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Can arbuscular mycorrhiza and fertilizer management reduce phosphorus runoff from paddy fields?

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ABSTRACT

Our study sought to assess how much phosphorus (P) runoff from paddy fields could be cut down by fertilizer management and inoculation with arbuscular mycorrhizal fungi. A field experiment was conducted in Lalin River basin, in the northeast China: six nitrogen–phosphorus–potassium fertilizer levels were provided (0, 20%, 40%, 60%, 80%, and 100% of the recommended fertilizer supply), with or without inoculation with *Glomus mosseae*. The volume and concentrations of particle P (PP) and dissolved P (DP) were measured for each runoff during the rice growing season. It was found that the seasonal P runoff, including DP and PP, under the local fertilization was 3.7 kg/ha, with PP, rather than DP, being the main form of P in runoff water. Additionally, the seasonal P runoff dropped only by 8.9% when fertilization decreased by 20%; rice yields decreased with declining fertilization. We also found that inoculation increased rice yields and decreased P runoff at each fertilizer level and these effects were lower under higher fertilization. Conclusively, while rice yields were guaranteed arbuscular mycorrhizal inoculation and fertilizer management would play a key role in reducing P runoff from paddy fields.

Introduction

Phosphorus (P) and nitrogen (N) concentrations of surface water are increasing (*i.e.* eutrophication), which typically promotes excessive growth of algae (Lewis et al., 2011). As algae die and are decomposed, the oxygen availability of water decreases, which leads to the death of other aquatic plants and animals. Therefore, it is crucial to control the availability of N and P in surface water to slow down the process of water eutrophication. As many algae are able to utilize atmospheric N (Saadatnia and Riahi, 2009), alga growth is not likely limited by N availability, suggesting that alga growth might depend on P availability and control of P load, rather than N load, and should be paid more attention to slow down water eutrophication (Cao and Zhang, 2004).

In agricultural ecosystems, P is often a major limiting nutrient for plant growth, requiring additional fertilizer application. As the demand for food is rising due to the increasing population, intensive P fertilization is very common to maintain high yields. However, it was reported that not all the P fertilizer applied could be taken up by crops, with 10%–20% of P fertilizer applied being recovered during the current growing season (Cordell et al., 2009). The remaining P fertilizer in soil has a high potential to be transported into surface water through runoff, indicating that the control of water eutrophication would benefit from cutting down P runoff from agricultural ecosystems. Studies on P runoff from paddy fields were carried out in central, southern and northern Korea (Cho, 2003; Kim et al., 2006; Sik Yoon et al., 2006) and it was found that the amount of P runoff was affected by weather, soil type and crops, which implies that studies on P runoff should

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cover various regions to calculate the contribution of P runoff from paddy fields to high P load of surrounding water. In China, many studies on P runoff have been conducted in Taihu Lake Region (Cao and Zhang, 2004; Guo et al., 2004) and Chaohu Lake Region (Yan et al., 1999); little information, however, is available for other regions of China, such as Songhua River region in northeast China. As one of the sub-watersheds of Songhua River, Lalin River basin is one of the major rice production bases for the whole country. Importantly, the rice growing season in this area extends from June to October each year which corresponds with the main rainy and hydrologically active period of the year. Therefore, Lalin River was vulnerable to pollution as paddy fields were characterized with the most intensive P runoff (Hao et al., 2012). As P runoff varied temporally, monitoring in field studies is usually costly and labor intensive, little information about P runoff from paddy fields is available in this region.

Phosphorus runoff from paddy fields was determined by both P input (*i.e.* application of P fertilizer) and self-purification of the paddy field. In terms of P input, the current application of P fertilizer caused P enrichment in soil, which in turn led to more P runoff (Carpenter et al., 1998; Cao and Zhang, 2004; Zhang et al., 2011). Importantly, rational fertilization was found to decrease P pollution from farmland and in Taihu Lake Region, the rational annual P application rate in theory is 13.5 kg/ha, only a half of the current annual P application rate, 25–35 kg/ha (Zhou and Zhu, 2003). As there is a difference in soil type, weather and farm operation from region to region, it is necessary to indicate the rational P application rate in Lalin River basin. In addition to the application of P fertilizer, self-purification of the farmland also alters P runoff. Self-purification in the farmland involves precipitation, adsorption, microbial assimilation and plant uptake. Importantly, precipitation and adsorption cannot last permanently as drought and flooding events led to P release by desorption of previously adsorbed P and higher mineralization of organic P (Song et al., 2007). These released P was discharged to the pore water and then to the flooding water (Young and Ross, 2001) and became vulnerable to be transported into surrounding water *via* runoff. This indicated that the effects of precipitation and adsorption were subtle at seasonally flooded farmlands, such as paddy fields. Given the unique water management, self-purification of paddy fields only involves plant uptake and microbial assimilation. Therefore, P runoff could be reduced by decreasing fertilization (*i.e.* lower input) and improvement in plant uptake and microbial assimilation (*i.e.* higher self-purification) at paddy fields.

Inoculation with arbuscular mycorrhizal fungi (AMF) has great potential to reduce fertilization and to enhance self-purification at paddy fields. Firstly, as one type of 'bio-fertilizer', AMF was reported to increase rice yield even under field experiment (Solaiman and Hirata, 1996), suggesting that AM inoculation could replace or partly replace chemical fertilizer (*i.e.* decrease P input) to maintain high yield of rice, with P runoff being cut down. Additionally, the positive effect of arbuscular mycorrhizal (AM) inoculation on rice P content has been well documented by many researchers (Solaiman and Hirata, 1996; Smith and Read, 2008), suggesting that AM inoculated plants are able to take up more P than non-inoculated plants (*i.e.* enhance self-purification). Thirdly, AM inoculation might play a critical role in improving microbial assimilation. It is known that microbes live and assimilate P in the space within soil aggregates. AMF were found to increase the amount and stability of aggregates in the soil by enmeshing soil particles with their hyphae (Rillig et al., 2001). Thus, AM inoculation improved microbial assimilation of P, that is, inoculation enhanced self-purification of paddy fields. Moreover, the improvement in the stability of soil aggregates due to AMF inoculation was also likely to reduce P runoff directly, especially in terms of particle P (Amézketa, 1999). Based on above analysis, AM inoculation has a great potential to slow down the eutrophication of surrounding water by decreasing P input and increasing self-purification of paddy fields. Few studies, however, have made efforts to estimate the contribution of AM inoculation to the control of P runoff from paddy fields.

Our study sought to quantify the contribution of P runoff from paddy fields to the P load of Lalin River. Additionally, it was estimated how P runoff from paddy fields in this area responds to reduction in fertilization and AM inoculation.

1. Methods and materials

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, Northeast China. The paddy soil contained 26 g/kg of organic matter, 125 mg/kg of hydrolysable N, 150 mg/kg of available P, and 18 mg/kg of available K (Zhang et al., 2012).

A split-plot design was conducted with fertilization in the main plots and inoculation in the split plots. There were two kinds of seedlings: 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 recommended fertilization applied. Each treatment was replicated three times. The main plots covered an area of 25 m^2 while the split plots covered an area of 1 m^2. 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 had one flow entry and one exit. All plots were provided with a water collector to collect runoff through a piping system.

1.2. Seedling production and transplantation

A mixture of soil, sand, vermiculite, root segments, hyphae and spores of *Glomus mosseae* was used as AMF inoculum. The number of spores in the inoculum was 33 g^{-1} and the percentage of root length colonization was 75% for *G. mosseae*. Wetland rice (*Oryza sativa* L.) was planted in a greenhouse. The nursery beds were established in plastic boxes filled with air-dried soil. The bedding soil in each box was mixed 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 of AMF inoculum was layered on top of soil, then 50 g of rice seeds, followed by 1000 g of air-dried soil. For the non-inoculated treatment (−M), 250 g of sterilized AMF medium (as mentioned above) instead of inoculum was added to each nursery bed.

The paddy fields were harrowed after basal fertilization in flooding condition. Seedlings from the nursery beds were transplanted into the field site six weeks after sowing. Seedlings for +M and −M, were transplanted in the same density (*i.e.* three seedlings per hill) and the space between hills was 30 cm × 13 cm apart.

1.3. Fertilization and water management

Six levels of fertilizer, labeled as F0, F20, F40, F60, F80, and F100, were provided at paddy fields. According to the local agricultural practice, 238 kg/ha of N, 106 kg/ha of P and 110 kg/ha of K are applied every year. N fertilizer is 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 P and K are applied as basal dressing. The basal dressing is nitrogen-phosphorus-potassium (NPK) compound fertilizer (NH_3, P_2O_5 and K_2O in ratio 16:17:12) while the first and third top dressings were ammonium sulfate, with the second top dressing was urea.

The study area was irrigated with surface water released from irrigation tunnel sourced from Lalin River. The paddy fields were submerged before the rice seedlings were transplanted. An overlying water layer of 3–5 cm was maintained during the whole growing season, except for two aeration period which was induced by artificial draining.

1.4. Sampling and measurements

For each runoff event, after the volume of the runoff water was recorded, 600 mL of runoff water from each plot was sampled from the water collectors during the whole rice growing season. The original water sample was used to determine total phosphorus (TP) concentration and the 0.45 μm filter membrane was used to determine dissolved phosphorus (DP) concentration. Particulate phosphorus (PP) concentration was calculated by subtracting the DP concentration from the TP concentration. Concentrations of TP and DP were determined by standard methods (Quality Standard of Surface Water Environment of China (GB3838-2002)).

AMF colonization was assessed at plant maturity. Fine roots were cut into 1.5 cm segments and mixed thoroughly; a 0.25 g (fresh weight) subsample was randomly taken for determination of root colonization. These subsamples were cleared with 10% KOH by heating in a beaker filled with water kept at a rolling boil for 30 min. Thereafter, samples were neutralized with 0.2% HCl and stained with 0.2% acid fuchsin (Aladdin Industrial Corporation, Shanghai, China) for 30 min at 90°C. Colonization by AMF was estimated by quantifying root length colonization (RLC) under a dissecting microscope (Eclipse E 200; Nikon Instruments, Beijing, China) using the grid line intersects method (Giovannetti and Mosse, 1980).

At plant maturity (*i.e.* early October), three hills were sampled randomly from each split plot within a given replicate block. As there were three replicate blocks, in total we harvested nine hills per fertilization/inoculation treatment combination. Rice yield was recorded on an air-dry basis including husk.

1.5. Data processing

The volume and P concentrations (*i.e.* concentration of TP, DP and PP) of runoff water were used to calculate individual TP runoff (*i.e.* the product of volume by P concentration), individual DP runoff (*i.e.* the product of volume by DP concentration) and individual PP runoff (*i.e.* the product of volume by PP concentration). Cumulative P runoff at each time point was the sum of individual P runoff occurred from DAT 0 to this time point. Seasonal P runoff was the sum of individual P runoffs during the whole rice growing season.

Two-way analysis of variance (ANOVA) based on a split-plot design were performed to assess the effects of AMF inoculation, fertilization and their interaction. All data were tested for normality and homogeneity of variance and in all cases, the data met the assumptions of normality and homogeneity. Significant interactions were followed up by simple main effect tests: one-way ANOVA was followed where there was a significant interaction. *T*-test was conducted several times for other comparisons. Statistical analyses were performed in SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

2. Results and discussion

The forms, causes and temporal fluctuation of P runoff from paddy fields were characterized in Lalin River basin. Our results highlighted how a combination of fertilizer management and inoculation with AMF affected P loss from paddy fields *via* runoff, which provided new insights into alleviating water pollution of Lalin River and then Songhua River.

2.1. Rainfall and water runoff

Large variations in the volume of runoff water during rice growing season were predominantly due to irrigation, rainfall and artificial draining (Fig. 1a). There were twelve rainfall events, with the total rainfall being 390.6 mm during rice growing season. The maximum rainfall was 52.0 mm on day 21 after transplantation (*i.e.* DAT21); while the minimum rainfall resulting in runoff was 18.4 mm on DAT 74. The rainfall events resulted in 82–236 m^3/ha of runoff water. Fig. 1b shows that the first runoff (3 days after transplantation; DAT 3) occurred two days after the first top dressing (DAT 1). There was no relatively heavy rainfall events causing runoff for 20 days afterwards and the second top dressing was carried out on DAT 11. Ten days later the first artificial draining caused 440 m^3/ha of runoff on DAT 21, followed by a 7.2-mm rainfall (DAT 22) without causing any runoff. The paddies were re-flooded and supplied with the third top dressing on DAT 28 after one week of aeration. Two days later (DAT 31 onwards), runoff occurred 9 times before the second artificial draining causing 390 m^3/ha on DAT 93. After that irrigation ended so that the paddies were not flooded when the last runoff was collected on DAT 120. During the whole rice growing season the total runoff water was 2296 m^3/ha, with 64% caused by rainfall and 36% by artificial draining.

2.2. Phosphorus runoff from paddy fields supplied with recommended fertilization

Our study suggested that P runoff from paddy fields was one of the potential causes of high P loading of the Lalin River. Firstly, TP concentrations of runoff water was in a range of 0.20–7.22 mg/L, with 100% of samples above the P threshold of Grade III (0.20 mg/L) and 36% above Grade V (0.40 mg/L) in

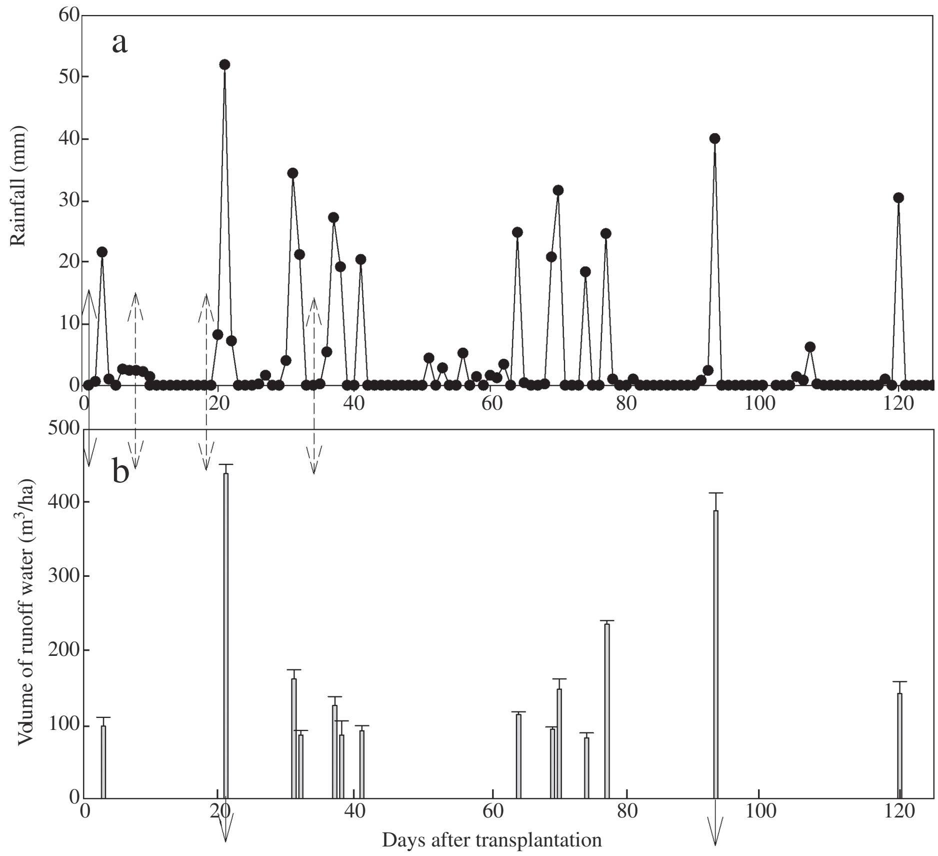

Fig. 1 – Rainfall and volume of runoff water from paddy fields. Solid line double arrow: time point for basal dressing; dashed arrows: time points for top dressings; solid line single arrows: time points for artificial draining.

Quality Standard of Surface Water Environment of China (GB3838-2002) (Fig. 2a). These results were higher than that obtained in Taihu Lake region (Cho, 2003) where TP concentrations were between 0.10 and 1.40 mg/L. Moreover, high TP concentrations (above 0.30 mg/L) were observed before DAT41, which suggested that the majority of P runoff was due to P fertilization. It was also found that the seasonal P runoff with the local fertilization was 3.7 kg/ha (Fig. 2d), accounting for 3.5% of P fertilizer applied. This indicated that the seasonal P runoff in Lalin River basin was much higher than that in Chaohu Lake region (0.69 kg/ha) (Yan et al., 1999) and Taihu Lake region (1.16 kg/ha) (Guo et al., 2004). Consequently, more attention should be paid on the contribution of P runoff from paddy fields to declining water quality of Lalin River.

There were several factors contributing to variations in P runoff from the paddy fields. Earlier researchers found P runoff depended on soil types, with more P runoff from permeable paddy soil than that from waterlogged paddy soil (Cao and Zhang, 2004). Also, P loss through runoff was dependent on the timing of fertilization and rainfall. Specifically, P runoff from paddy fields in Taihu Lake Region was found to be much higher when surface runoff occurred a few days after P application coupled with heavy rainfall (Zhang et al., 2007). Additionally, different methods applied for sampling altered the estimation of P runoff. Although the volume of runoff water was exactly determined using flow meters in earlier studies, the runoff water could not be homogenized before sampling and P concentrations measured just stood for instant P concentrations (Kim et al., 2006), which might lead to inaccurate estimation of P runoff. In our study, runoff water were collected and mixed properly before sampling in order to accurately reflect P concentrations of runoff water.

It was also observed that P runoff was in two forms, with one in dissolved form (DP) and the other in particle form (PP). We found that the difference between PP concentration and DP concentration was significant and PP concentration was higher than DP concentration ($p < 0.01$, Fig. 2b, c). This indicated that PP, rather than DP, was the main form of P loss by runoff, with the discharge percentage of PP varying from 60% to 80%. This was in agreement with other results where more P was observed to be transported as particles from paddy fields than dissolved forms (Guo et al., 2004). In the long term, this particle form of P would become available to algae as PP were easily absorbed and transferred (Carpenter et al., 1998). In addition, although artificial drainage caused 36% runoff in terms of runoff volume, 54% of P runoff was caused by artificial drainage. The main reason was that the TP concentration and the volume of runoff due to the first drainage were significantly higher than that because of rainfall ($p < 0.01$), with TP concentration being 3.9 mg/L and volume of runoff being 440 m^3/ha, respectively (Figs. 1b and 2a).

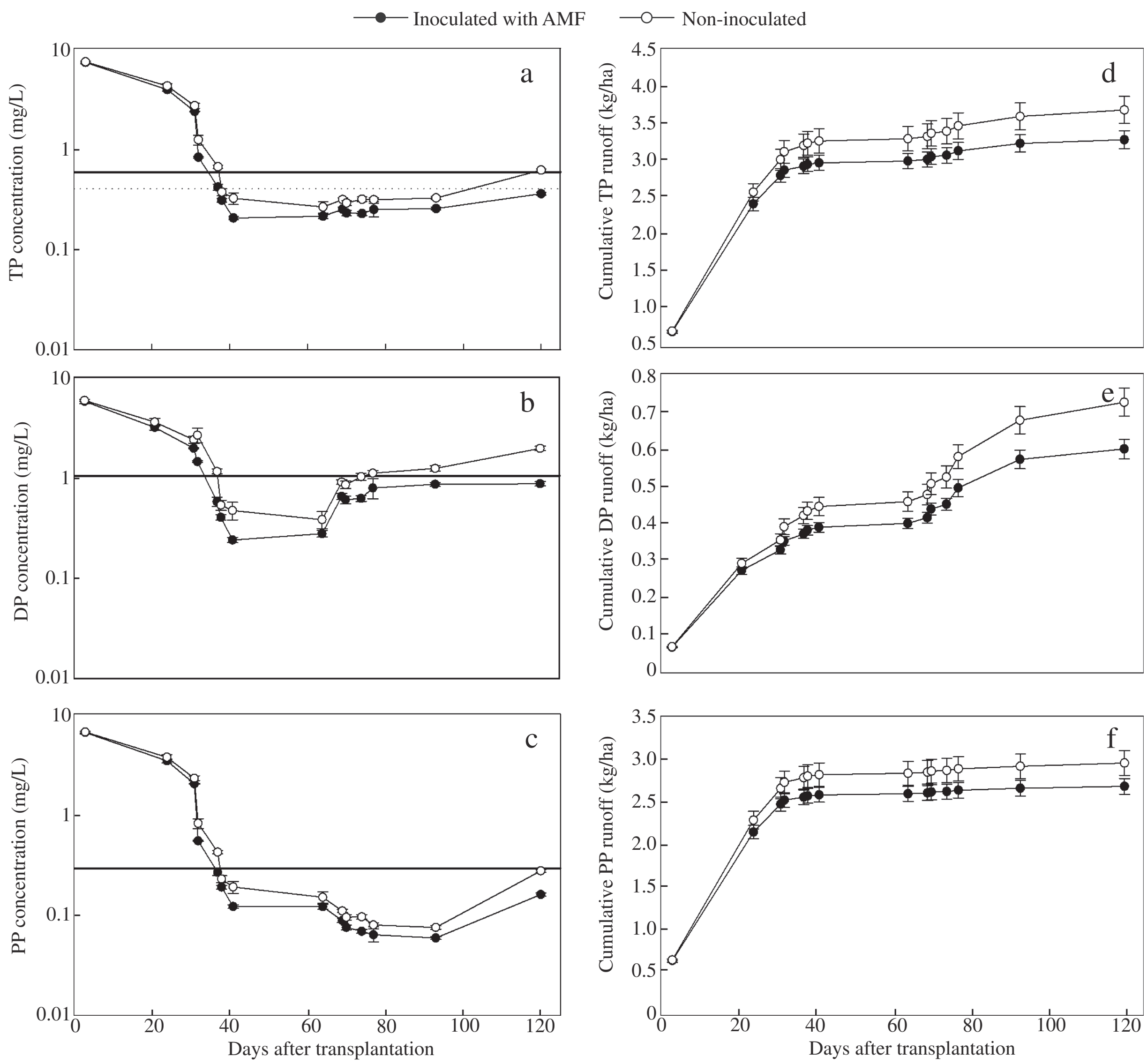

Fig. 2 – P concentrations of runoff water and the cumulative P runoff at the provided with 100% of the recommended fertilization. AMF: arbuscular mycorrhizal fungi; TP, total phosphorus; DP, dissolved phosphorus; PP, particle phosphorus. Solid line, the mean value of TP concentrations from non-inoculated treatment during rice growing season; dotted line, threshold value of surface water of Grade V (GB3838-2002).

2.3. Effects of reduction in fertilization on P runoff

Earlier researchers found that in Taihu Lake region, TP concentrations and TP loads during the rice growing season significantly increased as P fertilizer application rate increased (Cao and Zhang, 2004). Similar results were obtained in our study (Fig. 3a). There was a downtrend in the mean values of TP, DP and PP concentrations with decreasing fertilization ($p < 0.01$; Fig. 3a, b, c). In addition, the effect of fertilizer management was found to vary across rice growing season, with remarkable effect being at the initial stage of fertilization (*i.e.* before DAT 40), rather than the later stage (Fig. 2d, e, f; $p < 0.01$). This might be due to the method of fertilizer application. Unlike N fertilization which can be basal- and top-dressed during the growing season, P fertilization only entailed basal application in our study. Consequently, runoffs at the initial fertilization stage carried more P than that at the latter stage. This was in agreement with earlier research where 50%–98% of seasonal P runoff occurred when rainfall interacts directly with topsoil receiving recently applied fertilizers (Withers et al., 2003).

The seasonal TP runoff dropped with reducing fertilization (Fig. 4a, $p < 0.001$) as lower fertilization led to reduced mean P concentrations (Fig. 3a). Specifically, the seasonal TP runoff dropped only by 8.9% when fertilization decreased by 20% (*i.e.* at F80), while a drop in P runoff by 48% was associated with a decrease in fertilization by 40%. Moreover, TP concentration of more than 40% of runoff samples at F60 and F80 were significantly higher than the critical value of Grade V (GB3838-2002), and that percentage above *Grade III* was 100% for higher fertilizer levels (*i.e.* F60 and F80) ($p < 0.05$). This indicated that P runoff would not be reduced remarkably unless the fertilization was cut by 40%. In our study, rice yield

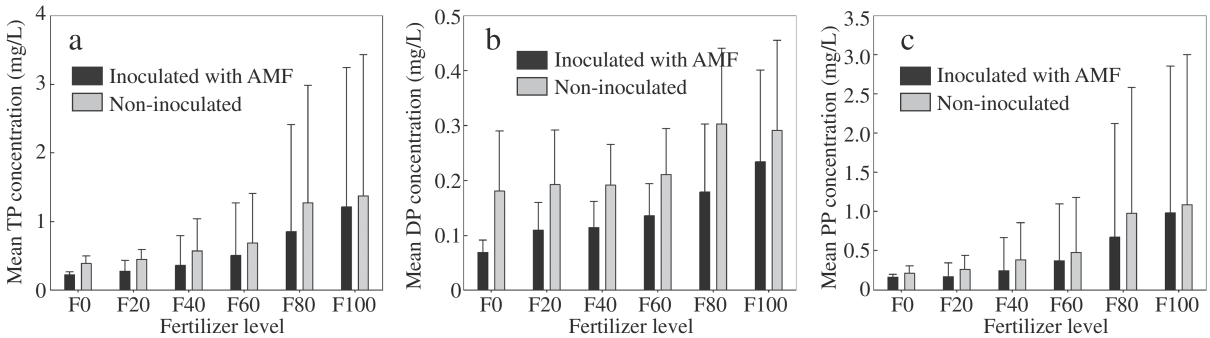

Fig. 3 – Mean P concentrations of runoff water under each fertilizer level. AMF: arbuscular mycorrhizal fungi; TP: total phosphorus; DP: dissolved phosphorus; PP: particle phosphorus; F0, F20, F40, F60, F80, and F100, provided with 0, 20, 40, 60, 80, and 100% of the recommended fertilization.

decreased with dropping fertilizer levels, especially where the fertilizer level was lower than F80 (Fig. 4b), suggesting that any drop in fertilization would lead to less rice yield.

2.4. Effects of AM inoculation on P runoff

At rice maturity, the overall root length of colonization (RLC) average was about 2.5% for non-inoculated rice, while that of inoculated rice ranged from 12.4% to 19.5% (Fig. 5) and this difference was significant ($p < 0.001$). For inoculated rice, RLC declined with rising fertilizer levels, while fertilizer level did not affect RLC of non-inoculated rice ($p > 0.001$). This further indicated that the effect of inoculation on RLC was more pronounced at the lower end of fertilization than that under higher fertilization. Therefore, inoculation improved AM colonization in rice roots, but this effect was reduced by high fertilizer levels.

As expected, AM inoculation played a pivotal role in decreasing P runoff. Firstly, inoculation decreased the mean values of TP concentrations ($p < 0.05$, Fig. 2a), leading to a reduction in seasonal TP runoff at each fertilizer level ($p < 0.05$, Fig. 4b). Specifically, a greater decrease in seasonal TP runoff, 1.5 kg/ha, was observed at F80 ($p < 0.\ 01$) while smaller ones (*i.e.* from 0.4 to 0.6 kg/ha) at other fertilizer levels (Fig. 4b, $p < 0.05$). Additionally, inoculation reduced the percentage of samples with TP concentration above the critical value of Grade V (GB3838-2002), from 43% to 21% at F60 and to 29% at F80. A drop by 7% of samples with TP concentration above Grade III (GB3838-2002) was caused by inoculation at F60 and F80. Moreover, the effect of AM inoculation on rice yields was significantly positive (Fig. 4a, $p < 0.001$) and this effect was greater under lower fertilizer levels.

The effect of AM inoculation on P runoff was affected by transplanting, artificial drainage, flooding and fertilization. Firstly, the external hyphae which were considered to involve P uptaking (Johnson et al., 2003) was damaged during transplanting (Lekberg and Koide, 2005) and was not able to work on DAT 3. But they grew gradually with rice growing and took up P for the host and themselves from DAT 21 to 38. And the two aeration periods from DAT 21 to 28 and from DAT 93 to 120 provided favorable condition for AMF so that there was a greater effect during these two periods. From DAT 29 to 92, the function of AMF was inhibited by flooding, support of which from the finding that conventional flooding depressed AM colonization in rice roots (Lumini et al., 2011). Furthermore, the effect of inoculation was altered by fertilizer levels (Fig. 2). In detail, the maximum reduction in seasonal TP runoff due to inoculation was 1.5 kg/ha at F20, while the minimum, 0.49 kg/ha was detected at F100.

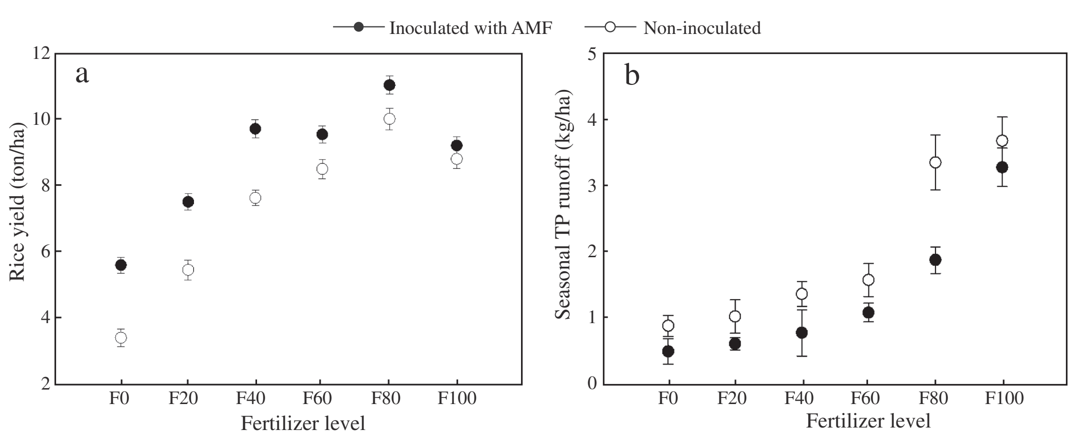

Fig. 4 – Effect of reduction in fertilization and arbuscular mycorrhizal (AM) inoculation on grain yield and seasonal TP runoff.

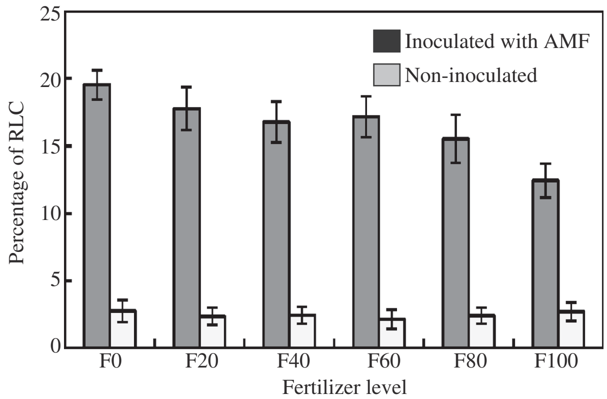

Fig. 5 – Impact of fertilization and inoculation on colonization by arbuscular mycorrhizal fungi. RCL: root length of colonization.

Combined with reducing fertilization by 20% (*i.e.* F80), AMF inoculation lowered P runoff by 44%. While the greatest effect of AM inoculation on rice yields and seasonal TP runoff was observed under F80, the subtle effect of inoculation on rice yields and seasonal runoff occurred under F100. This indicated that AM inoculation decreased P runoff by enhancing P uptake which could be indicated by improved rice yields. Therefore, the best combination was AM inoculation and 80% of recommended fertilization (*i.e.* F80), while the effect was enhanced by the aeration after artificial draining.

3. Conclusions

Under the local fertilization, the seasonal TP runoff from paddy fields was 3.7 kg/ha during the rice growing season in Lalin River Basin, with 80% being in particle form and 20% in dissolved form. In this region, rice yields decreased with decreasing fertilization and a reduction in fertilization by 20% only cut down P runoff by 8.9%, indicating that fertilizer management would not contribute much to reduction in P runoff without scarifying rice yields. Importantly, AM inoculation decreased P runoff at each fertilizer level, with rice yields being improved. Although this positive effect of inoculation was depressed by high fertilization, the combination of 80% of local fertilization and inoculation could cut down P runoff by 44% without scarifying rice yields. Taken together, this study highlighted that AM inoculation with appropriate fertilizer management is a potentially useful tool to slow down water eutrophication, although long-term monitoring is needed to determine how long the effects will last.

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