Effects of Winter Cover Crops on Rice Pests, Natural Enemies, and Grain Yield in a Rice Rotation System

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Abstract

Rotations that include winter cover crops are widely used in agricultural systems and can provide numerous agroecological and economic benefits. However, the effects of winter cover crops on arthropod diversity, specifically rice pests and related natural enemies in rice rotation systems, are still largely unknown. We compared the effects of three winter cover crops, rapeseed, Brassica napus L. (Brassicaceae), Chinese milkvetch, Astragalus sinicus L. (Fabales: Fabaceae), and garlic, Allium sativum L. (Asparagales: Amaryllidaceae), on arthropods species diversity and evenness, densities of populations of major rice pests and major natural enemies, and grain yield in an experimental double cropping rotational rice field in Jiangxi Province, China. We did not observe any effects of cover crops on arthropod species diversity and evenness. The presence of prior cover crops also had no effect on the number of plants infested by the two major rice pests, Chilo suppressalis Walker (Lepidoptera: Pyralidae) and Cnaphalocrocis medinalis (Gueneé) (Lepidoptera: Pyralidae). Our study did not show any effects of rapeseed and Chinese milkvetch on grain yield. However, grain yield was increased in the garlic treatment. Our results suggest that although the winter cover crops we tested in our study do not affect the number of rice plants infected by major rice pests, they do not negatively affect the arthropod community and grain yields in rice rotation systems. Therefore, planting of winter cover crops may increase agricultural land utilization and have an overall economic benefit in rice rotational systems.

Key words: winter cover crop, rotation, arthropod diversity, rice, garlic
Vigna unguiculata (L.) Walp. (Fabales: Fabaceae), hair vetch, Vicia villosa Roth (Fabales: Fabaceae), and white clover, Trifolium repens L. (Fabales: Fabaceae), as winter cover crops, no such effects were reported in predation levels on targeted pests (Woolwine and Reagan 2001). Therefore, the effects of winter cover crops on the population densities of pests and natural enemies seem to be dependent on the crop system and targeted pests. In addition, many agricultural management activities, such as tillage and flooding, could decrease overwinter population of insects (Lawani 1982, Ferr 1996).

Rice, Oryza sativa L. (Poales: Poaceae), is among the three most important grain crops in the world (Prasad et al. 2017). China ranks first in total rice production contributing about 35% of the global rice production in the world (FAOSTAT 2016). With double cropping systems utilized for maximizing land use, eastern and southern China are the major regions for rice production accounting for approximately 94% of rice planting area and 88% of rice production in China (Ma et al. 2013). Challenges to rice production include damage caused by insect pests, diseases, and weeds (Oerke and Dehne 2004), which has been estimated to cause 35% losses in rice production (Oerke 2006). In southern China, insect pests that affect rice agriculture are mainly in the family Pyralidae (Lepidoptera), including the rice striped stem borer, Ch. suppressalis and the rice leaf folder, Cn. medialis; and in the family Delphacidae (Hemiptera), including the brown planthopper, Nilaparvata lugens (Stål), the white backed planthopper, Sogatella furcifera (Horváth) and the small brown planthopper, Laodelphax striatellus (Fallen) (Lou et al. 2014). Biological control has been proposed as one of the most important IPM management strategies in rice (Lou et al. 2014).

Although winter cover crops have been reported to provide important environmental services for early rice crops such as reducing methane emission (Haque et al. 2012, Kim et al. 2012), as well as weed suppression (Dhima et al. 2006), to our knowledge there are no studies on the effects of winter cover crops on pest and natural enemy populations in a rice rotation system. It is very important to know whether these winter cover crops will affect the arthropod structure and increase the damage from major rice pests in a rice rotation system. Therefore, to determine the effects of three winter cover crops, rapeseed, Chinese milkvetch, and garlic (another locally important winter cover crop), on arthropod community and insect pest management, we investigated rice pests, related natural enemy populations, and grain yields in a rice rotation system for 2 consecutive years.

Materials and Methods

Field Survey
Experiments were conducted over 2 consecutive years from September 2012 to July 2014 at the experimental station of Jiangxi Agricultural University (28° 47′54″ N, 117° 04′43″ E) in Niejia, Wannian County, Jiangxi Province, China. Rapeseed, Chinese milkvetch, and garlic were used as winter cover crops before planting in a spacing of 2 m between plots. Although our experimental fields are double cropped in this study, we only focused on the direct and sustained effects of winter cover crops on arthropods in following season. Therefore, we only investigate the arthropods in winter cover crop season and early rice season, but not in late rice season.

Winter Cover Cropping and Rice Cultivation
Prior to our study, the field was planted with only rice for at least 3 yr without the inclusion of cover crops. All winter cover crops and rice were planted in accordance to local farmers’ practice. Chinese milkvetch was directly sown at the recommended rate (45 kg/ha) in early October 2012 and late September 2013, respectively. Rapeseed seedlings were prepared in a local nursery and transplanted (37 cm row spacing and 13 cm plant spacing) in mid-November 2012 and early November 2013, respectively. Garlic cloves (18 cm row spacing and 6 cm plant spacing; approximately 250 kg/ha) were planted in late November 2012 and early November 2013, respectively. For rapeseed seedlings and garlic cloves, the soil was ploughed to a depth of 20 cm before transplanting. In winter, the crops covered more than 70% area of the plots, while less than 20% plant coverage in fallow plots. For the surrounding area, 2 m ridges were partly covered by weeds in these two seasons but the weeds were regularly removed by ploughing and other farming practices. All winter cover crops were harvested at the beginning of April in 2013 and 2014. Overground portions of rapeseed plants and garlic cloves with root were removed from the experimental plots, and the soil was ploughed immediately after harvest. Chinese milkvetch, a green manure crop, was directly ploughed in the soil during harvest. All of the plots were flooded for 1 wk after ploughing, followed by rotary ploughing of the plots for rice cultivation. Rice was transplanted in a consistent pattern with 23 cm in row spacing and 17 cm in plant spacing in both 2013 and 2014. Seedlings of early rice (Var. ‘Xiamong 25’) were transplanted in May and harvested in July. Details of time schedule of crop cultivations can be found in Fig. 2, along with sampling dates. Seedlings of late rice (Var. ‘Tianyouzhuhan’) were transplanted in July and harvested in October. The cultural practices in all plots are the same in rice period.

Fertilizer Management
In this study, since different cover crops serve to different purposes, we followed local farmers’ practices of fertilizer management for different crops, instead of uniform fertilizer composition. To increase economic income, two major economic crops, garlic and rapeseed, require a higher amount of fertilizers, whereas milkvetch is used as a green manure crop which requires less fertilizers. During winter cover crop season, 375.0 kg/ha diammonium phosphate (45.0 kg/ha P2O5) were applied in milkvetch plots; 300.0 kg/ha three-component compound fertilizer (45.0 kg/ha N + 45.0 kg/ha P2O5 + 45.0 kg/ha K2O) and 150.0 kg/ha urea (52.9 kg/ha N) were applied in rapeseed plots; 900.0 kg/ha tea seed cake (41.4 kg/ha N + 22.3 kg/ha P2O5 + 12.6 kg/ha K2O), 750.0 kg/ha three-component compound fertilizer (112.5 kg/ha N + 112.5 kg/ha P2O5 + 112.5 kg/ha K2O), and 225.0 kg/ha urea (103.5 kg/ha N) were applied in garlic plots. In the early and late rice season, all treatments have similar fertilizer management practices. In the early rice season, 337.5 kg/ha formula fertilizer were applied as basal fertilizer, 105 kg/ha urea (48.3 kg/ha P, 412.5 kg/ha diammonium phosphate (49.5 kg/ha P2O5), and 150.0 kg/ha KCl were applied as top-dressed fertilizer. In the late rice season, 150.0 kg/ha urea (69.0 kg/ha P), 600.0 kg/ha diammonium phosphate (72.0 kg/ha P2O5), and 150.0 kg/ha KCl were applied as basal fertilizer. 150.0 kg/ha urea (18.0 kg/ha P) were applied as top-dressed fertilizer.
Arthropod Sampling

Due to low arthropod density and slow crop growth in winter, arthropods were sampled every 2 wk from early December 2012 to mid-April 2013 and from late November 2013 to early March 2014 in the winter cover crop season; and weekly from early May 2013 to early July 2013 and from mid-April 2014 to late June 2014 in the early rice season (Fig. 2). To account for the spatial differences in the habitats of arthropods, both sweep-net sampling and point sampling methods were used. Sweep-net sampling targets arthropods on the higher part of the crops, and point sampling targets arthropods on the lower part of the crops. From four different random starting points, 15 sweeps were conducted on each plot. For each sweep, the sweep net (48 cm diameter) was swung perpendicular to a single location in a figure-eight pattern. In each plot, five randomly sampled point quadrats of 1 x 1 m were inspected and all arthropods were counted and recorded. Arthropods collected by the two sampling methods were transferred to Ziploc plastic bags or tubes containing alcohol. These Ziploc plastic bags were then stored at −20°C for further arthropod identification under a stereoscopic microscope. Most arthropod species were overlapping in these two sampling methods, and therefore, all arthropods from the two sampling methods were pooled for each plot individually to calculate the abundance and diversity of arthropods. Based on arthropod identification, only rice pests and their predators were analyzed for further statistical analysis. Voucher specimens of all sampled species were deposited at the College of Agronomy in Jiangxi Agricultural University.

The striped stemborer (Ch. suppressalis) and the rice leaf folder (Cn. medinalis) were the major pests for rice but injuring different plant parts. The striped stemborers damage rice by boring in rice stems at larval stage and the larvae of rice leaf folders injure leaves by rolling itself inside (Padmavathi et al. 2013, Shamakhi et al. 2018). In addition to the aforementioned sampling methods, we investigated the numbers of infested rice stems and leaf rolls in each 1 x 1 m sampled point as indications of infestation levels caused by
the striped stemborer and rice leaf folder, respectively. Infested rice stems were identified by the presence of larval entry holes on the stem. Leaf rolls were identified as rolled leaves which were usually infested by the larva of the rice leaf folder. The numbers of infested stems and leaf rolls at the five sampled points in each plot were calculated respectively as the infestation rates for the striped stembomer and the rice leaf folder.

**Yield Determination**
Rice grains were threshed and weighed after manual harvesting at the ground level at the full maturity period. All grains from each individual plot were separately weighed on the day of harvest. For each plot, the grain yield was calculated as weight divided by the area (kg/ha).

**Data Analysis**
To investigate the effects of winter cover crops on the composition and abundance of arthropods, community evenness and species diversity were quantified using two ecological indices, diversity (Shannon–Weaver index $H'$) and evenness (Pielou's $J$) (Wilson et al. 2017). For the fields of winter cover crop and early rice crop in each treatment, the community evenness was determined for the pest and natural enemies, respectively, by calculating Pielou’s index $J' = H' / \ln(S)$, Pielou’s $J$ was derived from the Shannon–Weaver index $H': H' = -\sum_i (P_i \times \ln(P_i))$, $P_i = N_i / N$, where $P_i$ is the proportion of individuals in the $i$th species in the dataset and $S$ means species richness. The evenness ($H'$) indicates the number of individuals in each species, and the diversity ($J$) indicates the number of species in a community. Both Pielou’s $J$ and Shannon–Weaver index $H'$ were calculated using Microsoft Excel 2013 (Microsoft Corp., Redmond, WA). For each cropping season, all indexes, infested stems, infested leaf rolls, the number of natural enemies, and grain yield between the treatments of different winter cover crops were compared using one-way ANOVA in SPSS 17.0 (SPSS Inc., Chicago, IL). The post hoc analyses were conducted using Tukey’s HSD test at alpha = 0.05 if significant differences were found.

**Results**

**Arthropod Community**
In 2012–2014, 11,943 individuals of rice pests and 12,282 individuals of natural enemies were collected. Of these rice pests, most major rice pests (proportion > 1%) were investigated, including *Nephotettix cincticeps* (Uhler) (Hemiptera: Cicadellidae) (17.45%), *Hydrellia griseola* (Fallen) (Diptera: Ephydridae) (8.11%), *Chlorops oryzae* Matsumura (Diptera: Chloropidae) (7.91%), *Sitobion avenae* (Fabricius) (Homoptera: Aphididae) (7.82%), *Sogatella furcifera* (Horvath) (Homoptera: Delphacidae) (6.10%), *Lissioraptus oryzophilus* Kuschel (Coleoptera: Curculionidae) (5.19%), *Phaedon brassicae* Baly (Coleoptera: Chrysomelidae) (3.89%), *Inazuma dorsalis* (Motschulsky) (Hemiptera: Deltocephalidae) (3.38%), *Oxya chinensis* (Thunberg) (Orthoptera: Acrididae) (2.66%), *Oulema oryzae* (Kuwamura) (Coleoptera: Chrysomelidae) (2.52%), *Dolycoris baccarum* (L.) (Heteroptera: Pentatomidae) (1.66%), and *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) (1.08%) (Fig. 3a). The number of the two main rice pests investigated in this study, the striped stemborer (*Chilo suppressalis*) and the rice leaf folder (*Cn. medinalis*), was not included, because the numbers of infested rice stems and leaf rolls were reported in this study.

Six main arthropod groups were found in collected natural enemies. Natural enemies include spiders (Araneidae, Dolomedidae, Linyphiidae, Linyphiidae, Oxyopidae, Pholcidae, Salticidae, Tetragnathidae, and Thomisidae), rove beetles (*Paederus* spp.), dragonflies (Anisoptera and Coenagrionidae), lady beetles (*Micraispis discolor* (Fabricius) (Coleoptera: Coccinellidae), *Propylaea japonica* (Thunberg) (Coleoptera: Coccinellidae), and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)), Syrphus flies (Syrphidae, Tachinidae, and Pipunculidae), and parasitoids (Ichneumonidae, Braconidae, and Chalcididae). Spiders account for the majority of the total natural enemies at 87.81%, followed by 4.50% of rove beetles, 3.15% of dragonflies, 1.84% of lady beetles, 1.33% of Syrphus flies, and 0.91% of parasitoids (Fig. 3b). Abundances of spider species differ between the winter and rice period. *Hylaphantes graminicola* (Sundevall) (Araneae: Linyphiidae) is the most abundant spider species in the winter and *Tetragnatha maxillosa* (Thorell) (Araneae: Tetragnathidae) were the most abundant spider species in the rice period.

**Effects of Winter Crops on Arthropod Diversity and Evenness**
We did not observe differences in the diversity of rice pests and natural enemies between winter cover crop treatments and the control in both winter cover crop season (2013 pest: $P = 0.76$; 2013 enemies: $P = 0.07$; 2014 pest: $P = 0.12$; 2014 enemies: $P = 0.25$) and early rice season (2013 pest: $P = 0.02$; 2013 enemies: $P = 0.30$; 2014 pest: $P = 0.60$; 2014 enemies: $P = 0.05$) in 2013 and 2014 (Fig. 4a and b). We also did not observe differences in the evenness indexes of rice pests and natural enemies between winter cover crop treatments and control treatment in both winter cover crop season (2013 pest: $P = 0.01$; 2013 enemies: $P = 0.70$; 2014 pest: $P = 0.14$; 2014 enemies: $P = 0.13$) and early rice season (2013 pest: $P = 0.51$; 2013 enemies: $P = 0.23$; 2014 pest: $P = 0.28$; 2014 enemies: $P = 0.86$) in 2013 and 2014 (Fig. 4c and d). In short, planting of the winter cover crops did not affect the diversity and evenness of the rice pests and arthropod natural enemies in winter season and early rice season in both years.

**Effects of Winter Crops on Major Rice Pests and Natural Enemies**
We used the numbers of infested rice stems and leaf rolls of rice to estimate the infestation rates of the striped stemborer and the rice leaf folder, respectively. We did not observe any significant effects among treatments on the striped stemborer (2013: $P = 0.66$; 2014: $P = 0.99$) and rice leaf folder (2013: $P = 0.31$; 2014: $P = 0.56$) in early rice in either 2013 or 2014 (Fig. 5a). The average number of infested rice stems was the highest (7.73 ± 0.47 per plot in every sampling) in the rapeseed treatment in 2013 early rice season. In 2014, the control treatment had the highest number of infested rice stems (17.71 ± 7.12 per plot in every sampling) in early rice season. The average number of leaf rolls was the highest (3.06 ± 0.50 per plot in every sampling) in milkvetch treatment in 2013 early rice season. In 2014, the control treatment had the highest number of leaf roll (4.14 ± 0.44 per plot in every sampling) in early rice season. The most abundant natural enemy species that we observed are spider species such as *H. graminicola* and *T. maxillosa*. However, we did not observe any significant difference in abundance in either *H. graminicola* (2013: $P = 0.44$; 2014: $P = 0.82$) or *T. maxillosa* (2013: $P = 0.33$; 2014: $P = 0.42$) between winter cover crop treatments and control treatment in early rice season in both years (Fig. 5b).

**Effects of Winter Cover Crops on Grain Yield**
Winter cover crops had mixed effects on grain yields (Fig. 6). In 2013, the average grain yield of early rice following a garlic cover
crop was significantly higher than the grain yield in other treatments \( (F_{3,8} = 12.15, P < 0.01) \). The average grain yields of early rice from rapeseed treatment and milkvetch treatment were not different from the control treatments. In 2014, there were no differences between the control and winter cover crop treatments although yield was significantly higher in the garlic treatment as compared to the rapeseed and milkvetch treatment \( (F_{3,8} = 9.59, P < 0.01) \).

**Discussion**

In this study, 11,943 individuals of rice pests and 12,282 individuals of natural enemies were collected. We examined whether the use of three winter cover crops, rapeseed, milkvetch, and garlic, could affect species diversity, community evenness, and population densities of rice pests and related natural enemies in a rice rotation system, as well as the grain yields. In field experiments conducted from 2012 to 2014, we did not observe any effects of the infestation rates of two major rice pests, the striped stemborer and the rice leaf folder, and we also did not observe any effects of abundance in two major natural enemies, *H. graminicola* and *T. maxillosa*. This suggests that the three winter cover crops have no effect on the diversity and evenness of major rice pests and their arthropod natural enemies. However, all winter cover crop treatments did not show negative effects in grain yield of the early rice.

Cover crops have been shown to have mixed effects on natural enemy abundance and diversity in different crop systems (Woolwine and Reagan 2001, Fox et al. 2016, Damien et al. 2017, Gurr et al. 2017). While these studies mostly focused on the effects of cover crops on crops planted in the same season, effects of winter cover crops on arthropods in a rotational cropping system in the following season are less studied. Therefore, to explore how winter cover crops affect pest density and/or natural enemy abundance in the following crop season, we investigated the number and species of arthropods in winter cover crops in a rice rotation system for 2 yr. However, in our experiments, we did not observe any direct effects of winter cover crops on either the diversity and evenness of their arthropod natural enemies, or infestations of major rice pests. The effects of cover crops on arthropod diversity and population can be influenced by many factors, and results vary among systems (Landis et al. 2000). For example, in maize rotation cropping, winter cropped slender wheat grass increased the abundance of generalist predators and their predation rates and reduced the number of maize pests and the damage inflicted by these pests (Lundgren and Fergen 2010, 2011). However, using the cereal rye/ Austrian winter pea as winter cover crops showed neutral effects on the predators and pests in maize (Fox et al. 2016). In China, flooding of the plots before rice transplanting is usually done (Xiao et al. 2002). In our study, after the cover crops were ploughed, the plots were flooded before the rice was transplanted. The combination of ploughing and flooding...
likely causes a major disturbance that reduced the opportunity for arthropods carrying over into the summer crop. Indeed, these practices could decrease the positive effects on shelter provided by the winter cover crop for arthropods (Lawani 1982), not only pests but also natural enemies.

Winter cover crops had mixed effects on the rice grain yield in our experiments. We showed garlic had a positive effect on grain yield. One reason could be that garlic bulb extracts could increase bacterial counts, polyphenol oxidase, catalase, saccharase, and urease activities, and decrease fungal counts, which suggesting that using garlic may be effective in reducing some obstacles and increasing plant growth of continuous cropping as a preceding crop (Dong et al. 2012). In a watermelon rotation system, garlic has also been shown to decrease disease and increase available nutrients and

Fig. 4. Effects of winter cover crops on arthropod diversity and evenness. (a) The means of pest diversity in different crop periods in 2 yr; (b) the means of natural enemy diversity in different crop periods in 2 yr; (c) the means of pest evenness in different crop periods in 2 yr; (d) the means of natural enemy evenness in different crop periods in 2 yr. Winter: winter cover crop season, and early rice: early rice season. Bars represent means, and error bars represent standard error. Within each season, means followed by the same letter do not differ significantly at alpha = 0.05.

Fig. 5. Effects of winter cover crops on (a) major rice pests, *Chilo suppressalis* and *Cnaphalocrocis medinalis*; and (b) natural enemies, *Hylyphantes graminicola* and *Tetragnatha maxillosa*. In panel (a), the bars represent the means of infested rice stems of *Ch. suppressalis* and leaf rolls of *Cn. Medinalis* per plot. In panel (b), the bars represent the means of the number of *H. graminicola* and *T. maxillosa* per plot. Error bars represent standard error. For each year, the means labeled with same letter do not differ significantly at alpha = 0.05.

Fig. 6. Effects of winter cover crops on grain yields in early rice season. Bars represent means of yields, and error bars represent the standard error. Means followed by the same letter do not differ significantly at alpha = 0.05.
soil organic matter contents, which leads to higher watermelon yield (Yang et al. 2016). Another reason is that higher amount of fertilizer supplements may be involved in higher grain yield in garlic treatment. In our study, since different cover crops serve to different purposes, we followed local practices of fertilizer management for different crops, instead of uniform fertilizer composition. For example, garlic and rapeseed are widely planted as economic crops, while milkvetch is used as a green manure crop which requires less fertilizers. Different strategies can maximize the economic outcomes of these crops. Despite a potential difference in the cost from different managements, the sales of garlic and rapeseed could compensate the expenses in higher amount of fertilizers used, or even generate more economic benefits. Rapeseed and milkvetch had no effect on grain yield. This is consistent with a previous study showing rapeseed and milkvetch have no effect on rice yield (Yu et al. 2014). However, other studies have showed positive effects of rapeseed and milkvetch on rice yield (Fan et al. 2009, Yang et al. 2013). These different effects on grain yield may be also influenced by agricultural management, such as fertilizers, crop residues tillage, fire, and flooding (Whitbread et al. 2003, Zong et al. 2007, Schipanski et al. 2014).

In conclusion, three winter cover crops tested in this study have no effect on the population densities of rice pests and natural enemies, and they did not have any negative effects on rice grain yields in following rice period in a rotation system. This result is very important for evaluating the ecological effects of winter cover crops on arthropod community and insect pest management in a rotation system. In addition, considering other ecosystem services provided by winter cover crops, for example, improved soil quality (Ryder and Fares 2008, Yu et al. 2014) and/or extra economic benefits, the use of winter cover crops in the rice rotation system could be an important agricultural practice. We suggest that more crop species be tested in winter, as more detailed investigations are needed for better understanding of the biocontrol and ecosystem services of winter cover crops.

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