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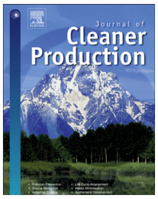

Ecological intensification of rice production through rice-fish co-culture

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ABSTRACT

Increased biodiversity can make valuable contributions to food production and security around the world. The role of plant species diversity for “ecological intensification” of agriculture has been widely recognised, but the potential contribution of multi-trophic-level production systems, such as rice-fish co-culture, has received less attention. A continuous 4-year experiment (2015–2018) was conducted comparing rice-fish (yellow finless eel and loach) co-culture, and mono-rice planting practices on the Chongming Eco-island of China. During the experiment, pests (insect herbivores and weeds), arthropods, pesticides, grain and marketable fish yield were sampled, soil quality (available nitrogen, phosphorus and potassium, total nitrogen, organic matter content and pH) and rice grain quality (protein content, chalkiness, gel consistency, amylose content) were evaluated, and an economic analysis were performed. Fish decreased herbivore insect abundance by 24.07%, reduced weeds abundance, richness and biomass by 67.62, 62.01 and 58.88% respectively, increased invertebrate predator abundance by 19.48%, and reduced the need for pesticide by 23.4%. Co-culture practice produced an average economic values 10.33% higher than in the mono-rice farming. In addition, rice-fish co-culture enhanced both soil and rice quality. Our results confirm that rice-fish co-culture can be an effective form of ecological intensification, incorporating and contributing ecosystem services in agricultural production and increasing sustainability.

1. Introduction

Agricultural intensification has been recognised one of the main reasons for biodiversity loss and related decline in ecosystem functioning due to the conversion of natural habitats into monoculture farming areas (Gagic et al., 2012; Batáry et al., 2017; Hass et al., 2018; Plue et al., 2018). Intensive use of agrochemicals is reducing environmental quality (Stehle and Schulz, 2015; He et al., 2017; Ruiz and MarÃa Dolores, 2018), damaging local plant communities (Cassman, 1999; Kremen et al., 2012), and reducing the abundance and richness of beneficial arthropods (Gagic et al., 2012;

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Kovács-Hostyánszki et al., 2017).

The concept of "ecological intensification" has been promoted to redesign agroecosystems, based on the increased use of ecological processes and biodiversity, using resource more efficiently, and decreasing anthropogenic inputs (Bommarco et al., 2013; Pywell et al., 2015; Bowles et al., 2016). In this framework, it has been showed that plant-diversified farming practices can contribute to ecological intensification of agriculture by providing multiple ecosystem services, promoting biological pest control (Redlich et al., 2018; Wan et al., 2018), decreasing the use of pesticides (Tscharntke et al., 2005; Gurr et al., 2016; Zhao et al., 2016), improving soil quality (Cassman, 1999), and enhancing crop yields (Tittonell and Giller., 2013; Gurr et al., 2016). The potential contribution of multi-trophic-level production systems, such as rice-fish co-culture, to ecological intensification has not received as much attention.

Rice-fish co-culture has been practiced in paddy fields for more than 2000 years in Asian countries (Anita et al., 2014; Islama et al., 2015), e.g., China (Xie et al., 2011a), Malaysia (Ali, 1990), Vietnam (Berg and Tam, 2018) and Bangladesh (Ahmed and Garnett, 2011). Previous studies have showed that Cyprinidae fish (e.g., local common carp, crucian and grass carp) ushered into paddy fields can provide multiple ecosystem services to rice ecosystems, i.e., decreasing pest abundance (Xie et al., 2011a), reducing agrochemicals inputs (Xie et al., 2011b; Berg and Tam, 2018), improving soil fertility and crop yield (Hu et al., 2013, 2016), and increasing economic benefit (Vromant et al., 2002; Feng et al., 2016). However, whether other functional group fish promotes ecological intensification, is not clear.

Rice-fish co-culture farming has been gradually increasing in China since 1990s, and have been recognised as globally important agricultural heritage systems (GIAHS) (Lu and Li., 2006; Xie et al., 2011a; Ren et al., 2018). To date, rice-fish co-culture farming has reached an area of 1.67×10^6 ha, which accounts for 4.48% of the total rice planting area (Hu et al., 2015). Chongming Eco-island, the third largest island in China, has the largest area and production of rice in the country, with 2.6×10^4 ha and 2.1×10^8 kg per year, respectively (Wan et al., 2015). Rice-fish co-culture production was introduced to the island in 2005, and now yellow finless eel (*Monopterus albus*), and loach (*Misgurnus* spp.), are the main fish species present in paddy fields. Whether such fish promotes ecological intensification is a scientific question for researchers and decision-makers to further apply rice-fish co-culture farming in Chongming Eco-island and China. Our hypotheses are that rice-fish co-culture decreases the densities of rice pests by hosting more arthropod predators, allowing reduced pesticide application without reducing rice grain yield and quality, thus enhancing economic profits. To test our hypothesis, we conducted a continuous 4-year experiment comparing rice-fish co-culture and mono-rice cropping on Chongming Eco-island. We also address further implications of rice-fish co-culture for ecological intensification.

2. Materials and methods

2.1. Study sites

The experiment was conducted at the Shanghai Lanhui Eco-agricultural Science and Technology Company Limited, in Sanxing Town, Chongming Eco-island, China (31°46′52″N, 121°15′17″E, elevation 4.0 m a.s.l.) (Fig. 1). The climate is characterised by southeast winds producing hot rainy weather during the summer season, and southerly winds causing winter monsoons during the winter season. Mean annual temperature is 15.3 °C, with 1003.7 mm of rainfall concentrated from April to September. Most of the rice cultivated in the area are "Qinggengxiangruan" varieties, developed by the Agricultural Technology Extension and Service Center in Qingpu District of Shanghai.

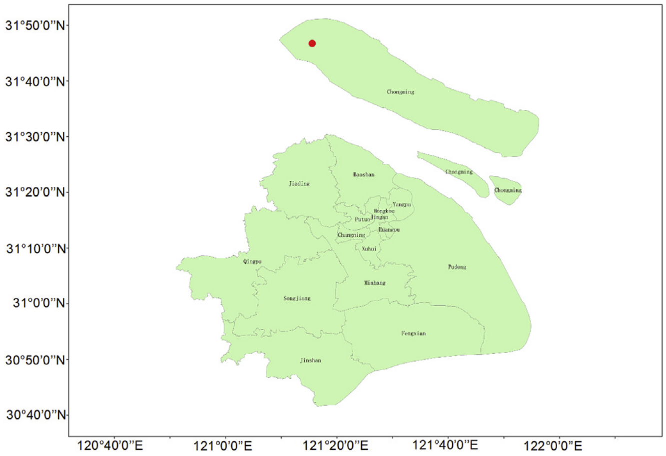

Fig. 1. A GIS map of Shanghai Municipality of China. The red dot represented the experimental site located in Sanxing Town, Chongming Eco-island, Shanghai, China (31°46′52″N, 121°15′17″E). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.1. Experimental design

The layout of the rice-fish co-culture areas (40.0 m × 62.5 m) consisted of a rice fields (38.2 m × 58.9 m) surrounded on three sides by a ditch (0.7m in depth and 1.8 m in width). The ratio of rice field area to surrounding water area was 9:1 (Fig. 2). Each year the co-culture and control plots received the same amount of fertilizers: 390 kg ha^{-1} of NPK fertilizer (15% Nitrogen, 15% Phosphorus, 15% Potassium) was used as the base fertilizer, and 75 kg ha^{-1} of urea (46% Nitrogen fertilizer) were used at the seedling, tillering, elongation and booting stages. After the construction of the co-culture plots (April 2015), the ditches were filled with irrigation water, and in June rice was seeded by mechanical dibbling at 0.07 m × 0.20 m space (row width by length, respectively).

Before rice reached the 6-leaf stage of growth and development (BBCH 16), the depth of irrigation water was kept at 2–3 cm in both co-culture and control rice fields. Water depth was measured from the surface of water to base of the rice plant. When the rice approached the 6 and 8 leaf stage (BBCH 16 and 18), and temperature started increasing gradually, the depth of the water was raised at 4–5 cm and 5–6 cm respectively (generally with one addition of irrigating water every 10–15 days), so that a more friendly environment was provided for the fish in the rice fields. In the control plot, when approaching the 6-leaf stage, the depth of irrigation water was kept at 2–3 cm (generally with one time of irrigating water per 7–10 days). If no rainwater was captured in the rice fields, water was pumped in the field from the irrigation channels around the experimental plots.

To control rice insect pest and diseases, insecticides and fungicides were applied over the course of the growing season when the abundance of insect pests or diseases were at the level recommended by the Shanghai Agricultural Technology Extension and Service Center (SATESC) publication "Pest Control Guidance". To exclude potential effects of herbicides on the fish, herbicides were not used in either co-culture or control rice fields. The insecticides applied are not known to affect fish. The kind and amount of insecticides sprayed was based on the pest abundance recorded in all experimental plots.

To estimate the economic threshold, a white stainless

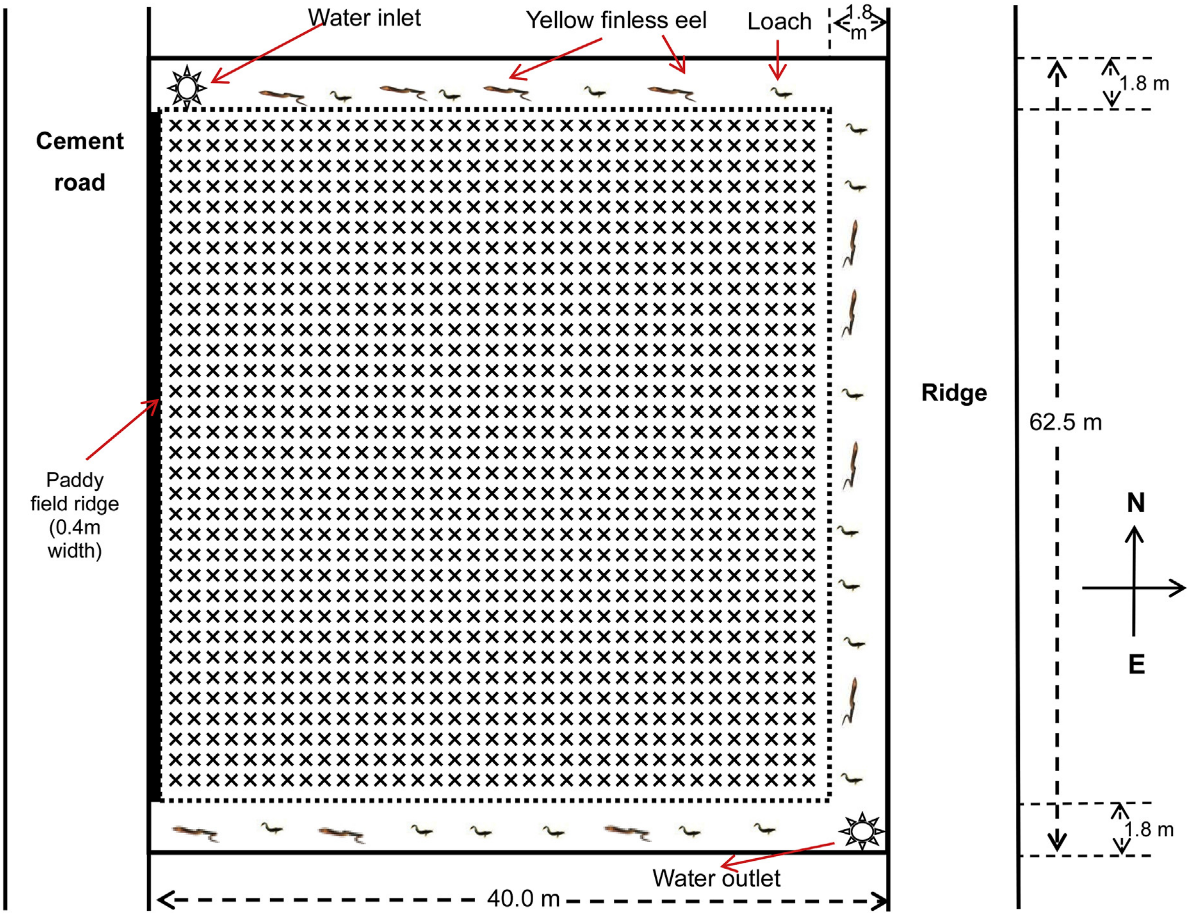

Fig. 2. The rice-fish co-culture plots consisted of a rice field (38.2 m × 58.9 m) surrounded on three sides by a ditch (0.7m in depth and 1.8 m in width). There were filter screens in the water inlet and outlet to prevent the fish from escaping. " × " denotes rice plants. The whole area = rice field area + water area of the ditch for the fish.

0.4m × 0.3m steel plate was placed at the base of rice plants. Rice plants were shaken, and pests and predators dropped onto the plate (Cheng, 2001). The numbers of pests and predators in the plate were then recorded. To survey the abundance of stem borers and leaf rollers in each plot, infested rice stems and leaves at each sampling point were examined, and the number of stem borers and leaf rollers was recorded. The total abundance of rice stem borers or leaf rollers at each sampling date per plot was then given by the sum of the values recorded in the plates and on the plants (Wan et al., 2018). For insect herbivores and invertebrate predators, three sampling dates at appropriately 15-20-day intervals were conducted from late August to middle October in 2016, 2017 and 2018.

The number of weed plants, weed species, and weed fresh biomass were collected at three sampling dates in early July, middle August, and late September, in 2016 and 2017. To scout weeds, seven sample points were sampled across each field in a zigzag pattern. At each sampling point, the number of individuals of all weed species was recorded in a 1 m^2 quadrat (1 m × 1 m). Weeds were removed and the fresh weight was measured in the laboratory.

Each year at the end of the growing season (from the middle to late October), rice yield was measured from each plot. Yield per square meter was measured from the fully mature rice plants as described by Wan et al. (2018). In each plot ten sample points were sampled in a zigzag pattern. At each sampling point, all rice plants in a 1 m × 1 m plot were cut at the base and collected. At the end of the field season, seven days after harvest, soil samples were collected with soil sampler auger (50 mm in diameter) at a depth of 0–0.20 m, in the co-culture and control rice field, using S-style sampling method as described by Chen et al. (2012), with 20 sub-plots in each rice field. The soil samples from each plot were then mixed and homogenised, and a sample of 1.0 kg of soil was send to the laboratory for analysis. The soils samples were dried at room temperature, crushed and grinded, and sieved with 20 mesh filters (0.85 mm in diameter). The soil analyses were soil pH, available nitrogen, available phosphorus and available potassium using the electrode method, alkali diffusion method, molybdenum antimony colorimetric method, and flame photometry method, respectively (Bao, 1999). To measure total nitrogen and organic matter content, soil samples were additionally sieved with 100-mesh filters (0.15 mm in diameter), and analyses were conducted using the kjeldahl method and potassium dichromate capacity method (Lu, 2000). Soil samples were collected at end of every season from 2015 to 2018.

Fish in the ditch around the rice field were captured using a "L" shape pipe trap (Fig. 3(h)), before rice reached the harvest stage. Fish trapping continued from late September to early October in 2016, 2017, and 2018. During the first year (2015), fish were not captured as they had not reached marketable size. During 2017, 2018, after the harvest, rice was sun dried, and 15–20 days later rice grain quality was measured. Rice quality indicators included appearance quality (chalkiness degree, chalky kernel percentage, particle length, length-width ratio, transparency), nutritional quality (protein and amino acid content), cooking quality (amylose content, gel consistency and alkali spreading value), and processing

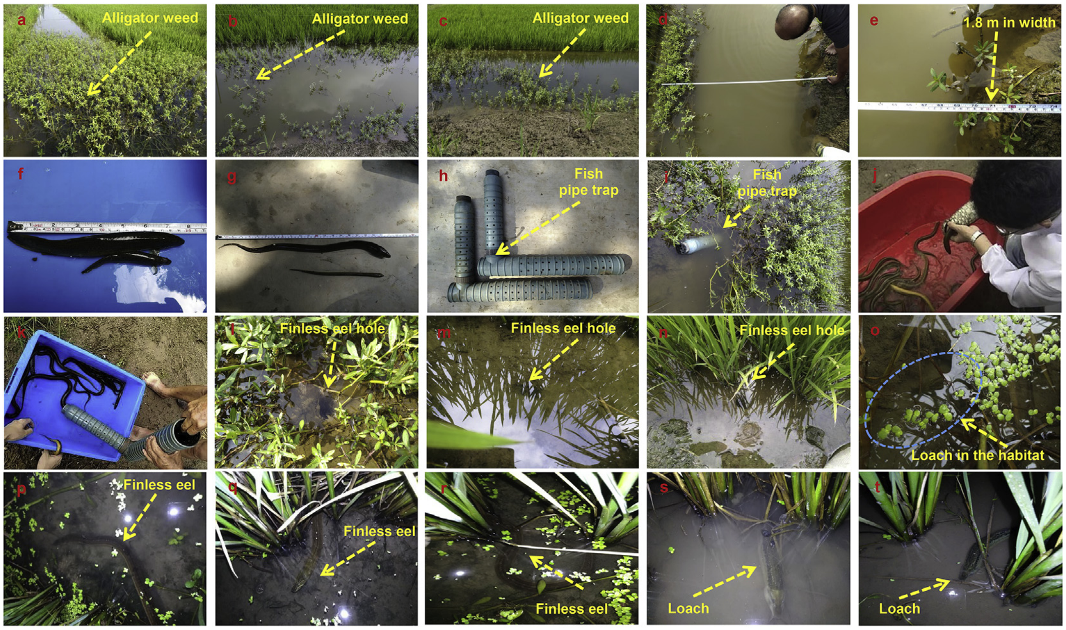

Fig. 3. The Photos for rice-fish co-culture plots. (**a**), (**b**) and (**c**) Ditches with alligator weeds around rice field; (**d**) Measure of ditch width; (**e**) 1.8 m in ditch width; (**f**) Marketable loach and loach fry; (**g**) Marketable yellow finless eel and yellow finless eel fry; (**h**) Fish L shape pipe trap; (**i**) Fish pipe trap placed in the ditch; (**j**) and (**k**) Captured fish from the ditch; (**l**) Finless eel hole in the ditch; (**m**) and (**n**) Finless eel hole in rice field; (**o**) Loach in the habitat in rice field; (**p**), (**q**) and (**r**) Finless eel activated in rice field; (**s**) and (**t**) Loach activated in rice field. Photos of Fig. 3(p–t) were taken in evening with cell phone lamplight. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

quality (brown rice rate, head rice rate and milled rice ratio) (The Ministry of Agriculture of the People's Republic of China, 2010) (additional information on experimental design is available in Note S1).

2.3. Data analysis

Normal distribution and homoscedasticity of all data were checked using the Kolmogorov-Smirnov test and Levene's test, respectively. Two-way ANOVA with GLM (General Linear Model) was used to analyse the interactive effects of years and rice types (rice-fish co-culture and mono-rice) on the average abundances of pests (insect herbivores, rice plant-hoppers, rice leaf rollers, rice stem borers and weeds), the density of the invertebrate predators, the number of weed species and the biomass of the weeds. Two-way ANOVA with GLM was also adopted to analysis the six soil environmental indicators (available nitrogen, phosphorus, potassium, total nitrogen, organic matter content and pH), six pesticides use indicators (listed in Note S2), two grain yield indicators (grain yield per land use area and grain yield per rice area), and six indicators of economic cost–benefit analysis (listed in Note S2) and rice quality indicators. Statistical analyses were performed using SPSS 16.0 software (SPSS Inc, Chicago, IL).

Due to the high number of rice quality indicators, we tested the difference between rice-fish co-culture and mono-rice plots with partial least squares–discriminant analysis (PLS–DA), using the software program SIMCA-P v13.0 (Umetrics AB, Umea, Sweden). Because of the alkali spreading value or the value of transparency observed was the same in both rice-fish co-culture and mono-rice plots, these two indicators were excluded by the PLS–DA. Thus, 10 indicators (listed in Note S2) were analysed in the PLS–DA.

PLS–DA result were visualized in score plots, which represent the sample structure according to the model components, and loading plots, showing the contribution of the tested variables. Cross Validation Analysis of Variance (CV-ANOVA) was used to evaluate the significance of treatment effect by comparing the goodness of fit (R^2) and the predictive value (Q^2) of the extended model (including the treatment parameter) with that of the reduced model. An economic cost–benefit analysis was conducted according to Gurr et al. (2016) and Wan et al. (2018) (detailed information on economic cost–benefit analysis is given in Note S3).

3. Results

Rice-fish co-culture practice decreased herbivore insect abundance, reduced weeds abundance, richness and biomass, increased invertebrate predator abundance, reduced the need for pesticide and increased economic values (the magnitudes of differences between rice-fish co-culture and mono-rice systems were presented in Fig. 4).

3.1. Rice pests and arthropod predators

Two-way ANOVA showed that the rice field types affected significantly the invertebrate densities of five groups (insect herbivores, rice plant-hoppers, leaf rollers, stem borers, and predators) and three weeds (number of weed species, number of weed plants, and fresh weight of weeds). The effect of year and the interaction of year × rice field type showed also significant effects on pest and predators (Table s1).

The three sampling dates on which pest abundances was measured each year from 2016 to 2018 showed significant lower pest recruitment in the co-culture plots than in the mono-rice plots (insect herbivore: mean percent decrease = 24.07, SD [standard deviation] = 7.37, IQR [interquartile range] = 21.25–28.24; rice plant-hopper: mean percent decrease = 24.21, SD = 4.35, IQR = 22.15–26.48; rice leaf roller: mean percent decrease = 18.27, SD = 5.45, IQR = 15.13–20.11; rice stem borer: mean percent decrease = 25.55, SD = 5.70, IQR = 22.41–27.98). Predator abundance was higher in the co-culture plots than in the mono-rice

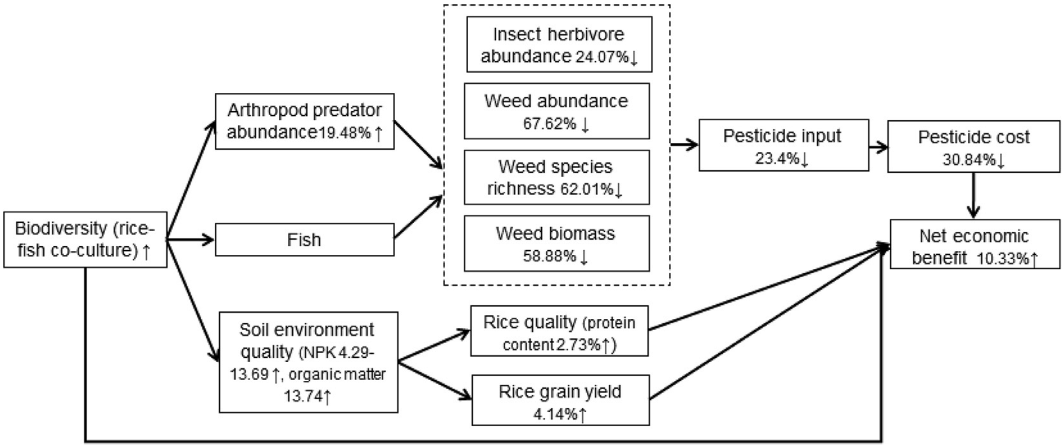

Fig. 4. A summary graph illustrating the magnitudes of differences between rice-fish co-culture and mono-rice systems. "↑" means increased level and "↓" denotes decreased level.

plots (mean percent increase = 19.48, SD = 0.69, IQR = 19.08–19.70; Fig. 5(a–e)).

The two-year data collected on weed species, number of weed plants, and weight of weeds, showed a significant decrease in the co-culture plots compared with the mono-crop plots (weed species: mean percent decrease = 62.01, SD = 0.52, IQR = 61.85–62.22; number of weed plants: mean percent decrease = 67.62, SD = 1.00, IQR = 67.27–67.97; fresh weight of weeds: mean percent decrease = 58.88, SD = 1.21, IQR = 58.45–59.31) (Fig. 5(f and g)).

3.2. Soil environment quality

Two-way ANOVA showed that the rice field type significantly affected soil qualities except total nitrogen, while for year was a significant effect of year for all soil qualities except available potassium. There was a significant treatment × year interaction (Table s1).

Four-year data showed that available nitrogen, phosphorus and potassium ($mg\bullet kg^{-1}$), total nitrogen ($g\bullet kg^{-1}$) and organic matter content ($g\bullet kg^{-1}$) were higher in co-culture than mono-crop fields, respectively (available nitrogen: mean percent increase = 6.54, SD = 7.56, IQR = 1.18–9.69; available phosphorus: mean percent increase = 13.69, SD = 7.46, IQR = 10.62–18.77; available potassium: mean percent increase = 4.50, SD = 3.49, IQR = 2.08–6.22; total nitrogen: mean percent increase = 4.29, SD = 1.03, IQR = 3.48–4.90; organic matter: mean percent increase = 13.74, SD = 6.31, IQR = 11.70–18.02; Fig. 6(a–e)). Co-culture decreased soil pH value (mean percent decrease = 1.02, SD = 0.10, IQR = 0.96–1.07 (Fig. 6(f)).

3.3. Rice quality

The last two years of the 4-year experiment (2017–2018), showed that rice field type had significant effects on rice qualities except length-width ratio and particle length. Furthermore, the PLS-DA showed that the rice-fish co-culture field was clearly separated from the mono-crop field in 2017, and three principal components were observed ($R^2X = 0.937$, $R^2Y = 0.998$ and $Q^2 = 0.996$). Of these three components, the first and second components explained 84.50% and 9.17% of the variance (Fig. 7(a and b)). Data from 2018 showed also a clear separation ($R^2X = 0.922$, $R^2Y = 0.995$ and $Q^2 = 0.987$) and the two components explained 82.10% and 10.10% of the variance (Fig. 7(c and d)).

3.4. Pesticide use and grain yield

Both rice field types and year showed a significant effect for the six pesticides and two grain yield indicators in a two-way ANOVA. The interaction of the two factors did not affect the values of above eight indicators (Table s1).

Averaging 4-year (2015–2018) data, the pesticide use decreased in the rice-fish co-culture plots during the whole experiment (amount of commercial pesticide sprayed per rice area: mean percent decrease = 23.40, SD = 7.37, IQR = 19.00–27.36; amount of commercial pesticide sprayed per land use area: mean percent decrease = 31.06, SD = 6.63, IQR = 27.10–34.63; amount of active ingredient in pesticide sprayed per rice area: mean percent decrease = 16.29, SD = 2.17, IQR = 14.57–18.16; amount of active ingredient in pesticide sprayed per land use area: mean percent decrease = 24.66, SD = 1.95, IQR = 23.11–26.34; number of pesticide sprays per rice area: mean percent decrease = 28.60, SD = 5.74, IQR = 28.43–31.58; number of pesticide sprays per land use area: mean percent decrease = 35.74, SD = 5.2, IQR = 35.59–38.42). Grain yield per land use area was lower in the co-culture (mean percent decrease = 6.62, SD = 1.15, IQR = 6.21–7.00), while grain yield per rice area increased (mean percent increase = 4.14, SD = 0.78, IQR = 3.71–4.21; Fig. 8).

3.5. Economic cost–benefit analysis

Two-way ANOVA showed that both rice field types and year had a significant effect on the six economic cost–benefit analysis indicators, while no significant effect was observed on the total cost per land use area. Furthermore, the interaction of the two factors was significant only on the total benefit per land use area, and total cost per land use area (Table s1).

The value of grain yield decreased in rice-fish co-culture plots when averaged over both experiments per land use area, and for economic cost–benefit analysis, during the whole period (2015–2018; mean percent decrease = 6.27, SD = 0.70, IQR = 6.21–6.66) (Fig. s1(a)), and the same trends occurred for the cost of pesticide entities sprayed (mean percent decrease = 30.84, SD = 4.85, IQR = 28.23–34.67; Fig. s1(c)), and for the cost of labour required to spray the pesticides (mean percent decrease = 35.74, SD = 5.16, IQR = 35.59–38.42; Fig. s1(d)). In 2015, fish were not captured in co-culture plots as the fish had not yet reached the marketable weight, so no fish economic value was calculated, no cost for fish capture devices, and no cost of the labour required to trap the fish. Consequently, during 2015, total benefit, total cost and

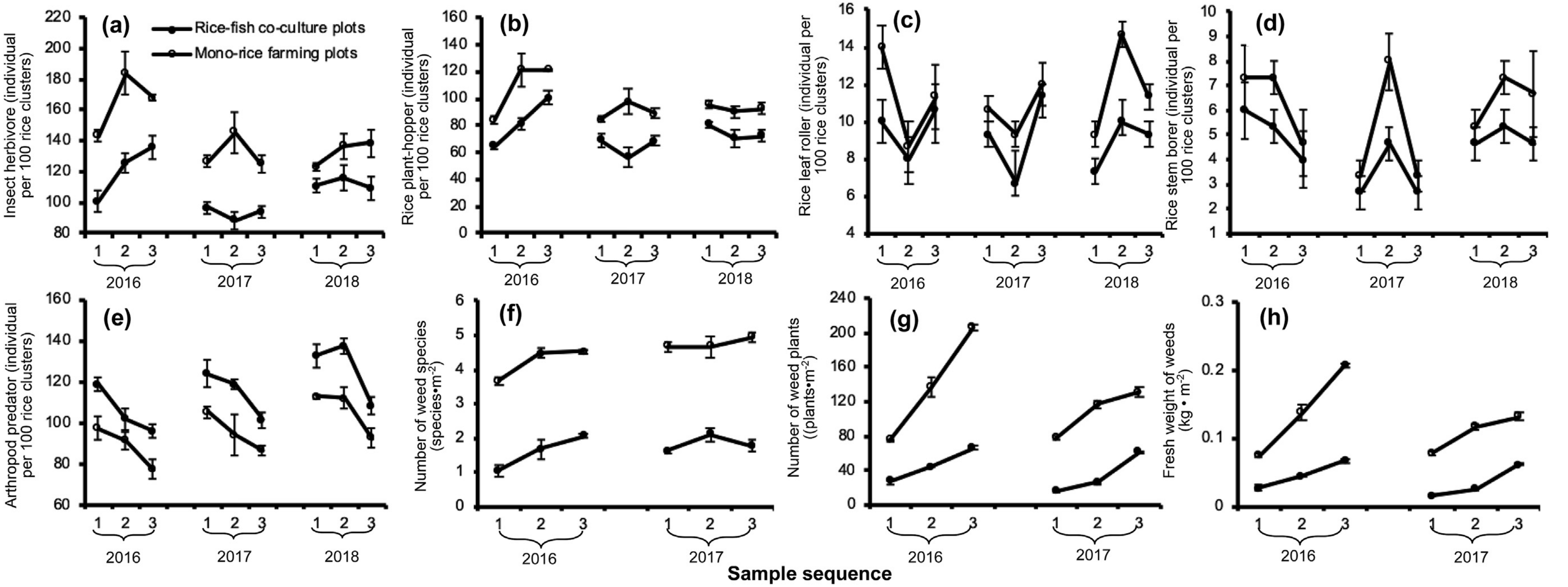

Fig. 5. Temporal changes of the insect herbivores, insect predators and weeds in rice fields with rice-fish co-culture plots and with mono-rice farming plots at the same location (Sanxing Town, Chongming Eco-island, Shanghai, China). Vertical bars shows SE. (a) Density of insect herbivores (individual per 100 rice clusters); (b) Density of rice plant-hoppers (individual per 100 rice clusters); (c) Density of rice leaf rollers (individual per 100 rice clusters); (d) Density of rice stem borers (individual per 100 rice clusters); (e) Density of the invertebrate predators (ladybird beetles, lacewings and spiders) (individual per 100 rice clusters); (f) Number of weed species sampled in rice field plots (species$\bullet$m^{-2}); (g) Number of weed plants sampled in rice field plots (plants$\bullet$m^{-2}); and (h) Fresh weight of weeds sampled in rice field plots (kg$\bullet$m^{-2}). The numbers on the *X*-axis indicate the sampling times, the 3 samplings for insect herbivores, rice plant-hoppers, leaf rollers, stem borers and invertebrate predators, and the 3 samplings for number of weed species, number of weed plants and fresh weight of weeds.

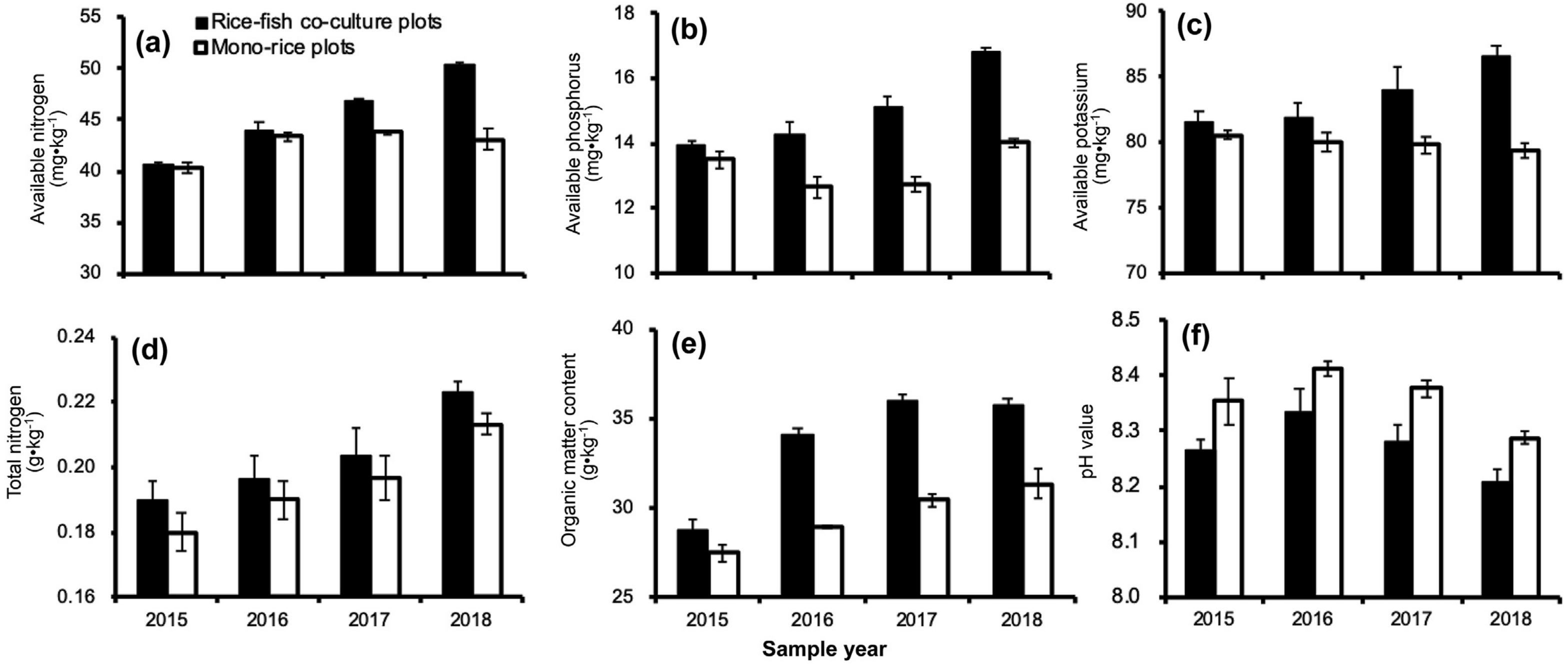

Fig. 6. Comparison of the soil environment indicators between rice-fish co-culture and mono-rice field plots at the same location (Sanxing Town, Chongming Eco-island, Shanghai, China) from 2015 to 2018. (a) Available nitrogen (mg•kg^{-1}); (b) Available phosphorus (mg•kg^{-1}); (c) Available potassium (mg•kg^{-1}); (d) Total nitrogen (g•kg^{-1}); (e) Organic matter content (g•kg^{-1}); and (f) pH value. Vertical bars denote SE.

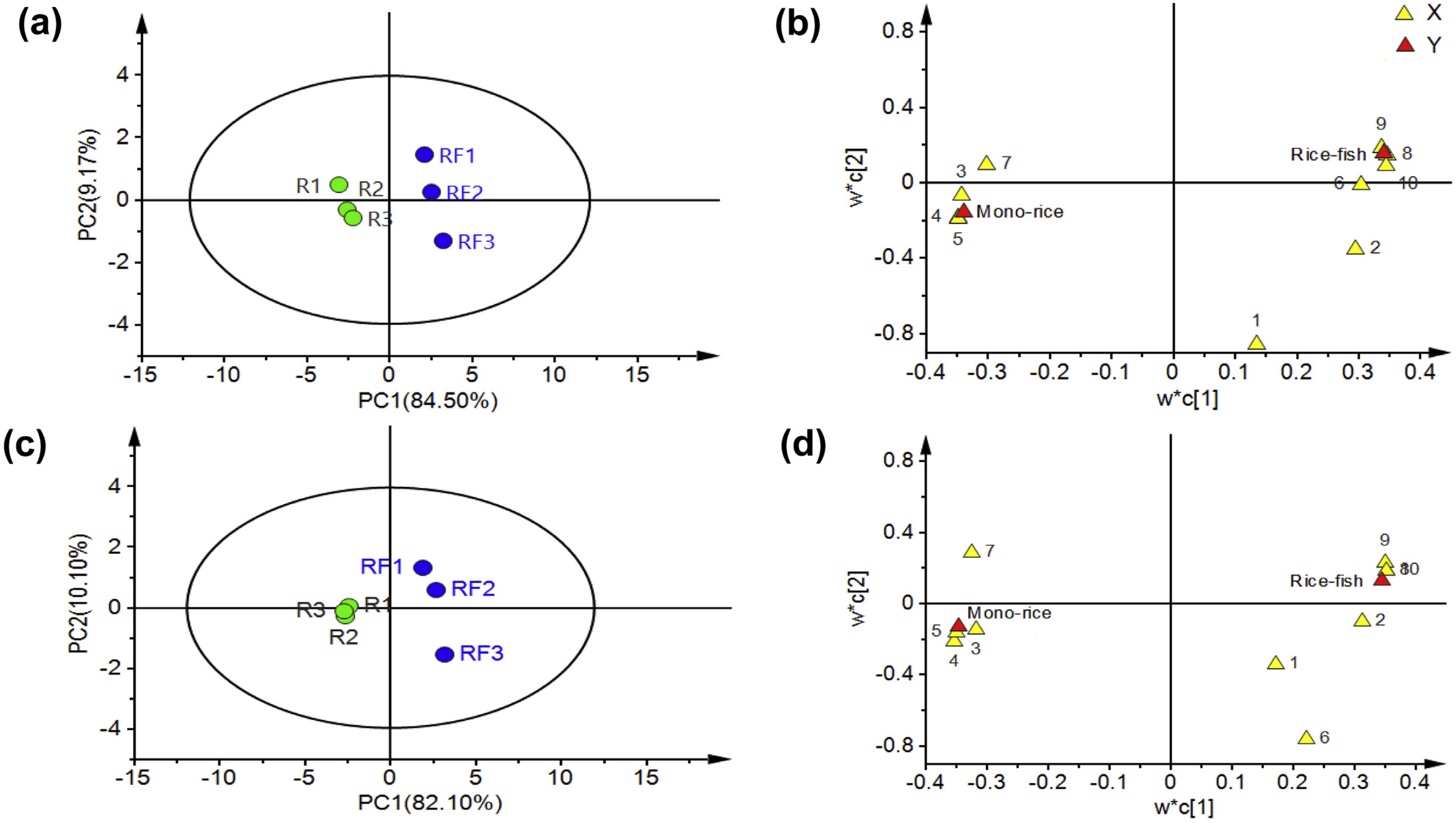

Fig. 7. Partial least squares–discriminant analysis (PLS–DA) of rice quality in rice-fish co-culture (RF1, RF2 and RF3) and mono-rice (R1, R2 and R3) farming plots in 2017 and 2018. Score plots for 2017 (a) and 2018 (c), and loading plots for 2017 (b) and 2018 (d) of the first two principal components with the explained variance in brackets. The ellipse defined the Hotelling's T2 confidence region (95%). Yellow triangles (*X*) in (b) and (d) represent rice quality indicators of 1: length-width ratio; 2: protein content (%); 3: chalkiness degree (%); 4: chalky kernel percentage (%); 5: gel consistency (mm); 6: particle length (mm); 7: amylose content (%); 8: brown rice rate (%); 9: head rice rate (%); 10: milled rice ratio (%). Red triangles (*Y*) in (b) and (d) are dummy variables that appoint the samples to rice-fish co-culture or mono-rice plots. The w*c [1] and w*c [2] values represent the contributing weights of each rice quality indicator to principal components 1 and 2 of the PLS-DA model, respectively. The alkali spreading value and the value of transparency are 7 and 1 in both rice-fish co-culture and mono-rice plots respectively, so the values of these two indicators were not analysed in this figure. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

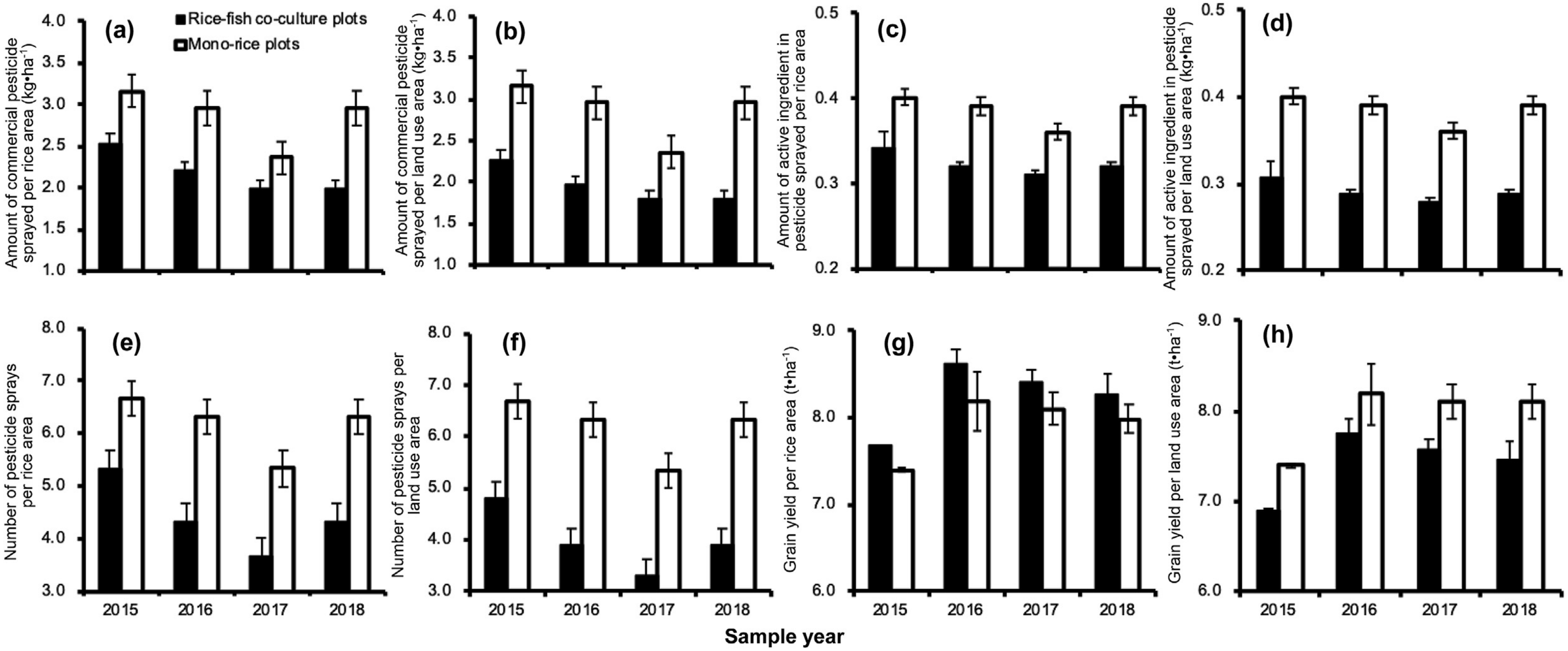

Fig. 8. Comparison of pesticide use and grain yield between rice-fish co-culture and mono-rice field plots at the same location (Sanxing Town, Chongming Eco-island, Shanghai, China) from 2015 to 2018. (a) Amount of commercial pesticide sprayed per rice area (kg•ha^{-1}); (b) Amount of commercial pesticide sprayed per land use area (kg•ha^{-1}); (c) Amount of active ingredient in pesticide sprayed per rice area (kg•ha^{-1}); (d) Amount of active ingredient in pesticide sprayed per land use area (kg•ha^{-1}); (e) Number of pesticide sprays per rice area; (f) Number of pesticide sprays per land use area; (g) Grain yield per land use area (t•ha^{-1}); and (h) Grain yield per rice area (t•ha^{-1}). Rice area denotes the field was only planted with rice without ditch area, and the land use area includes the rice area plus ditch area for the fish. In rice-fish co-culture, 90% of the total area used had rice planted. Vertical bars denote SE.

net benefit, were all observed lower in rice-fish co-culture plots than mono-rice plots. From 2016, fish reached the marketable weight and fish trapping was conducted. The total and net profit increased in co-culture plots. Co-culture plots produced a net profit of 22.82 (±0.13) − 23.66 (±0.35) x 1000 RMB • ha^{-1} • $year^{-1}$), which was on average 10.33% higher than in the control plots (SD = 2.78, IQR = 8.94–11.71; Fig. s1(f)).

4. Discussion and conclusion

Analysis of ecological intensification of agriculture has focused on ecosystem service providers and service-providing units (Gurr et al., 2016; Wan et al., 2018). In this study, we observed that finless eels are able to dig holes and reside in rice fields (Fig. 3(m and n)), while loach can utilize the grooves and rice plant roots as shelters and habitats in the paddy fields (Fig. 3(o)). Both fish species were able to forage for food (i.e. insect herbivores and pest weeds; Fig. 3(p–t)). Accordingly, we found evidence that finless eel and loach can provide ecosystem services in rice fields, and that rice-fish co-cultural practices can help to reduce the abundance of different pest groups. The decrease in the use of pesticides, which lead to a positive effect on arthropod predators, also improved crop management, with a reduction in pesticide inputs and labour cost. These changes resulted in an increase in the economic performance (Fig. s1).

The presence of fish in rice fields also resulted in a decrease in insect herbivores. Previous research (Xie et al., 2011b) showed that herbivores (rice plant-hoppers) often fell into the water surface when fish hit the rice stems when swimming. The lower abundance of rice plant-hoppers could also be due to the water level, which was deeper in the co-culture fields than mono-rice fields. The deeper water had a direct effect on the feeding areas of rice plant-hoppers, who feed at the base of rice plants. However, Xie et al. (2011b) showed that both the abundances of rice stem borers and leaf rollers did not significantly differ between rice-fish co-culture fields and mono-rice fields, mainly because the two pest groups only attacked the upper part of the rice plants and did not fall into the water surface when fish hit the rice stems. Our study showed that the abundances of the two groups decreased significantly (stem borers, $F_{2,\ 12} = 15.385$, $P = 0.002$; leaf rollers, $F_{2,\ 12} = 35.636$, $P < 0.001$). Another interpretation for the reduction in the abundance of stem borers and leaf roller can be attributed at the different oviposit habits that the two species have. The deeper water level decreased the oviposition space of stem borers, who usually oviposit at the base of rice plants (Xiao et al., 2001). The reduction in abundance of the leaf roller may be due to their habit of ovipositing one egg on the middle and top sections of the rice leaves. As a result, the rice stems hit by the fish cause the eggs and 1st-instar larvae to fall into the water, while not affecting the 2nd-5th-instar larvae, which are able to roll into the rice leaves (Liu, 2007). Our observations suggested that leaf rollers falling into the water are eaten by fish in co-culture fields, keeping the population abundance of leaf rollers at a lower level.

Other fish species, such as local common carp and grass carp, introduced to paddy fields, have been shown to reduce number, species (Du and Zhang, 2000) and biomass (Rothuis et al., 1999) of weeds present. The reason was mainly attributed to the higher palatability of weeds (Wang et al., 2007). Finless eel and loach rarely eat weed plants, but loach eat weed seeds. During the night time foraging, loach dig holes in the bottom of the paddy field, likely digging up and eating weed seeds. Additionally, the higher water level of the rice-fish co-culture creates an unfavourable habitat for the emergence and growth of weeds compared to mono-rice production.

Our study also showed that rice-fish co-culture enhanced soil environment quality (or soil nutrients), as previously reported with other fish species (Xie et al., 2011b). Fish excrement increase both organic matter content and total N (Ning, 2007), and through their activities on water perturbation, on the high level of microorganisms in fish excrements, they can improve soil permeability and soil microorganism metabolism, promoting nutrient cycling, increasing available N, P, K in the soils (Zhang et al., 1991). The presence of loach in rice fields has also been showed to promote the growth of soil microorganisms (i.e. nitrogen fixing bacteria, cellulose decomposing bacteria, nitrobacteria, sulphur bacteria and ammonifying bacteria), which are beneficial for improving the supply of soil nutrients (Sun et al., 2008). In addition to the soil nutrient increase, our study showed that the presence of fish decreased the average pH of the soil, probably due to the increased redox potential stemming from the stirring effects of fish on soils (Frei and Becker, 2005).

The effect of fish on the nutrient utilization efficiency of paddy field previously observed rice yield (Sun et al., 2008) was confirmed in our study (Fig. s1(g)). This higher rice yield was probably due to fish excrement, which is high in phosphate and ammonia, an N form that rice can utilize directly (Chakraborty and Chakraborty, 1998), and to potassium in a form easily taken up by rice (Zhang et al., 1991).

The rice variety utilized in this study was milled round-grain glutinous rice, which is able to synthesize the characteristic of milled medium to short-gain, non-glutinous rice and waxy rice, which has an elliptical form. Our study showed that rice-fish co-culture improved rice quality as observed by the increased values in length and length-width ratio, and the chalkiness degree and chalky kernel percentage reduction in co-culture rice fields. According to advisory documents issued by Ministry of Agriculture of the People's Republic of The Ministry of Agriculture of the People's Republic of China (2010), rice quality grade is superior if the values of length, length-width ratio, and transparency of rice particles are higher, or if the chalkiness degree, chalky kernel percentage and alkali spreading value is lower. Interestingly, we found that both the transparency, and alkali spreading value, did not differ between the co-culture and mono-rice fields.

Enhancing total protein in human food is a means of promoting nutritional quality of rice and improving human nutrition and health (Peng et al., 2014), and our study showed that rice-fish co-culture can promote protein content in rice particles. Amylose content in milled, medium to short-grain non-glutinous rice is usually less than 20%, and lower amylose content associated with softer characters, greater viscosity and better taste, is appreciated by consumers. Additionally, gel consistency, another cooking quality indicator, is generally more than 80 mm in milled round-grain glutinous rice. Consumers are fond of lower gel consistency of rice as it could result in better texture (Du et al., 2010). Thus, we conclude that rice-fish co-culture promoted rice quality, as both amylose content and gel consistency decreased.

The ditch around the co-culture rice fields used for breeding fish took up some land area that could have been used to cultivate more rice (Feng et al., 2016). However, our results on pests and pesticide application, soil quality, rice quality and economic benefit resulting from the introduction of finless eel and loach, suggest that the potential for ecological intensification through rice-fish co-culture is high. Similar research on rice-carp, rice-crab and rice-lobster co-culture) warrant for further research to promote sustainability and ecological intensification of agriculture.

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Appendix A. Supplementary data

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