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Reducing nitrogen runoff from paddy fields with arbuscular mycorrhizal fungi under different fertilizer regimes

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ABSTRACT

Nitrogen (N) runoff from paddy fields serves as one of the main sources of water pollution. Our aim was to reduce N runoff from paddy fields by fertilizer management and inoculation with arbuscular mycorrhizal fungi (AMF). In northeast China, Shuangcheng city in Heilongjiang province, a field experiment was conducted, using rice provided with 0%, 20%, 40%, 60%, 80%, and 100% of the local norm of fertilization (including N, phosphorus and potassium), with or without inoculation with *Glomus mosseae*. The volume, concentrations of total N (TN), dissolved N (DN) and particulate N (PN) of runoff water were measured. We found that the local norm of fertilization led to 18.9 kg/ha of N runoff during rice growing season, with DN accounting for 60%–70%. We also found that reduction in fertilization by 20% cut down TN runoff by 8.2% while AMF inoculation decreased N runoff at each fertilizer level and this effect was inhibited by high fertilization. The combination of inoculation with AMF and 80% of the local norm of fertilization was observed to reduce N runoff by 27.2%. Conclusively, we suggested that the contribution of AMF inoculation combined with decreasing fertilization should get more attention to slow down water eutrophication by reducing N runoff from paddy fields.

Introduction

As the third biggest river in China, Songhua River serves as a major freshwater source for three provinces, including Heilongjiang, Jilin and Inner Mongolia municipality (He et al., 2008). However, the possibility of polluting Songhua River was reported (Guo et al., 2007b). Especially the total nitrogen (TN) load was 113×10^4 tons in Songhua River, with 84% caused by anthropogenic activities (Ma et al., 2011) which was aggravated by the long stay of pollutants due to the long icebound season (Guo et al., 2007b). Lalin River is one of the first tributaries of Songhua River and its water quality would contribute directly to the pollution of Songhua River (Guo et al., 2007a). The water quality of upper reaches of Lalin River was better than the threshold value of surface water of Grade I in *Quality Standard of Surface Water Environment of China* (GB3838-2002) while that of the lower reaches was much worse than that of Grade V (Cai and Shang, 2009; Ma, 2006), with high load of TN being one of the characteristics. N runoff from paddy fields was suspected to be one of the factors contributing to the poor water quality of the lower reaches of Lalin River (Kaushal et al., 2011) as rice production was dominated on its middle and lower reaches. In rice production, paddy fields are intensively fertilized for high rice yields (Liu, 2012), contributing the largest amount of N load to the surface water (Kawara et al., 1996; Zhao et al., 2012). In addition, the rice growing season in this area extends from June

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to October and corresponds with the main rainy and hydrologically active period of the year, which makes Lalin River be more vulnerable to be polluted. Furthermore, the shallowly ponded paddy fields (with a depth of 3–10 cm) experience irrigation, artificial draining and random rainfalls, increasing the possibility of N export via runoff (Kim et al., 2006). Collectively, the great contribution of N runoff from paddy fields to the deterioration of water quality was realized but little information of Lalin River is available.

There is growing concern about the environmental impacts of fertilizer applied in agricultural systems on water quality. The fertilization in intensive agricultural areas of China has resulted in serious environmental problems because of atmospheric, soil, and water enrichment with reactive N of agricultural origin (Tian et al., 2007). A number of studies have concluded that the large amount of chemical N fertilizer used in rice production was one of the major sources of N pollutants in water bodies (Kim et al., 2006; Zhao et al., 2012). Furthermore, it was reported that in Taihu Lake Region of China, the current application rate of N fertilizer was excessive (Guo et al., 2004) and the recommended rate could decrease from 10% to 40% to enable optimum economic and ecological results (Xia and Yan, 2012). However, the contribution of fertilizer applied in paddy fields to N runoff from paddy fields has not been well documented in Lalin River basin. The changing dynamics of N runoff, including dissolved N and particle N, during rice growing season is not well characterized in this area either. The lack of this comprehensive information has hampered the development of effective nutrient management policies, which led to the fact that the practice of high inputs of fertilizers is still underway in Lalin River basin while the deterioration water quality of Lalin River continues with little sign of improvement. Therefore, more information on N export via runoff from paddy fields supplied with different levels of fertilizers is required to improve the water quality of Lalin River and then Songhua River.

Besides fertilizer management, developing a safe alternative bio-fertilizer to replace or partly replace chemical fertilizer is another way to relieve water pollution or to prevent water deteriorations. Specifically, arbuscular mycorrhizal fungi (AMF) can be used as such a safe bio-fertilizer since AMF improved the capability of plants for nutrient uptake (Smith and Read, 2010). Potentially beneficial effects of AMF colonization on rice production systems has received much attention in scientific literature and a range of positive responses have been observed even under anaerobic conditions (Purakayastha and Chhonkar, 2001; Wangiyana et al., 2006), including an increase in plant size, tissue nutrient concentration (Solaiman and Hirata, 1996, 1997), photosynthetic rates (Black et al., 2000) and grain yields (Liu et al., 2013). Importantly, these beneficial effects of AMF were persistent until the second year after inoculation (Pellegrino et al., 2011). Although AMF inoculation was hypothesized to slow down the deterioration of surrounding water systems, few research make efforts to estimate the role of practical application of AMF in cutting down N runoff from paddy fields so that there is no conclusive evidence to prove this hypothesis.

The water environment of China suffered seriously and the water quality of many rivers, streams, reservoirs and lakes often does not meet the established standards, with periodic algal blooms occurring in most reservoirs. This implied that further efforts to safeguard the water quality of these water bodies are still needed (Meng, 2009).There is an abundance of evidence demonstrating the negative effect of N runoff due to intensive fertilization in rice production on surface water quality in Korean (Kim et al., 2006), Taihu Lake region of China (Zhao et al., 2012) and Lake Biwa basin of Japan (Kawara et al., 1996). This gave rise to the need for an urgent reduction in fertilizer rate (Xia and Yan, 2012) and application of arbuscular mycorrhizal fungi (AMF) as a safe biofertilizer (Zhang et al., 2010). Our study sought to investigate the dynamics, forms and accumulation of N runoff from paddy fields under different fertilizer regimes, both in the absence and presence of AMF inoculation. We try to address the following questions: Q1: Under the local norm of fertilization, what is the temporal dynamics in two forms and seasonal N runoff from paddy fields in Lalin River basin? Q2: How much N runoff can be cut down by fertilizer management? and Q3: Can N runoff be reduced by AMF inoculation?

1. Methodology

1.1. Site description and experimental design

The experiment site was located on the lower reaches of Lalin River (45°13.82′N, 126°22.61′E) in Songhua River basin, Heilongjiang Province of China. The mean annual temperature at the site is 4.3°C, while the annual temperature range (comparing the average temperature of the hottest and coldest month) is 42.2°C. The frost-free period lasts 135 days and annual rainfall is near 500 mm. The hydromorphic paddy soil contains 26.3 g/kg of organic matter, 125.3 mg/kg of hydrolysable N, 150.6 mg/kg of available phosphorus (P) (Bray-P No.1), and 17.6 mg/kg of available potassium (K) (Zhang et al., 2012). Soil analysis methods used in this study are described in a soil analysis manual (Sparks et al., 1996).

The experimental design was a split-plot design with fertilization (including N, P and K) in main plots and inoculation in split plots. There were two levels for inoculation: inoculated (+M) and non-inoculated (−M) while there were six fertilizer levels, labeled as F0, F20, F40, F60, F80, and F100 which indicated 0%, 20%, 40%, 60%, 80%, and 100% of the local norm of fertilization. Each inoculation and fertilizer combination was replicated three times. The main plots covered an area of 25 m^2 (*i.e.*, each 5 × 5 m) while the split plots covered an area of 1 m^2 (*i.e.*, each 1 × 1 m). A vertical geomembrane (extending 0.5 m above and below ground) was placed around the perimeter of each main and split plot to prevent the movement of surface and ground water.

Each plot, main and split, had one flow entry and one exit. Nutrients added to one plot could not be transferred to its neighbors either by diffusion or by splash. The main water channel ran across the experimental area. All the split plots were provided with water collectors connected with the exits. The runoff collectors were plastic storage boxes (1.5 m × 0.75 m × 0.65 m) fixed beside the main plots to collect runoff water through a piping system. Because the depth of water layer in flooding stage differed from that in artificial draining stage, there were two holes for the collecting pipe; the top one was used to collect runoff water during flooding stage and the lower one was used to collect runoff water when artificial draining occurred. The top hole was 5 cm above the soil surface so that only the water above 5 cm could overflow; the lower one was on the soil surface so that there was no surface water after artificial draining.

1.2. *Inoculum and plant material*

In our study, the AMF isolate was *Glomus mosseae* HDSF1 which was provided by Professor Fuqiang Song and has been deposited in China General Microbiological Culture Collection Center (CGMCC) (Beijing), with the deposition number being CGMCC No. 3012. A mixture of soil, sand, vermiculite, root segments, hyphae and spores of *G. mosseae* was used as inoculum. The number of spores in the inoculum was 33–35 spores/g and percentage of root length colonization (percentage of RLC) was 74.8% for *G. mosseae*. The inoculum was developed in pots by cultivating AMF on roots of *Trifolium repens* L. in autoclave sterilized soil diluted with sand and vermiculite (volume ratio of soil, sand, and vermiculite was 2:5:3). After three months of growth in a controlled-environment room, the aboveground was cut off and the mixture in the pots was the inoculum. The inoculum was air dried, homogenized and refrigerated at 4°C or one to two months before use.

The wetland rice (*Oryza sativa* L.) was Hejing No. 1 and the seeds were provided by Heilongjiang Academy of Agricultural Sciences. Rice was planted in a greenhouse (91% of seeds were germinated after three days at 27°C in distilled water). The nursery beds were established in plastic boxes (58 cm long × 28 cm wide × 3 cm height) with air-dried soil. Twenty identical nursery boxes were set up to provide plants for subsequent transplanting into the field (ten for each inoculation and non-inoculated treatment). The bedding soil in each box was fertilized with 160 mg N as urea, 800 mg P as calcium magnesium phosphate fertilizer and 160 mg K as KCl. For the inoculated treatment (+M), 250 g inoculum was layered on top of the air-dried soil, then 50 g rice seeds followed by 1000 g air-dried soil above the seeds. For the non-inoculated treatment (−M), 250 g growth medium (as mentioned above, volume ratio of soil, sand, and vermiculite was 2:5:3) instead of inoculum was added to each nursery bed.

1.3. *Transplantation*

The surface paddy soils were puddled (or mixed with standing water) after fertilization in flooding condition to reduce percolation and to suppress weed growth just prior to transplanting. The seedlings from the nursery beds were transplanted into the field site six weeks after sowing. The seedlings for two inoculation treatments, +M and −M, were transplanted in the same density (three seedlings per hill). Hand transplantation was adopted to ensure an even number of seedlings per hill and regular hill spacing. The space between hills was 30 cm × 13 cm apart.

1.4. *Fertilization*

Six levels of fertilizer (labeled as F0, F20, F40, F60, F80, and F100) were provided: 0%, 20%, 40%, 60%, 80%, and 100% of the local norm of fertilization (*i.e.*, 238 kg N/ha, 106 kg P/ha and 110 kg K/ha). N fertilizer was applied four times, with 60% as basal dressing, 5% as first top dressing, 25% as second top dressing and 10% as third top dressing while all of P and K were applied as basal dressing. The basal dressing was nitrogen–phosphorus–potassium (NPK) compound fertilizer (weight ratio of N, P_2O_3 and K_2O was 16:17:12) while the first and third top dressings were ammonium sulfate, with the second top dressing was urea. The basal dressing was carried out before transplanting and the first and third top dressings were added on the day of transplanting (DAT 1) and 28th day after transplanting (DAT 28), respectively. The second top dressing was conducted on the 11th day after transplantation (DAT 11) (Table 1).

1.5. *Water management*

The study area was largely irrigated with surface water released from the irrigation tunnel originated from Lalin River and the discharge flows were drained back to the same river. The paddy fields were submerged before the rice seedlings were transplanted. An overlying water layer of 3–5 cm was maintained during the rice growing season, except for two aeration periods which were induced by artificial draining. The first aeration last seven days (from June 21 to 28, *i.e.*, DAT 21 to DAT 28) to control tiller number and algae growth and the piping systems were connected to the top holes after the first artificial draining finished. The second one was carried out thirty-four days before the harvest (from September 1 to October 3, *i.e.*, DAT 93 to DAT 125) to facilitate mechanized combine harvesting (Table 1). And the piping systems were connected with the lower holes.

1.6. *Sampling*

The amount of rainfall was recorded daily by a rainfall collector. After the volume of the runoff water was recorded, the runoff water from each plot was sampled from the water collectors soon after every runoff event occurred during the whole growing season. The runoff water was mixed completely before the sampling was carried out. Samples for water quality were collected using a plastic bottle and then immediately transported to the laboratory for analysis. Upon arrival in the laboratory, the water samples were filtered with a 0.45 μm filter membrane and the filtered water was for dissolved N (DN) concentration and the original water sample was used to

Table 1 – Dates of cultivation and management during the growing season in 2011.

Item	Date
Flooding	May 28
Basal fertilization and harrowing	May 31
Transplanting and first top dressing	June 1 (DAT 1)
Second top dressing	June 11 (DAT 11)
First draining	June 21 (DAT 21)
Re-flooding and third top dressing	June 28 (DAT 28)
Second draining	September 1 (DAT 93)
Harvesting	October 3 (DAT 125)

DAT: date after transplanting.

determine total N (TN) concentration using the ultraviolet spectrophotometry method with potassium peroxydisulfate. Particulate N (PN) concentrations were calculated by subtracting the DN concentrations from the corresponding TN concentrations. There were twelve runoffs caused by rainfall events and two runoffs were due to artificial draining.

The volume and N concentrations (*i.e.*, concentrations of TN, DN and PN) of runoff water were used to calculate individual TN runoff (*i.e.*, volume by TN concentration), individual DN runoff (*i.e.*, volume by DN concentration) and individual PN runoff (*i.e.*, volume by PN concentration). The cumulative N runoff was the sum of individual N runoffs. The average concentrations of TN, DN, and PN in irrigation water were 2.60, 1.54, and 1.03 mg/L, respectively, during the experimental period.

1.7. Statistical analysis

All data were tested for normality and homogeneity of variance. Two-way analysis of variance (ANOVA) based on a split-block design was performed to assess the effects of AMF inoculation, fertilization and the interaction between them. Significant interactions were followed by simple main effect tests to examine specific differences between groups. One-way ANOVA was used to compare multiple groups with Turkey's HSD test as post hoc test. Independent *T* tests were conducted to compare N concentrations with the threshold of Grade V in *Quality Standard of Surface Water Environment of China* (GB3838-2002) (Meng, 2009). Paired *T* tests were carried out to examine the difference in the mean values of N concentration between +M and −M groups. Statistical analyses were performed in SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

2. Results and discussion

2.1. Runoff during rice growing season

As the surface water in the paddy fields was maintained by irrigation, large variation in runoff water from paddy fields during rice growing season was predominantly due to variable natural rainfall events and artificial draining. The total rainfall of this year was 243.6 mm which was a half of the mean value of the whole year (near 500 mm). There were twelve rainfall events, with each resulting in 82–236 m^3/ha of runoff water during this season (Fig. 1).The first runoff (DAT 3) which produced 98 m^3/ha of runoff water occurred two days after the first top dressing (DAT 1). There was no relatively heavy rainfalls causing runoff event for 20 days afterwards and during this period the second top dressing was carried out (DAT 11). Ten days later the first artificial draining occurred on DAT 21, which led to 440 m^3/ha of runoff water. This was followed by a 7.2-mm rainfall (DAT 22) but did not cause any runoff. The paddy fields were re-flooded and fertilized for a third time (the third top dressing) on DAT 28 after one week of aeration. Two days later (DAT 31 onwards) rainfall events occurred frequently and the runoff water was collected nine times before the second artificial draining (DAT 93) which resulted in 390 m^3/ha of runoff water. After that, the irrigation ended and the paddy fields were not flooded when the last runoff, 142 m^3/ha, was collected on DAT 120. During the rice growing season the cumulative runoff water was 2296 m^3/ha, with 64% caused by rainfall events and 36% by artificial draining.

2.2. N runoff from paddy fields supplied with the local norm of fertilization

Total nitrogen (TN) concentration is regarded as one of major indexes of environmental quality standards of surface water. We found that across the rice growing season TN concentration of runoff water varied from 4.16 to 12.29 mg/L at F100. Specifically, 100% of samples above the TN threshold of Grade V in *Quality Standard of Surface Water Environment of China* (GB3838-2002) (Meng, 2009) ($p < 0.01$, Fig. 1a), indicating a high risk of contamination of Lalin River. In addition, the cumulative N runoff from paddy fields with the local norm of fertilization was 18.9 kg/ha (Fig. 1d) at the end of the season, which was close to the finding (18.8 kg/ha) obtained from large sized paddy fields in Japan (Yoshinaga et al., 2007) and the outcome (19.4 kg/ha) of field investigation of Taihu Lake region (Guo et al., 2004). However, it was higher than 10.5 kg/ha estimated during rice season in a rice-wheat rotation system in Taihu Lake region of China (Gao et al., 2004). This indicates that the growing system contributes to the difference in N runoff from paddy fields. Our result was also much higher than 3.9 kg/ha in Chungbuk of South Korea where lower (172 kg/ha) N was applied (Kim et al., 2006), suggesting the dosage of N fertilizer applied also played a role in altering N runoff from paddy fields. Besides growing system and N fertilization, different methods applied for sampling possibly altered the results. Although the volume of runoff water was exactly determined by using flow meters in previous studies, the runoff water were not homogenized before sampling. Therefore N concentrations measured using these samples just stood for instant N concentrations (Kim et al., 2006; Zhao et al., 2012), which might lead to inaccurate estimation of N runoff. In our case runoff water was collected completely and mixed properly before sampling, which enables N runoff to reflect the real conditions of the micro-environment. No matter which factor dominates, more attention should be paid on the contribution of N runoff from intensively fertilized paddy fields to the worsen water quality of Lalin River.

There were two forms of N available in runoff water: dissolved N (DN) and particle N (PN). It was found that the difference between DN and PN concentrations was significant and DN concentration was higher than PN concentration ($p < 0.01$, Fig. 1b, c). This indicated that DN, rather than PN, was the main form of N loss by runoff. Furthermore, the accumulative DN runoff at the end of the season accounted for 60% to 70% of the accumulative TN runoff (Fig. 1e, f), which was consistent with previous reports (Tian et al., 2007). However, this was different from phosphorus (P) runoff as our previous results suggested that particle P, rather than dissolved P, was the main form of P loss via runoff from paddy fields (Zhang et al., 2015). This was likely due to that most of P fertilizer applied was fixed in soil via biological, chemical and physical processes (Cao and Zhang, 2004; Carpenter et al., 1998; Zhang et al., 2011) so that a greater portion of P would be lost with soil particles whenever runoff events occurred. In

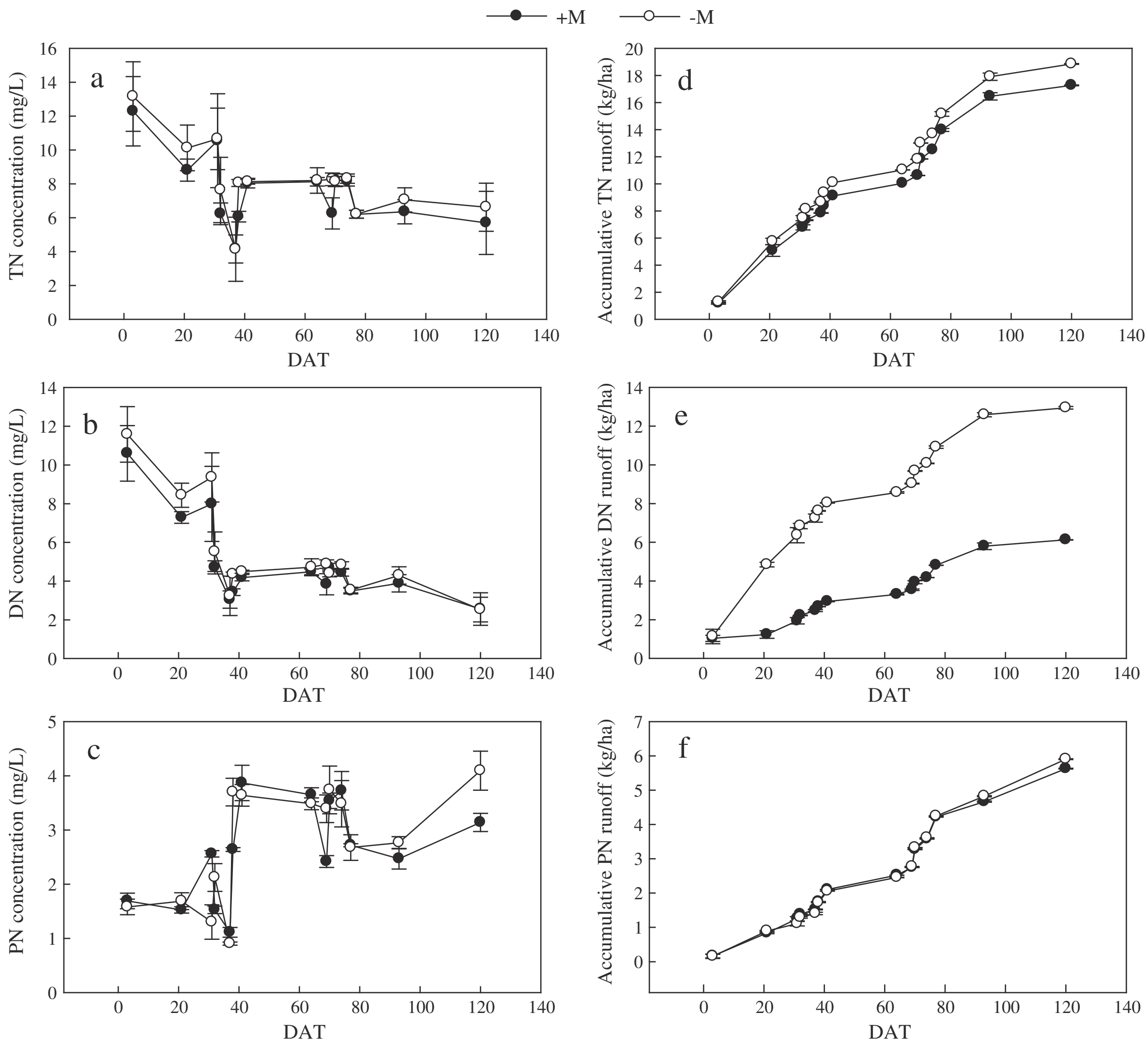

Fig. 1 – N concentrations and the accumulative N runoff at F100. +M: inoculated with arbuscular mycorrhizal fungi (AMF); −M: non-inoculated; TN: total nitrogen; DN: dissolved nitrogen; PN: particle nitrogen; F100: provided with 100% of the local norm of fertilization; DAT: days after transplantation.

terms of N, NH_4^+ and NO_3^- are usually the main forms of N fertilizer. Although, the mobility of NH_4^+ was not as good as that of NO_3^-, NH_4^+ could be transformed into more mobile forms with nitrification, such as NO_2^- and NO_3^- (Edwards and Daniel, 1993; Lee et al., 2009). Therefore, most N fertilizer applied was unlikely to be fixed in soil and had a high potential to be lost in a dissolved form, rather than in a particle form via runoff.

To explore the temporal dynamics of N concentration in runoff water, we performed one-way ANOVA tests for DN and PN concentrations. The difference in DN concentrations among separate runoff events was significant and DN concentration decreased during rice growing season ($p < 0.01$, Fig. 1b). The variation in DN concentrations at the earlier stage was predominantly governed by the timing of fertilization and runoff events (Fig. 1b). The DN concentration of the first runoff (DAT 3) was the highest as the first runoff event happened soon after the basal and first top dressing (DAT 1). A similar phenomenon was also observed for the third runoff event which (DAT 31) occurred three days after the third top dressing (DAT 28). In addition, the second runoff (DAT 21) exhibited lower DN concentration as it delayed for ten days after the second top dressing (DAT 11), which was consistent with previous research (Somura et al., 2009). There was a downward trend in DN concentration at the later stage of the experiment period ($p < 0.05$, Fig. 1b), which might result from the fact that the retention water in paddy fields inducted a purification mechanism, including denitrification, sedimentation or ammonia volatilization (Edwards and Daniel, 1993; Lee et al., 2009). Also, it may be due to a dilution effect induced by an influx of irrigation water from Lalin River. The average concentrations of DN and PN of irrigation water were 1.54 and 1.03 mg/L during the experiment period which were low enough to dilute runoff water from paddy fields. Similar statistical analysis were conducted for PN concentrations. Although there was no significant differences in PN concentration across rice growing season ($p > 0.05$, Fig. 1c), PN concentration of the last runoff event was significantly greater than others ($p < 0.05$, Fig. 1c). This was likely due to that the paddy fields were not flooded so that rain eroded the surface soil and the generated runoff certainly carried a great amount of soil particles, which led to high PN concentration. Therefore, the variation in DN and PN concentrations were attributed to different factors.

2.3. Effect of reduction in fertilization on N runoff

In our study, 86% of all TN concentration of runoff water from non-fertilized blocks (F0) was above 2 mg/L, indicating that the water quality was worse than surface water of Grade V in *Quality Standard of Surface Water Environment of China* (GB3838-2002) (Meng, 2009). That percentages offertilized ones (F20, F40, F60 and F80) were 100% (in all cases $p < 0.05$). However, the outcomes of one-way ANOVA suggested that mean TN concentration declined significantly with decreasing fertilizer levels ($p < 0.05$, Fig. 2a). Specifically, mean TN concentrations decreased from 7.85 mg/L at F100 to 2.44 mg/L at F0 (Fig. 2a). Similar trend was also found in Taihu Lake region (Tian et al., 2007). Regarding different forms of N in runoff water, decreasing fertilization was observed to lower the mean DN concentrations of each treatment ($p < 0.05$, Fig. 2b). By contrast, the mean PN values from fertilized paddy fields were similar with each other ($p > 0.05$) but a bit higher than that from non-fertilized treatments ($p > 0.05$, Fig. 2c). Put together, the effect of reducing fertilization on DN concentrations was more remarkable than that on PN concentrations.

One-way ANOVA analysis indicated that the seasonal TN runoff dropped significantly with reducing fertilization ($p > 0.05$, Fig. 2d). Specifically, the difference in seasonal TN runoff between any of the two adjacent fertilizer levels was 1.5–2.8 kg/ha, indicating that every 20% of decrease in fertilization resulted in a decline in N runoff by 8.2%–15.2%. Our published results showed that the highest yield was observed at F80 and from F0 to F80 every 20% of decrease in fertilization caused a drop in grain yields by 10.1%–36.7% (Zhang et al., 2015). So, in order to keep high grain yield, it would be wise to reduce the rate of fertilization by 20%

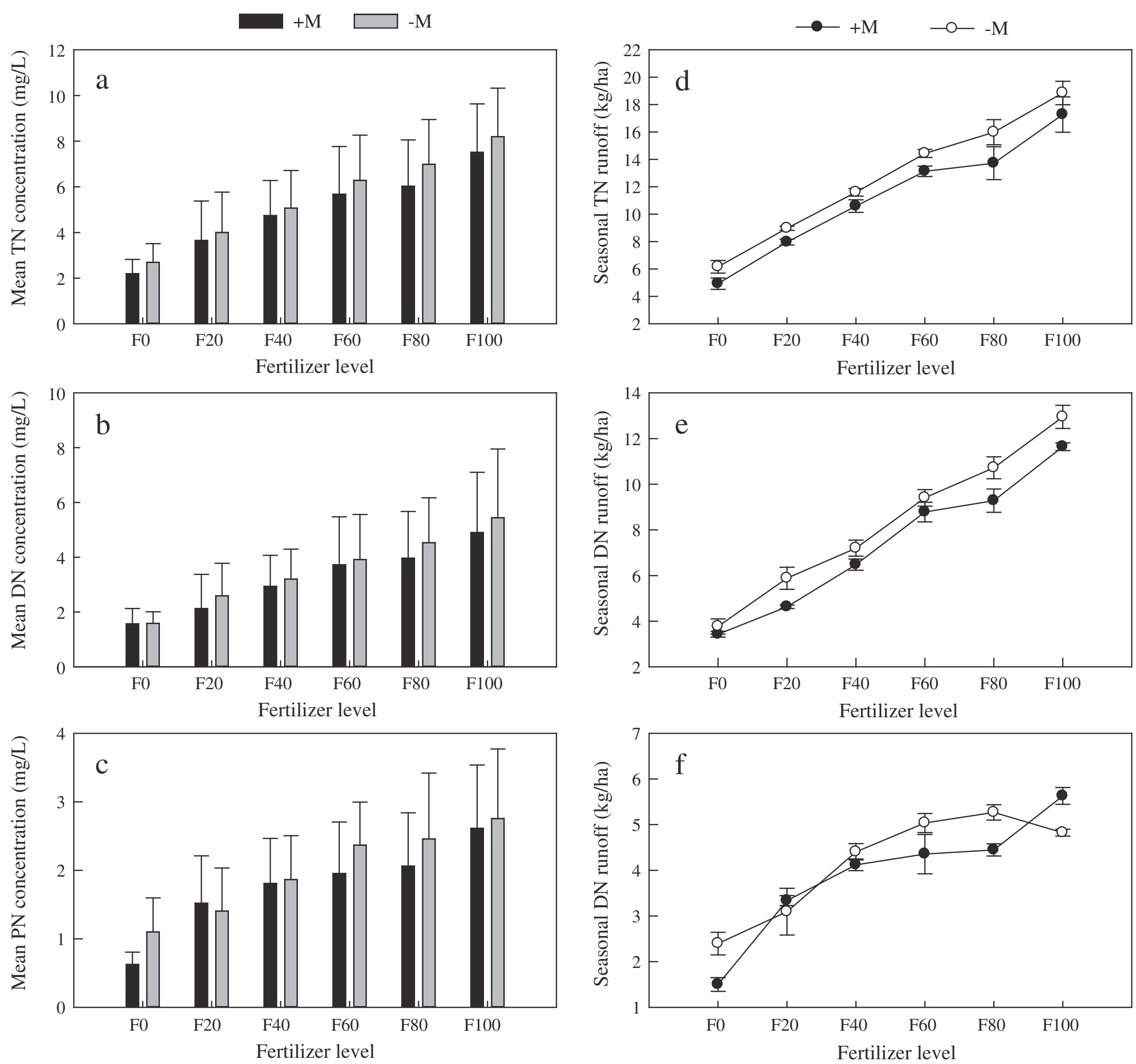

Fig. 2 – Mean N concentrations and seasonal N runoff under each fertilizer level. F0, F20, F40, F60, F80, and F100, provided with 0%, 20%, 40%, 60%, 80%, and 100% of the local norm of fertilization.

(to F80), which fell within a range of decrease from 10% to 40% recommended by other researchers to enable optimum economic and ecological results (Xia and Yan, 2012). Exactly, we found that the TN runoff was reduced by 2.86 kg/ha, *i.e.*, 15.2%, when the fertilization was reduced by 20%. This was lower than 39.4%–47.6% obtained in a previous study due to alternate wetting and drying (AWD) and site-specific nutrient management (SSNM) (Liang et al., 2013). The divergent results were due to the differences in water and fertilizer management: in our study, an overlying water layer of 3–5 cm was maintained during the whole growing season, except for two aeration period while AWD allowed for floodwater levels in the field to drop below the soil surface before the next irrigation is needed (Liang et al., 2013). In addition, the SSNM approach commonly included target yield selection, estimation of plant nutrient uptake requirements and nutrient use efficiencies to select the optimal N rate, and in-season adjustment of fertilizer N application timing and/or rate using a chlorophyll meter (Liang et al., 2013). These comparisons indicated that combined with nutrient management, water management should be paid attention to reduce TN runoff from paddy fields.

In our study, the seasonal DN and PN runoff responded to the varying fertilization in separate ways, with the seasonal DN runoff being more sensitive than the seasonal PN runoff (Fig. 2e, f). Specifically, the seasonal DN runoff decreased with declining fertilization ($p < 0.05$, Fig. 2e) and compared with that from paddy fields under F100 the seasonal DN runoff from paddy fields under F0 was 9.18 kg/ha lower ($p < 0.01$, Fig. 2e). This may be due to that N fertilizers applied were in dissolved forms so that the reduction in fertilization inevitably caused greater decrease in DN runoff than PN runoff. In addition, puddling was carried out in fertilized treatments but not in non-fertilized ones, which caused the difference in PN runoff between the fertilized and non-fertilized treatments ($p < 0.01$, Fig. 2f) (Somura et al., 2009). Therefore, we suggested that the forms of N fertilizer and puddling might contribute to the difference in the responses to varying fertilization between DN and PN runoff.

2.4. Effect of AMF inoculation on N runoff

At maturity, the root length of colonization (RLC) by AMF was quantified for +M and −M plants and it was found that RLC of +M was significantly greater than that of −M, indicating that AMF inoculation significantly increased root colonization by AMF in our study ($p < 0.01$). Specifically we found that in roots of −M rice, the average RLC was 2.5% while it was in a range of 12.4%-19.5% for +M plants.

To gain insights into whether AMF inoculation played a role in reducing N runoff, we carried out paired *T*-test for the mean values of N concentrations. We found that AMF inoculation decreased the mean values of N concentrations of runoff water (in all cases $p < 0.05$, Fig. 1a–c). Additionally, the outcomes of two-way ANOVA indicates that the impacts of AMF inoculation on TN and DN concentrations fluctuated dramatically with rice growing, with no effect on DAT 3 and during the period from DAT 41 to DAT 93 ($p < 0.05$). However, announced effect of inoculation was observed during the remaining period (Fig. 1a, b). These results were due to that the effect of AMF inoculation on cutting down N runoff was affected by agricultural practices. Firstly, the external hyphae which were considered to involve nutrient uptake (Johnson et al., 2003) were damaged during transplanting so that they were not able to take up N for themselves and their hosts (*i.e.*, rice) (Solaiman and Hirata, 1996, 1997). Therefore, there was no effect of AMF inoculation on reducing N concentrations on DAT 3. They grew gradually with rice growing and took up N for the host and themselves, which was support by our previous study where we found that AMF inoculated plants took up more N but accumulate less biomass than their non-inoculated counterparts (Zhang et al., 2015). This led to a negative effect on TN concentrations from DAT 21 to DAT 38. During the period from DAT 41 to DAT 93 which was in between the two aeration periods, the paddy fields were flooded and the function of AMF was inhibited, support of which from the finding that conventional flooding depressed AMF colonization in rice roots (Lumini et al., 2011). However, during the two aeration periods, AMF were provided favor condition for their growth so that there was a greater effect on reducing N concentrations. Taken together, transplanting and flooding inhibited the effect of AMF inoculation while the effect was enhanced by the aeration after the artificial drainage.

Two-way ANOVA was conducted for seasonal N runoff, with one-way ANOVA followed. We found that there was a significant interaction between inoculation and fertilization in our study ($p < 0.05$). This suggested that AMF inoculation effectively lowered the seasonal TN runoff from paddy fields ($p < 0.01$) and the negative effects depended on fertilizer levels, with the greater one (2.3 kg/ha) observed at F80 and smaller ones (from 1.0 to 1.6 kg/ha) at other fertilizer levels (Fig. 2d). The greater effect of inoculation on TN runoff at F80 might result from that AMF assist rice in taking up more N under this fertilizer regime. This was supported by our previous study where the greatest effect of inoculation on grain yields and then N uptake were observed under F80 (Zhang et al., 2015). The effect of inoculation on the accumulative DN runoff was likely attributing to that both ammonium and nitrate were found to be taken up by the external hyphae which transported N into plant roots by the internal hyphae (Govindarajulu et al., 2005) (Fig. 1e). In terms of PN, negative effect was only found on DAT 120 (Fig. 1f). This negative effect on PN was due to their critical role in the formation and maintenance of soil aggregates (Rillig et al., 2001), which was essential for minimizing soil erosion (Amezketa, 1999) and then minimizing the amount of PN in the runoff water from paddy fields. In sum, inoculation with AMF reduced both DN and PN runoff with separate mechanisms.

Our previous study found that AMF inoculation significantly lowered plant biomass but increased grain yields. Additionally, large decrease in fertilization decreased plant biomass and grain yields (Zhang et al., 2015). In order to gain insight into the integrated effect of inoculation, we plotted the seasonal TN runoff against the grain yields of rice for +M and −M rice (Fig. 3). We found that there were two separate fitted lines for the two sets of plants (+M: $y = 1.73 \times$, $R^2 = 0.8374$, $r = 0.786$, $p < 0.05$; −M: $y = 1.30 \times$, $R^2 = 0.553$, $r = 0.9154$, $p < 0.05$), with the spread of data cloud being lower in +M treatment. This indicated that with a given grain yield guaranteed, AMF

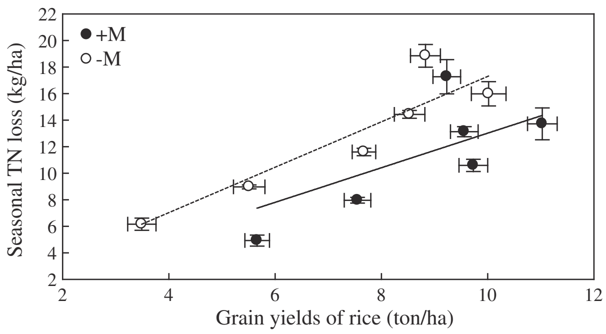

Fig. 3 – Relationship between grain yield and seasonal N runoff.

inoculation was able to reduce the season TN loss from paddy fields. Therefore, in order to keep high grain yield, it would be wise to reduce the rate of fertilization by 20% (to F80), which fell within a range of decrease from 10% to 40% recommended by other researchers to enable optimum economic and ecological results (Xia and Yan, 2012). Exactly, the N runoff was reduced by 2.86 kg/ha, *i.e.*, 15.2%, when the fertilization was reduced by 20% in our study which was also lower than 39.4%–47.6% obtained in earlier study due to alternate wetting and drying and site-specific nutrient management (Liang et al., 2013). On balance, besides the combination between AMF inoculation and fertilizer management, other strategies are required to further cut down N runoff from paddy fields.

3. Conclusions

Lalin River, in the northeast of China, exhibited high N loading–given this, it is vital to reduce the contribution of N loss via runoff from paddy fields in Lalin River basin. In our study, we found that the TN runoff from paddy fields was 18.9 kg/ha during the rice growing season in Lalin River basin, with dissolved N (DN) accounting for 60%–70%. In addition, reduction in fertilization by 20% could cut down TN runoff by 8.2%–15.2%. And, inoculation with arbuscular mycorrhizal fungi (AMF) reduced both DN runoff and PN runoff, with 8.4% to 20.0% of N runoff cut down at each fertilizer level. The best combination was AMF inoculation and 80% of local norm of fertilization, which led to a reduction by 27.2% in TN runoff without sacrificing rice grain yield. As a whole, although long-term monitoring is required, it is promising to combine AMF inoculation with fertilizer management to cut down N runoff from paddy fields.

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