Summary of Doctoral Thesis

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Title	Utilization of Azolla in rice cultivation for climate change adaptation and mitigation

Rice (*Oryza sativa L.*) is one of the most important crops in the world. And with a predicted population increase in the near future, an increase in rice production to meet its demands is inevitable. This projected rise in production however faces several threats occasioned by climate change. These threats mainly include; (1) irrigation water shortages as a result of competition for water from other uses as well as the threat of droughts, (2) soil fertility and organic matter loss as a consequence of chemical fertilizer overuse and low manure amendments, and (3) increasing global warming as a result of anthropogenic greenhouse gas (GHGs) emissions. However, rice is not just a victim of climate change but also a major contributor, as paddy fields are considered the most important sources of anthropogenic GHGs; methane (CH_4) and nitrous oxide (N_2O) emissions. Therefore, adaptation and mitigation strategies are needed to cope with the effects of climate change on rice production as well as reduce water loss and GHGs emissions from rice fields. Azolla is a genus of small aquatic ferns that is naturally found in temperate and tropical regions worldwide, particularly in constantly flooded paddy ecosystems. Through its symbiosis with the cyanobacterium *Anabaena azollae*, Azolla is capable of fixing atmospheric nitrogen (N) and has thus been successfully utilized as green manure in lowland rice fields. However, the mitigation efficiency of Azolla on GHGs emissions from rice paddies remains contradictory. Furthermore, literature on its influence on water loss remains scarce. Within this context, this research study aimed to determine the potential of Azolla application not only as a promising alternative to chemical fertilizers but also as a possible water saver and a likely management practice to decrease the CH_4 and N_2O emissions from flooded paddy ecosystems.

First, to determine the influence of the rapidly growing Azolla cover on evapotranspiration (ET), two independent pot experiments were conducted in an incubation chamber (experiment 1) and greenhouse (experiment 2). The results showed that Azolla cover significantly decreased ET losses on average by 17.3% (experiment 1) and 20.0% (experiment 2) compared with open water

surfaces and both open water surfaces and green polyester covered mats (analogous to plant cover), respectively. The ET reduction potential by Azolla in both experiments was attributed to, but not limited to, its anatomy, horizontal placement of its leaves, and smaller leaf area, which possibly restricted simultaneous evaporation-transpiration losses by shielding much of the water surface.

Second, to investigate the effect of Azolla cover on simultaneous CH_4 and N_2O emissions from the constantly flooded rice paddies, an outdoor pot experiment was setup in a single rice-growing season in 2016. Two treatments, control (rice plant only) and Azolla cover (rice plus Azolla covering on the flooding water) were established in four replications. The bulk alluvial soil used in this experiment was collected from a rice field at the University Farm. Results showed that dual cropping of Azolla with rice significantly suppressed CH_4 emissions by 34.7% compared with the control, likely due to an increase in dissolved oxygen concentration and redox potential at the soil-water interface between the flooding water and soil surface. However, the Azolla cover did not significantly affect N_2O emissions from both treatments. This implied that Azolla cover did not affect extra N_2O flux from dual Azolla and rice cropping ecosystems.

Third, to investigate the influence of Azolla incorporation as green manure and its subsequent growth as a dual crop in conjunction with chemical fertilizers, on CH_4 and N_2O emissions from flooded paddy soil planted with rice, an outdoor pot experiment was setup in a single rice-growing season in 2017 with three treatments, chemical fertilizer (NPK) as control, incorporation of Azolla as green manure (AGM), and AGM plus basal chemical fertilizer (NPK + AGM). Results showed that AGM and NPK + AGM treatments significantly increased seasonal CH_4 emissions by 31.5% and 43.5%, and decreased seasonal N_2O emissions 3.4 and 4.6 folds compared to NPK, respectively. Both the CH_4 increase and N_2O decrease were attributed to the effect of the incorporated Azolla particularly at the early rice growth stages. Significantly higher grain yields were observed under AGM (32.5%) and NPK+ AGM (36.3%) compared to NPK. There were no significant differences in the CH_4 emissions per grain yield among treatments, however, compared to NPK, AGM and NPK+ AGM treatments significantly reduced N_2O emissions per grain yield by 78.7% and 84.1%, respectively.

Fourth, in the same batch of experiments as highlighted in the third section above, we investigated the effects of poultry-litter biochar (hereinafter biochar) amendment and its co-application with NPK and AGM (i.e., NPK + biochar and AGM + biochar) on the simultaneous CH_4

and N_2O emissions. The main objective was to determine the influence of AGM (incorporated as green manure and its successive growth as a cover) co-applied with biochar in lowland rice paddies on simultaneous CH_4 and N_2O emissions. Results showed that compared with AGM and NPK + biochar treatments, AGM + biochar did not significantly influence cumulative CH_4 emission during the whole rice growth period. Conversely, AGM + biochar significantly reduced N_2O emissions by 76.4%-95.9% compared with the other treatments, with a significantly high interaction ($P < 0.01$) between biochar and fertilizer amendments. Additionally, compared with all other treatments, AGM + biochar significantly increased rice grain yield by 27.3%–75.0%, and consequently, decreased both yield equivalent CH_4 emissions by 24.7%–25.0% and N_2O emissions by 81.8%–97.7%. These results suggest that the co-application of Azolla and biochar offers a novel approach to increase yield while mitigating CH_4 and N_2O emissions.

Fifth, to determine the effect of biochar application and its co-treatment with NPK and/or Azolla as green manure on rice yield, N uptake, and N use efficiency, eight treatments were compared; no amendment (control), NPK, AGM, NPK+ AGM, without and with biochar amendment. Biochar was the main factor, with fertilizer N sources (NPK and AGM) as the sub-factors. Results showed biochar amendment significantly increased grain yield (32.4%), grain N uptake (23.9%), apparent N recovery efficiency (28.1%), agronomic N efficiency (50.0%), and internal N utilization efficiency (35.9%), and decreased the soil N dependence rate (-15.2%) compared with the without biochar amended treatments. No significant synergistic interactions between biochar and the fertilizer N sources were observed on all determined parameters in this study setup.

In conclusion, the utilization of Azolla in rice cultivation has the potential to reduce chemical fertilizer application needs and irrigation water, increase rice yield, and reduce and/or mitigate CH_4 and N_2O emissions. However, these results were based on pot experiment setups in the laboratory and glasshouse, and on in situ outdoor setups during single rice cropping systems. Thus, long-term field studies should be carried out to validate the findings.