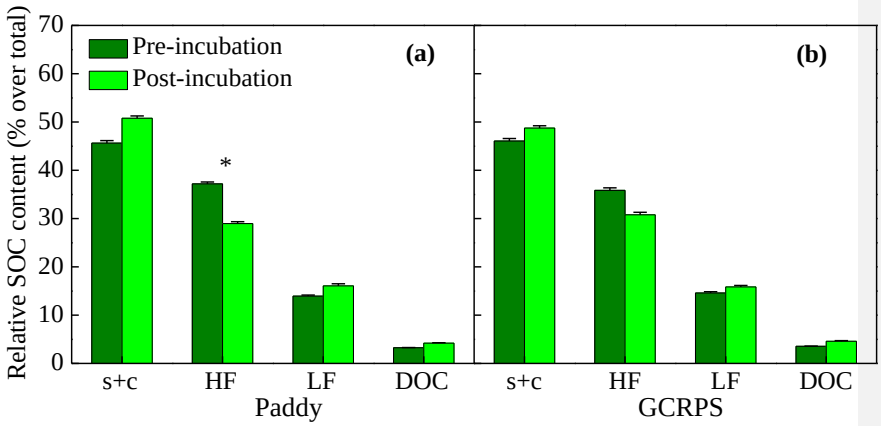

Relative SOC content (% over total)
70
60
50
40
30
20
10
0
Pre-incubation
Post-incubation
(a)
(b)
*
s+c
HF
LF
DOC
s+c
HF
LF
DOC
Paddy
GCRPS

Figure 7

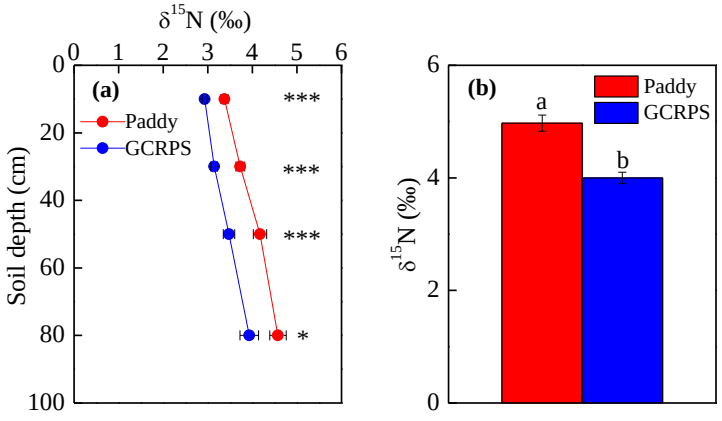
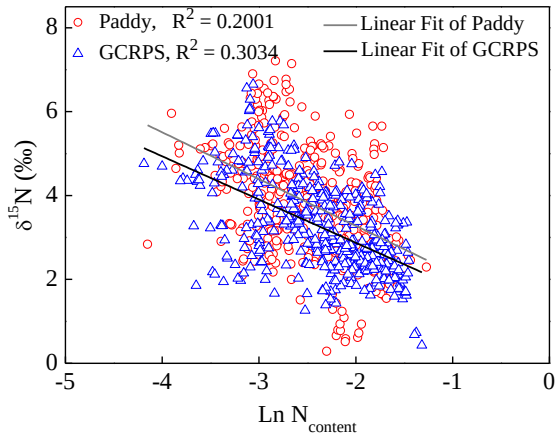

δ15N (‰)
0 1 2 3 4 5 6
(a)
Paddy
GCRPS
Soil depth (cm)
0
20
40
60
80
100
***
***
***
*
(b)
Paddy
GCRPS
a
b
δ15N (‰)
0
2
4
6

Figure 8


Paddy, R2 = 0.2001
GCRPS, R2 = 0.3034
Linear Fit of Paddy
Linear Fit of GCRPS
δ15N (‰)
Ln Ncontent
8
6
4
2
0
-5
-4
-3
-2
-1
0