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Enhanced nitrogen cycling and N_2O loss in water-saving ground cover rice production systems (GCRPS)

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ABSTRACT

An alternative to conventional cultivation of rice on submerged paddy soil is the ground cover rice production system (GCRPS), in which soil is covered with a plastic film to reduce the use of irrigation water. However, reduced soil water, increased aeration and temperature under GCRPS could promote soil nitrogen (N) mineralizing, nitrifying and denitrifying microbes and thus enhance soil N turnover and environmental losses e.g., through emission of the potent greenhouse gas nitrous oxide (N_2O). At two sites with paired GCRPS and conventional paddy fields in Central China, we followed the abundance and activity of N-mineralizers, nitrifiers, denitrifiers and N_2-fixing microbes based on qPCR from DNA and RNA directly extracted from soil. With decreasing soil water during the growing season, GCRPS strongly increased N mineralization as illustrated by several fold increased transcript levels of *chiA*. Furthermore, GCRPS reduced the *nifH* transcripts (encoding for nitrogenase) by 38% to 70% but increased the *qnorB transcripts by* 160% and archaeal *amoA* (AOA) transcripts by one order of magnitude (encoding for nitric oxide reductase and ammonia monooxygenase). This indicated a higher potential for N losses due to decreased biological N_2 fixation, increased N leaching and increased N_2O emission in GCRPS. The latter was confirmed by increased in situ N_2O emissions. In addition, the N_2-fixing and denitrifying microbial community composition as measured by a community fingerprinting approach was strongly influenced by GCRPS cultivation. Hence, our study reveals the microbial mechanisms underlying the risks for increased N mineralization, nitrification and N_2O emissions and decreased biological N fixation in GCRPS. However, analysis of topsoil N stocks provided evidence that at least under N fertilizer application, GCRPS might overall maintain soil N stocks. This might result from a GCRPS-induced increase in fertilizer N use efficiency, root development and C and N return via residues, which appear to outbalance the observed effects on nitrification, gaseous N losses and biological N fixation, thereby preventing a net loss of total soil N.

1. Introduction

Rice is the major staple food for almost half the global population, and about 90% of rice in the world is produced in Asia (FAO, 2011). To meet the food demand of a growing population, an annual increase in rice production in the range of 8–10 million tons is needed over the next 20 years, equaling to a global annual increase in rice production of 1–2%. Due to the need for irrigation, conventional paddy rice systems require about 2.5 m^3 water per kg of grain (Bouman and Tuong, 2001; Tuong et al., 2005). Consequently, an increase in rice production currently goes along with increased demand for irrigation water (GRISP, 2013; Ray et al., 2013). This is in contrast to declining water availability due to climate induced water scarcity and increased domestic and industrial water demands, which challenges overall food security in the world. Approx. 15–20 million hectares of irrigated rice fields suffer from water scarcity (Bouman, 2007). Thus, reducing the irrigation water demand of rice production systems has been a key research area (Bouman and Tuong, 2001; Qu et al., 2012; Yuan et al., 2014). One of the most promising technologies is the ground cover rice production system (GCRPS), which was introduced and promoted in

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Table 1
Site characteristics. Soil data are for 0–20 cm depth and originate from Liu et al. (2015) for the 10yr site. Data for the 3yr site originate from this study.

Field	Location	Mean annual temperature (°C)	Mean annual precipitation (mm)	Elevation (a.s.l.) (m)	Soil texture (sand/silt/clay) (%)	Bulk density (g cm^{-3})	Soil pH	Soil total organic C (g C kg^{-1})	Soil total N (g N kg^{-1})
3year GCRPS	N32°07′ E110°43′	14.7	856	440	20/60/20	1.36	6.0	12.4	1.31
10year GCRPS	N32°38′ E110°37′	15.3	834	234	36/47/17	1.05 (Paddy); 1.42 (GCRPS)	6.2	10.3	1.20

China roughly two decades ago and now has been widely adopted across China (Lin et al., 2002).

In GCRPS the soil surface is covered with a 5- to 7-μm thick plastic film and modern, high-yielding lowland rice cultivars are used and grown at soil water content nearby 80–90% of water holding capacity with no standing water layer during the entire growth period (Qu et al., 2012). This reduces evaporation by 32–54% and alleviates temperature limitations because the soil temperature is increased by 3–5 °C during early stages of rice cultivation. GCRPS cultivation requires an initial flooding of the field only, while during later crop growing stages the soil water filled pore space is kept at 80–90%. Using the GCRPS technique significantly increases rice yields where the water and temperature are limiting factors compared to the traditional paddy rice cultivation (Liu et al., 2013; Qu et al., 2012). The combination of increased temperatures and aerobicity in GCRPS soils compared to soils being permanently flooded for traditional paddy cultivation could stimulate mineralization of soil organic matter with losses of SOC and total N (TN). However this view was recently challenged by a regional study pointing to the opposite by revealing a net increase in SOC and TN stocks due to GCRPS cultivation (Liu et al., 2015). While these authors could provide some hints on the underlying mechanisms such as increased C and N input by roots and decreased fertilizer NH_3 losses, a detailed investigation of the response of key soil N turnover processes such as mineralization, nitrification, denitrification and biological N_2 fixation is missing. This still impedes a functional understanding of the response of the soil N cycle and associated N loss processes to GCRPS.

In a recent study it was shown that compared to conventional paddy systems rice production via GCRPS leads to decreased CH_4 emissions but increased N_2O emissions (Xu et al., 2004; Yao et al., 2014). Nitrous oxide can be produced by both aerobic nitrification and anaerobic denitrification pathways. While the underlying microbial mechanism has not yet been addressed in detail, it is commonly hypothesized that GCRPS promotes soil nitrification activity, as increased soil nitrate (NO_3^-) availability has been observed. However, increased nitrification and NO_3^- availability may also affect denitrification rates and denitrification N_2O yields. Hence several, potentially interacting microbial mechanisms may account for increased N_2O emissions observed under GCRPS cultivation. Thus in the frame of this study we analyzed the effects of GCRPS systems on microbes driving key N turnover processes affecting the soil N balance such as mineralization, nitrification and denitrification as well as biological N fixation.

The soil microflora responsible for processes of N turnover might be characterized by analyzing genes encoding for specific enzymes catalyzing specific N conversions within e.g. the nitrification or denitrification processes (Braker and Conrad, 2011). However, a decoupling between gene abundance at DNA levels and N turnover rates was found in many studies. This could be due to the fact that most of the N cycling genes might carry out multiple biogeochemical processes. E.g., the NO_3^- reduction genes *narG* and *napA* might conduct both denitrification and dissimilatory nitrate reduction to ammonium (NH_4^+). Furthermore, the abundance of genes is not necessarily related to the biogeochemical activity of the related microorganisms, as DNA levels only show the presence of such microorganisms in soil, but do not allow to conclude on activity levels (Sessitsch et al., 2002). Recently, it has even been shown that the DNA abundance of some genes catalyzing selected steps of the N cycle can be inversely related to the biogeochemical activity patterns as indicated by the analysis of the corresponding transcript levels (RNA) and/or associated gross N turnover rates (Chen et al., 2015; Di et al., 2009; Liu et al., 2014).

Here we examined changes in abundance and activity of microbial communities involved in soil N cycle processes for rice management is changed from paddy to GCRPS. We also explored consequences for soil N_2O emissions, dissolved C and N availability in soil as well as multi-year effects on SOC and TN concentrations. Hence, both the abundance of marker genes encoding for enzymes catalyzing key steps in N turnover as well as their transcripts were quantified in this study using quantitative real-time PCR (qPCR) based on DNA and mRNA extracted from soil. In the light of the regional study of Liu et al. (2015), which reported a positive effect of GCRPS on SOC and TN stocks, we hypothesized that GCRPS would not significantly increase N mineralization and rarely affect biological N fixation. However we expected that increased oxygenation of GCRPS soils would promote nitrification and increased microbial N_2O production.

2. Material and methods

2.1. Sites and soil sampling

Two experiments comparing GCRPS and conventional rice production on submerged paddy soil were conducted in a typical mountainous rice growing region of Central China - Shiyan County in Hubei Province. One experiment was sampled in 2012, 10 years after the initiation of GCRPS in 2003. The other experiment was sampled in 2014, 3 years after the initiation of GCRPS in 2012. Both field sites are located on the floor of small valleys in the upper Han River basin. However, the sites differ in soil texture and SOC content (Table 1) so that they were not regarded as a chrono sequence. The region is mountainous and has a humid subtropical climate with low temperatures and severe seasonal and regional water scarcity, both limiting rice yields (Liu et al., 2013). The location, soil physical and chemical properties of the two sites are provided in Table 1. The weather data during the growth period were collected at a nearby meteorological station (Fig. 1). Soil temperature at the 5-cm depth was recorded every 2 h by data loggers (EBI-20T, Ebro Instruments, Germany). Over 7 monitored years, the mean daily air temperature during the growing season ranged from 19 ± 1.3 to 28 ± 0.7 °C (Qu et al., 2012).

In both field experiments, we deployed two fertilizer treatments under the two rice production systems since GCRPS initiation in a full factorial design: P0 (paddy with no N fertilization), P150 (paddy with 150 kg urea-N ha^{-1}), G0 (GCRPS with no N fertilization) and G150 (GCRPS with 150 kg urea-N ha^{-1}). The treatments were arranged in a completely randomized block design with three replications and plot sizes of ca. 40 m^2 (10 years GCRPS) and 90 m^2 (3 years GCRPS) (Qu et al., 2012; Tao et al., 2015). All plots were completely isolated by levees with plastic coverings. As N fertilizer urea was applied once at a rate of 150 kg ha^{-1} y^{-1}, all treatments received 45 kg P_2O_5 ha^{-1} $y^{-1}$and 45 kg K_2O ha^{-1} y^{-1} as basal fertilization just before transplanting of rice plants. More details on the experimental setup are provided by (Qu et al., 2012; Tao et al., 2015).

In agreement with the local water management, the experimental