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Strip intercropping peanut with maize for peanut aphid biological control and yield enhancement

Qian Ju[a,b,1], Fang Ouyang[a,1], Shimin Gu[a], Fei Qiao[a], Quanfeng Yang[a], Mingjing Qu[b], Feng Ge[a,*]

[a] State Key Laboratory of Integrated Management of Pest and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China
[b] Shandong Peanut Research Institute, Qingdao, 266100, China

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ABSTRACT

Increasing crop biodiversity, such as by strip intercropping, is recognized as an effective biological control measure. However, few studies have focused on the process of strip intercropping to increase natural enemy abundance, reduce pests and subsequently reduce crop damage. In the context of organic peanut (*Arachis hypogaea* L.) production, maize (*Zea mays* L.) intercropping with peanut was proposed to provide habitat for predators that suppress peanut aphid to enhance pest control in peanut. To test this hypothesis, we compared predator communities across monoculture and intercropping systems, investigated shifts of predators in response to strip intercropping systems, and evaluated their prey consumption via PCR-based gut content analysis under realistic field conditions. Last, we assessed the biological control of peanut/maize strip intercropping in peanut production. Our results demonstrated that intercropping significantly increased ladybeetle density and significantly reduced the number of peanut aphids. In the peanut/maize strip intercropping plots, peanut aphid density was significantly related to ladybeetle density. Moreover, in the intercropping plots, more than 90% of the predators prefer to inhabit on maize, and less than 10% of the predators inhabit on peanut. Further molecular gut-content analysis revealed that the ladybeetles inhabited on maize exhibited significantly higher predation on peanut aphids in the intercropping system. Visualization of the food chains indicated that peanut aphid population in intercropping system was effectively suppressed by predator abundances in the early season. In the yield experiments, compared with the monoculture systems, the strip intercropping system presented significantly higher peanut production. Linear regression analysis revealed that peanut aphid significantly reduced the pod maturity index and peanut yield. Our results indicated that peanut/maize strip intercropping could enhance the predator number, suppress pest and reduce peanut loss. This research incorporating field studies and molecular tools demonstrates the successful conservation and biological control of peanut aphids.

1. Introduction

Crop biodiversity in farmlands has rapidly declined as a result of intensified agricultural production (Rayl et al., 2018; Tomasetto et al., 2017; Tschumi et al., 2018). A decline in biodiversity reduces both the abundance of natural enemies and suppressive effects on pests (Gurr et al., 2017; Jacobsen et al., 2019; Muneret et al., 2018; Shapira et al., 2018). Letourneau et al. (2011) used a meta-analysis of 552 experiments in 45 articles published over 10 years and concluded that enemy enhancement, herbivore suppression, and crop damage reduction were significantly stronger on diversified crops than on crops with no or few associated plant species. Supporting natural enemies with shelter, nectar, alternative prey/hosts, and pollen (SNAP) has emerged as a major research topic (Gurr et al., 2017; Liu et al., 2018; Skidmore et al., 2017). Specifically, strip intercropping is an effective strategy to increase crop biodiversity (Brennan, 2013; Brooker et al., 2015; Hong et al., 2017). Intercropping is an important farming practice involving two or more crop species that are grown together and coexist on the same piece of land at the same time (Brooker et al., 2015; Liu et al., 2018). With respect to strip intercropping, maize (*Zea mays* L.) is a frequently studied crop because of its biological control services. Our research group has demonstrated that maize can provide benefits to predators to potentially enhance the biological control of insect pests in cotton (Ouyang et al., 2012), which implies that maize may provide habitat shelter for natural enemies to suppress pests on other crop species. However, the effects of maize on the biological pest control of other crop species are still unknown.

Peanut (*Arachis hypogaea* L.) and maize are the main agricultural

* Corresponding author at: Institute of Zoology, Chinese Academy of Sciences, Beichenxilu 1-5, Chaoyang District, Beijing, 100101, China.
E-mail address: gef@ioz.ac.cn (F. Ge).
[1] These two authors contributed equally to this work.

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crop species in northern China. Peanut is one of the most economically important legumes. Peanut seeds contain a rich source of edible protein and represent the major oilseed crop, accounting for 30% of the total crop production in China (Xiong et al., 2013). The widespread adoption of monoculture peanut has ultimately led to adverse ecological consequences for peanut production, as peanut yields suffer great loss due to pest damage (Awal et al., 2006; Xiong et al., 2013). Subsequently, the use of pesticides has increased, which is not conducive to a healthy environment. In the context of organic peanut production, it is necessary to develop effective biodiversity control approaches (Röös et al., 2018). The peanut aphid (*Aphis craccivora* Koch) is one of the most challenging pests in organic peanut production. It attacks many plant species—most often leguminous crops—and acts as a vector of numerous viral diseases (Angelella et al., 2016; Rayl et al., 2018). Data from China's Ministry of Agriculture show that the peanut aphid occurrence area in China covers an average of 1797.24 million ha $year^{-1}$ and that peanut losses due to peanut aphids amounted to an average of 28,735.81 million tons $year^{-1}$ from 2000 to 2014 (see Appendix S1 in Supplementary material). Strip intercropping peanut with maize is clearly an effective agronomic mechanism; it is a widespread practice in northern China because maize can improve the Fe nutrition of peanut by rhizospheric interactions (Awal et al., 2006; Li et al., 2018; Zuo and Zhang, 2009). There have been many studies on plant nutrition (Li et al., 2018; Zhang and Li, 2003), but none have focused on the biological control services of crop biodiversity.

Biological control services provide an effective strategy for pest suppression and natural enemy promotion, and the trophic structure of natural enemy or food webs in agri-ecosystem become the key issue for biological control (Kamenova et al., 2018; Perović et al., 2018; Schmidt et al., 2014; Yang et al., 2017). Understanding trophic structures requires research on natural enemy communities and their impacts on pest population dynamics. Molecular ecology techniques can provide an evaluation process that extends beyond the assessment of abundance and diversity and can provide a specific mechanism to assess the interactions of natural enemies and pests (González-Chang et al., 2016). Furthermore, molecular gut content analysis can help determine the trophic structure of natural enemy food webs and can be used to improve the management of main pests by measuring the interaction between predator and prey (Hrček and Godfray, 2015; Kamenova et al., 2018; Peterson et al., 2018; Schmidt et al., 2014; Wang et al., 2017). For instance, using molecular gut analysis, Chen et al. (2000) identified key cereal aphid predators, and by measuring the predator-prey trophic relationships in organic cucurbit production, Schmidt et al. (2014) identified a suite of potentially effective biological control agents. In this study, in the field in 2016, we found the largest proportion of predators to be members of the Coccinellidae family (53.11%), with a total of 1207 predators screened for predation on peanut aphids (Appendix S2 in Supplementary material). Therefore, we used molecular gut content analysis to measure peanut aphid and coccinellid predation by *Harmonia axyridis* and *Propylea japonica* (Coleoptera: Coccinellidae) and to obtain evidence of peanut aphid predation directly from the field.

In this study, we assessed both the predator-prey trophic structure in peanut/maize strip intercropping systems and the frequency of prey consumption for the biological control of peanut aphid. Specifically, we aimed to (1) investigate population numbers of aphids and predators, and the shifts in predators in response to strip intercropping systems; (2) evaluate prey consumption in response to intercropping systems via a PCR-based gut content analysis under realistic field conditions and confirm the dominant predator of peanut aphids during the growth period; and (3) confirm the biological control services of strip intercropping on the basis of peanut yield. We hypothesize that the maize element of the strip intercropping can serve as a habitat for natural enemies, suppress peanut aphids and increase peanut yields.

2. Materials and methods

2.1. Feeding and sampling of predators and prey

The experimental fields were located at the research station of the Shandong Peanut Research Institute (N 36.809, E 120.498), Qingdao City, Shandong Province, China. The average temperature was 28.7 °C during the growing season (May–September). The fields were managed according to the management practices of local farmers, with no crop rotation. No pesticides were applied during the growing season. Prey and predators used for DNA decay analysis were collected from the peanut field, and colonies were established in the laboratory on *Vicia faba* L. plants. The insects were reared under controlled conditions of 25 ± 2 °C and a 16:8 light: darkness cycle. A Malaise trap was used in the same field in 2016 to estimate the abundance and diversity of predators on peanut aphids on the basis of taxonomic criteria, feeding behavior and trophic morphology, and all arthropods were frozen at −20 °C for primer screening (see Appendices S2 and S4 in Supplementary material).

2.2. Field sampling: experimental design and investigation

Both peanut (Huayue 36 breed) and maize (Zhengdan 958 breed) were planted in May 2017 in 120 m × 20 m fields. The fields were divided into 12 cells: each cell size was 15 m × 5 m, and the distance between cells was 5 m. Three experimental treatments were applied to each cell: (a) peanut monoculture, (b) peanut/maize intercropping (planted in a 3:2 planting pattern, or 3 rows of peanut and 2 rows of maize), and (c) maize monoculture. There were four replications of each treatment (five plots, with ten plants per plot). The pest and predator populations were measured throughout the growing season from early May to the end of August every ten days to coincide with the reproductive generations of peanut aphids. The date of each survey, field cell number, row number and plant number was recorded, and the number of peanut aphids and ladybeetles on each surveyed date was recorded to provide an estimate of their abundance and diversity.

2.3. Field sampling: molecular gut content analysis for estimating predation

Approximately 20 ladybeetles were collected by hand every ten days in the cells of each field after experimental investigations. The sample size for each treatment and period depended on the availability of predators in the field. The sampled ladybeetles were immediately placed individually in 95% EtOH in a 2 ml microcentrifuge tube on ice to avoid regurgitation and then stored at −20 °C until DNA extraction.

2.4. DNA extraction, PCR and primer design

DNA was extracted using a QIAGEN DNeasy Blood and Tissue Kit (QIAGEN Inc., Chatsworth, CA, USA) according to the manufacturer's animal tissue protocol. DNA integrity was evaluated by PCR amplification, with the reference primers of ladybeetle (Appendix S3 in Supplementary material) designed according to the *H. axyridis* 18S sequence obtained from the GenBank database (GenBank Accession No. GU073689.1). The final volume of 50 μL consisted of 25 μL of Premix Taq (Ex Taq Version 2.0 plus dye), 1 μL of template DNA, 1 μL of each primer and 22 μL of ddH_2O. The PCR cycling conditions were 30 cycles of 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s, with a final 72 °C 10 min extension period.

Cytochrome *c* oxidase subunit I (COI) primers targeting peanut aphid were designed to test the predation of natural enemies on peanut aphids. To obtain sequences for primer design, all available peanut aphid COI sequences (18 sequences, including GenBank Accession No. EF591594.1, FJ965670.1-FJ965670.1, HM062843.1, HQ528252.1, JX559638.1, KC897556.1, KC897559.1, KF362037.1, KJ803177.1, KJ803177.1, KJ803181.1, KJ803182.1, KJ814962.1, KJ814963.1,

KY323016.1, and KY846650.1) in GenBank were downloaded and aligned. According to the sequences above, HS-F1: 5′-GGAATAATTGG ATCTTCACTTAGTATT-3′ and HS-R1: 5′-AAGGTAGTTCTGAATATGAA TGTTCTA-3′ were designed to obtain a sequence for primer design. Peanut aphid DNA was used as a template, and PCR was conducted in conjunction with HS-F1 and HS-R1, after which sequencing was performed. According to the sequencing results, seven pairs of primers were designed via the Primer 3 website (see Appendix S3 in Supplementary material). The primers were screened for cross-reactivity against other nontarget arthropod, mollusk and nematode species (88 nontarget taxa, replicates and treatments for a total of 212) occurring in the field and collected by Malaise traps and by hand (see Appendix S4 in Supplementary material). Extraction, PCR and sequencing of field-collected predators or predators used in the feeding experiments followed the protocols described above. Peanut aphid DNA extractions were used as a positive control to identify peanut DNA in predator guts, whereas ddH_2O was used as a negative control. After preliminary experiments, the primer pair AC_F2: 5′-TTGTTACAATTCA TGCTTTCATTAT-3′ and AC_R2: 5′-TGAAATACCTGCTAAATGAAGAG-3′ was selected and produced a 299 bp amplicon. The primers exhibited specificity for peanut aphids when screened for cross-reactivity against 212 nontarget DNA extractions (see Appendix S4 in Supplementary material).

2.5. DNA detectability feeding experiments and molecular gut content analysis for estimating DNA decay rates

The methods used were modified from articles published in 2014 (Greenstone et al., 2014; Schmidt et al., 2014). Prior to the feeding experiment, ladybeetles were maintained in the laboratory without exposure to peanut aphid prey for a minimum of two weeks, and the ladybeetles fed on the pea aphid *Acyrthosiphon pisum*. After the 2-week feeding period, the ladybeetles were transferred to new individual plastic Petri dishes (3.5 cm diameter), which were kept moist, and starved for 24 h. After the starvation period, all the beetles were transferred to a new Petri dish and provided with one fresh 1 st or 2nd instar peanut aphid. Predators were observed feeding, and at the end of the feeding period (t < 2 h), when the predators discarded the peanut aphid or nothing remained, time zero was recorded for all predators (t = 0 h). The freeze times were 0, 1, 2, 4, 6, 8, 16, and 20 h after feeding for *P. japonica* and 0, 2, 4, 8, 12, 16, and 24 h for *H. axyridis*. At each freeze time following feeding, the predators were transferred individually to sterilized 2.0 ml microcentrifuge tubes containing −20 °C prechilled 95% EtOH. The sample size for each treatment and period depended on the success of the experiment, and more than 15 ladybeetles were recorded at each freeze time. The time periods were selected for predators on the basis of previous studies. All samples were stored at −20 °C for DNA extraction to estimate DNA decay rates.

2.6. Pod maturity index (PMI) and peanut yield

Peanut and maize plants in each plot were harvested, and a total area of 15 × 5 m^2 was harvested from each treatment when the peanut and maize plants were mature. The peanut pods were removed from the plants and air dried to approximately 8% moisture content, after which the pod dry weight was determined. The PMI was calculated as the number of mature pods divided by the number of pegs per plot (ten plants) for each treatment.

2.7. Statistical analysis

All descriptive statistics (means and standard errors) and tests of differences were conducted in R version 3.2.0 and theSPSS software version 21.0 package (IBM, Armonk, NY, USA). GraphPad 7.0 (GraphPad Software Inc., La Jolla, CA) was used to construct the graphs.

To determine the effects of the three cropping patterns (peanut monoculture, peanut/maize intercropping and maize monoculture) on the densities of peanut aphid, four steps were taken. First, differences in the densities of peanut aphid on peanut patches between the peanut monoculture and peanut/maize intercropping systems were analyzed with ANOVA, and the effects of sampling date (Date), cropping pattern (Pattern: peanut monoculture and peanut/maize intercropping) and their interaction (Date × Pattern) on the densities of peanut aphid were tested with repeated measures and the general lineal model. Second, differences in the densities of peanut aphid on peanut patches between the peanut monoculture and peanut/maize intercropping systems were analyzed on the sampling dates with a *t*-test. Third, the differences in the densities of ladybeetles (*H. axyridis* and *P. japonica*) on treatment plots between the peanut monoculture, peanut/maize intercropping and maize monoculture systems were analyzed with ANOVA, and the effects of sampling date (Date), cropping pattern (Pattern: peanut monoculture and peanut/maize intercropping) and their interaction on the densities of ladybeetles were tested with repeated measures and the general lineal model. Fourth, differences in the densities of ladybeetles (*H. axyridis* and *P. japonica*) on treatment plots between the peanut monoculture, peanut/maize intercropping and maize monoculture systems were analyzed on the sampling dates with a *t*-test. The numbers of peanut aphids were converted to numbers per 100 plants and then $\log_{10}(x + 1)$ transformed. With respect to ladybeetle density, we summed the numbers of adults and larvae within each time period (Yang et al., 2018), and the numbers of ladybeetles were converted to densities per square meter of the area sampled.

Using exponential regression analysis, we determined the DNA detectability half-life of the proportion positive for peanut aphids. The detectability half-lives were compared between species using the recommended 95% fiducial confidence limits. To analyze and display the molecular gut content data, we adjusted the raw proportions by weighting the proportion of predators positive for peanut aphid by the DNA detectability; those predators that had relatively long detection periods were assigned lower weights than those that had relatively short detection periods (Chen et al., 2000). Gut content positives were weighted by the number of predators tested multiplied by the DNA decay adjustment. Linear regression analysis was also performed between the variables. We used the R package 'plotweb-bipartite' to reflect the trophic linkage and quantitative relationships from prey (peanut aphid) to predator (*H. axyridis* and *P. japonica*) (Schmidt et al., 2014).

3. Results

3.1. Population dynamics of aphids and predators

Peanut aphid density throughout the whole growth period (from 31-May to 19-August) significantly differed between treatments ($F_{2,\ 378} = 72.1$, $P < 0.0001$, Fig. 1a). *A. craccivora* density increased over time in the peanut monoculture and intercropping systems before 10-Jul ($F_{8,\ 315} = 92.33$, $P < 0.0001$), and intercropping significantly lowered aphid densities ($F_{1,\ 315} = 35.21$, $P < 0.0001$). Aphid densities in the intercropping system were lower than those in the monoculture peanut system on three dates: 10-Jun ($t = 4.115$, $df = 38$, $P = 0.0002$), 30-Jun ($t = 4.535$, $df = 38$, $P < 0.0001$) and 10-Jul ($t = 4.082$, $df = 38$, $P = 0.0002$) (Fig. 1a). The peak occurrence period of peanut aphid during three sampling date was coincided with the seedling stage and the flower-pegging stage of peanut.

Compared with the peanut monoculture system, the maize monoculture and intercropping systems significantly increased *P. japonica* density ($F_{2,\ 513} = 22.67$, $P < 0.0001$). The *P. japonica* density increased over time in the intercropping and maize monoculture systems before 20-July ($F_{8,\ 513} = 22.92$, $P < 0.0001$, Fig. 1b). Few *P. japonica* insects were found in the peanut monoculture before 20-July. The abundant of *P. japonica* in both the maize monoculture and intercropping systems were significantly more than that in the peanut

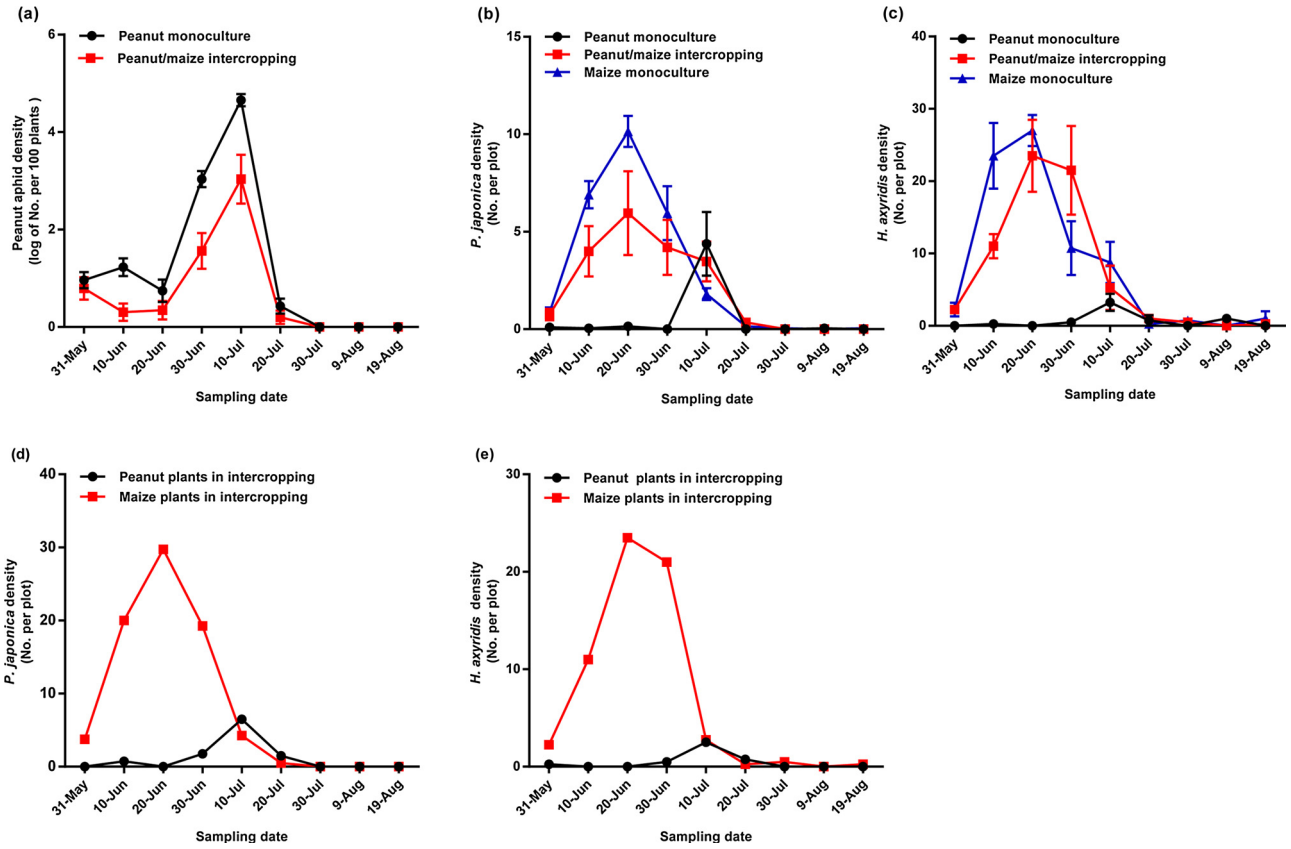

Fig. 1. Temporal changes in the abundance patterns of peanut aphids and predators: (a) densities of peanut aphids on peanut plants in peanut monoculture and peanut/maize intercropping systems; (b) *P. japonica* and (c) *H. axyridis* densities among peanut monoculture, peanut/maize intercropping and maize monoculture systems; and (d) *P. japonica* and (e) *H. axyridis* densities on peanut and maize plants in the peanut/maize intercropping system.

monoculture system before 30-June ($t = 3.055$, $df = 6$, $P < 0.05$ and $t = 3.365$, $df = 6$, $P < 0.05$, respectively), while no significant difference between treatments after 30-Jun ($F_{2,\ 285} = 1.186$, $P = 0.3070$). *H. axyridis* density increased over time in the maize monoculture and intercropping systems before 20-Jul ($F_{8,\ 513} = 13.91$, $P < 0.0001$, Fig. 1c). Few *H. axyridis* were found in the peanut monoculture before 20-July. Compared with the peanut monoculture system, the maize monoculture and intercropping systems had significant positive effects on *H. axyridis* density ($F_{2,\ 513} = 21.56$, $P < 0.0001$). The numbers of *H. axyridis* in both the intercropping and maize monoculture systems were significantly more abundant than that in the peanut monoculture system before 30-June ($F_{1,\ 152} = 129.8$, $P < 0.0001$; $F_{1,\ 152} = 23.23$, $P < 0.0001$), while no significant difference between treatments after 30-June ($F_{2,\ 285} = 1.723$, $P = 0.1804$). Moreover, there were no significant differences between the maize monoculture system and the intercropping system ($F_{1,\ 342} = 0.02281$, $P = 0.8800$).

More than 90% of the predators occurred on the maize in the intercropping system (Fig. 1d and e). In addition, the dates of peak abundances for *P. japonica* (20 June, Fig. 1b) and *H. axyridis* (20 June, Fig. 1c) in the intercropping system occurred prior to that of peanut aphid in the monoculture peanut system (10 July, Fig. 1a). The normal distribution test was conducted to estimate the relationship between the population densities of ladybeetle and peanut aphid, and the test showed that all data presented continuity variance and followed a normal distribution (the same below). A linear relationship was found between the population density of ladybeetle (X) on 30-June and peanut aphid (Y) on 10-July in the peanut monoculture and intercropping ($Y = -0.08851X + 4.567$). The number of peanut aphids decreased with the increase in ladybeetles, and peanut aphids were significantly negatively affected by the ladybeetle population (Fig. 2, $F_{1,\ 62} = 5.678$, $P < 0.05$, $R^2 = 0.0839$).

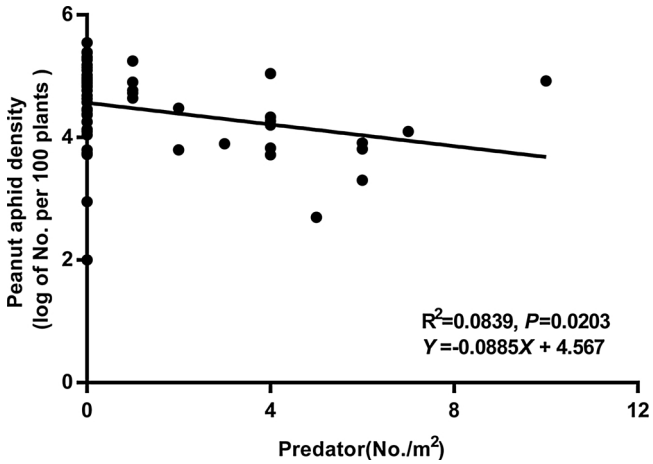

Fig. 2. A linear regression was completed to determine the linear relationship between predator and pest densities.

3.2. Direct evidence of predation on peanut aphid under realistic field conditions on the basis PCR analysis

Analysis of the feeding trial specimens generated a predicted curve ($R^2 = 0.9337$) with a DNA detectability half-life of 4.355 h for *P. japonica* and 3.284 h for *H. axyridis* (Table 1 and see Appendix S5 in Supplementary material). The estimated DNA detectability half-life values were used to calculate predator importance weighting values (PIWVs) (Table 1), which were used in subsequent analyses to adjust raw field predation results by weighting the proportion positive by the DNA decay half-life. The PIWVs of *H. axyridis* and *P. japonica* were 1.00

Table 1
Results of DNA detectability analysis of peanut aphid PCR to detect DNA consumed by predators held under laboratory conditions.

Predator group	n	DNA detectability half-life (hours)	95% fiducial confidence limits	Predator importance weighting value
H. axyridis	137	3.284	1.702–11.11	1.00
P. japonica	169	4.355	1.995–20.11	0.75

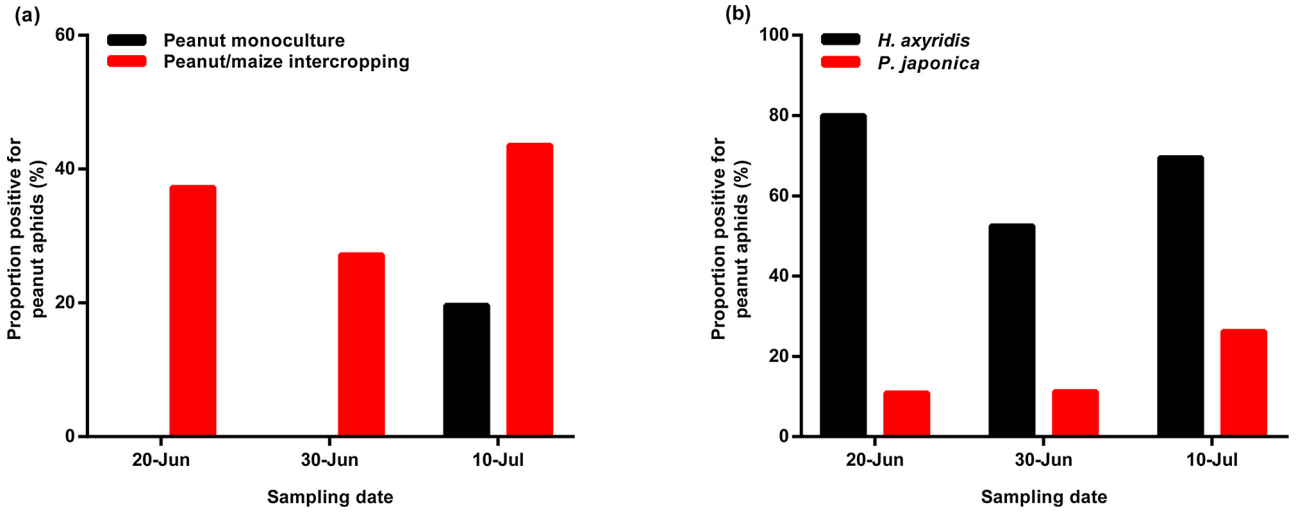

Fig. 3. Temporal changes in the frequency of the detection of peanut aphid adjusted proportion positive for peanut aphid DNA in predator gut contents in relation to (a) the peanut monoculture or intercropping system (Note: proportion positive for peanut aphids (PPPA) = ($PPPA_{P.\ japonica} \times 0.75 + PPPA_{H.\ axyridis} \times 1.00$)/2) and (b) the intercropping system to represent predation patterns in relation to predator groups and time.

and 0.75, respectively.

To evaluate peanut aphid predation under realistic field conditions, the data were analyzed on three separate dates: at the onset of the population growth of peanut aphids (20-June and 30-June) and during the peak period of peanut aphid abundance (10-July). The results showed that peanut aphid predations were significantly influenced by experimental treatment (peanut monoculture and intercropping system), as reflected by the sampling dates ($F_{1,\ 2} = 53.70$, $P < 0.05$, Fig. 3a). The predations of the two ladybeetle predators were significantly different in the experimental fields in three dates ($F_{1,\ 18} = 25.00$, $P < 0.0001$, Fig. 3b). The proportion of predators positive for peanut aphid DNA adjusted by DNA decay varied from 0 to 19.64% in the peanut monoculture system and from 27.19 to 43.57% in the intercropping system. Predation by *H. axyridis* was significantly greater than that by *P. japonica* on peanut aphids at all sampling dates ($t = 5.067$, $df = 22$, $P < 0.0001$).

Visualization of the food chains was plotted using bipartite food web diagrams, which represented the trophic linkage at each of the sampling dates under field conditions (Fig. 4). The levels of peanut aphid predation in the intercropping system were greater than that in the peanut monoculture system at all the sampling dates (Fig. 3a), and the ladybeetles consumed peanut aphids were detected in the intercropping system during the early season (20-June and 30-June) (Figs. 3 and 4) when peanut aphid abundances were low (Fig. 1a). No peanut aphid predation was observed in the peanut monoculture in the early season (Figs. 3 and 4).

3.3. Peanut yield enhancement via peanut aphid predation by ladybeetles

Significant variation in peanut yield was observed across the different treatments (peanut monoculture system and intercropping system). The PMI was significantly greater in the intercropping system (with an average of 0.56%) than in the peanut monoculture system (with an average of 0.75%) (Fig. 5a, $t = 2.712$, $df = 14$, $P < 0.05$). On

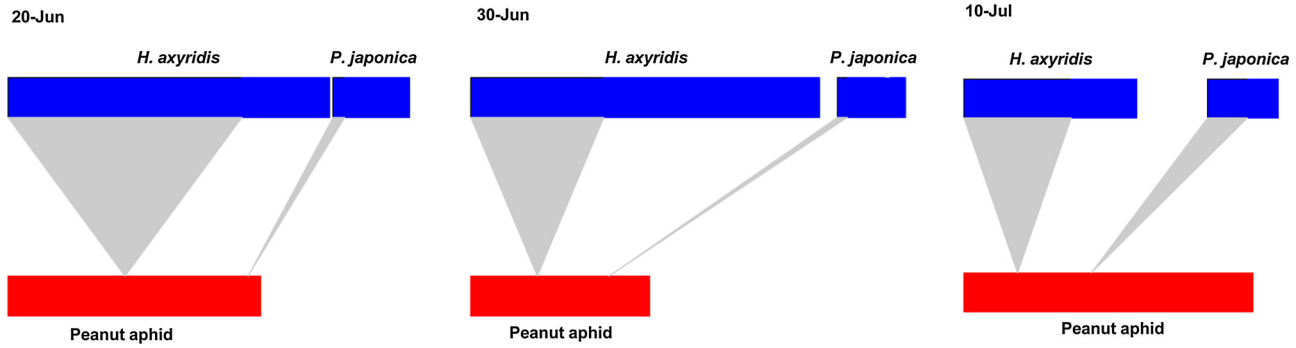

Fig. 4. Visualization of food chains structure representing the trophic linkage at each of the sampling dates under field conditions. The width of each predator group box (blue shaded upper boxes) and peanut aphid box (red shaded lower box) represent the predator and prey abundance, respectively. The lines connecting predators to peanut aphids represent the peanut aphid predation in the diets of the predator populations in realistic field conditions, as determined by molecular gut-content analysis and adjusted by predator importance weighting values (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

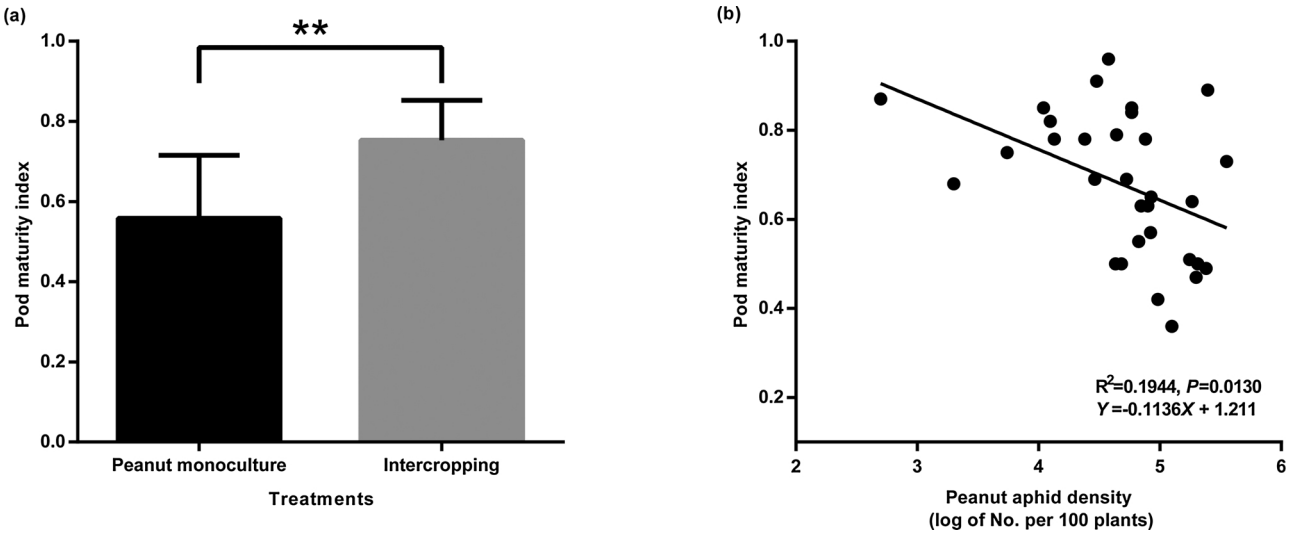

Fig. 5. (a) Comparison of the pod maturity index in the peanut monoculture system and intercropping system and (b) linear regression analyses of pest density and the pod maturity index. The asterisks indicate statistically significant differences at the 0.05 test level (*t*-test).

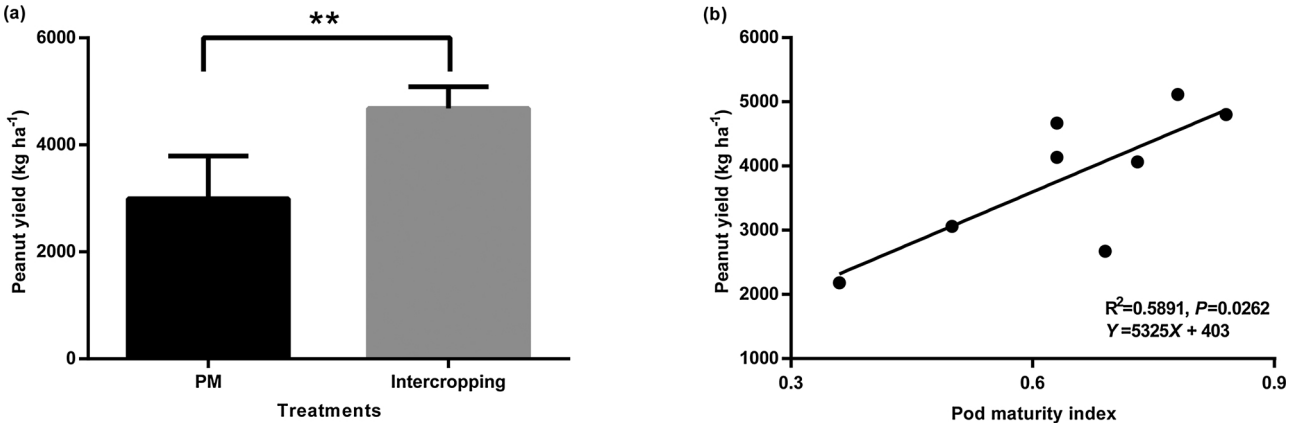

Fig. 6. (a) Comparison of peanut yields in the peanut monoculture system and intercropping system and (b) linear regression analyses of the pod maturity index and yield. The asterisks indicate statistically significant differences at the 0.001 test level (*t*-test).

the basis of this phenomenon, a linear regression analysis was performed to confirm the effects of peanut aphid density on the PMI. A linear relationship was found between peanut aphid density (X) and the PMI (Y) ($Y = -0.1136 \times X + 1.211$). The PMI decreased with increasing aphid density, indicating that the PMI was significantly negatively affected by aphid density (Fig. 5b, $F_{1,\ 29} = 6.997$, $P < 0.05$, $R^2 = 0.1944$). Compared with the peanut monoculture system (with an average of 2994.84 kg ha^{-1}), the intercropping system (with an average of 4680.12 kg ha^{-1}) presented a significantly greater peanut yield (Fig. 6a, $t = 3.757$, $df = 6$, $P < 0.05$). The linear regression analysis revealed that peanut yield increased with increasing PMI values ($Y = 5325 \times X + 403$), indicating that peanut yield was significantly positively affected by the PMI (Fig. 6b, $F_{1,\ 6} = 8.601$, $P < 0.05$, $R^2 = 0.5891$).

4. Discussion

Predator abundance and community structure can affect the suppression of low trophic levels; however, there are relatively few studies on these specific processes under field conditions (Ali et al., 2018; Lundgren and Fergen, 2014). Our results showed that strip intercropping peanut with maize contributed to increased ladybeetle abundance, decreased peanut aphid and, consequently, reduced peanut losses. On the basis of PCR gut content analyses, our studies have also provided direct evidence of peanut aphid predation by *H. axyridis* and *P. japonica* under realistic field conditions. Research incorporating molecular tools and field studies can therefore provides useful information for the successful conservation and biological control management of peanut aphids.

Increasing crop biodiversity, such as by strip intercropping, can promote biological pest control in agroecosystems (Liu et al., 2018; Roubinet et al., 2017). However, strip intercropping has agronomic constraints (Gurr et al., 2017). Optimizing strip intercropping systems requires a design that is based on local soil and climate conditions, crop combinations, sowing date, strip width, row spacing and cultivar selection to maximize the synergistic effects (Feike et al., 2012). There are many different peanut-maize strip intercropping patterns in use on the North China Plain. From an ecological perspective, peanut and maize can be planted in a 3:2 planting pattern, which was used in our study. Strip intercropping maximizes resource capture, yields and economic profits in the existing patterns on the North China Plain (unpublished results). In our study, compared with the monoculture systems, the intercropping system significantly increased the density of the most important natural enemies of peanut aphids before 30-June. More than 90% of the predators occurred on maize in the intercropping system (Fig. 1e and d). Further study via gut content molecular analysis showed that, compared with those in the monoculture systems, the ladybeetles sampled from maize in the intercropping system exhibited

significantly greater predation on peanut aphids. Therefore, a predator shift from maize to peanut in response to strip intercropping was confirmed. In addition, the function of peanut/maize intercropping for the biological control of peanut aphids was confirmed. In our study, the period of high abundance of predators was consistent with the small trumpet stage, the large trumpet stage, the tassel stage, the flowering powder stage and the silky stage of maize. The increased number of ladybeetles may be related to the habitat and feeding behavior of the natural enemies, and the maize in this planting pattern can provide natural enemies with food such as nectar and pollen and can provide shelter, such as a moderated microclimate that enhances natural enemy survival (Landis et al., 2000; Peterson et al., 2018; Root, 1973; Yang et al., 2018).

In very high-pest-pressure systems, management is needed to boost predator abundance relatively early during the season, which is a critical time because the ratios between predator and pest are highest and because the pest population is most likely to be suppressed by predators (Athey et al., 2016; González-Chang et al., 2016; Layman and Lundgren, 2015; Liu et al., 2018; Toju and Baba, 2018). We found that periods with high predator abundance matched the early season of peanut aphid occurrence with strip intercropping. Roubinet et al. determined the key period for aphid biological control and asserted that if appropriate farm system strategies are implemented during periods when pest abundance is low, then a positive response in terms of an increase in predator abundance would likely result in improved biological control (Roubinet et al., 2017; Verschut et al., 2018). In addition, in our study the trophic linkage of ladybeetles and peanut aphids was determined by molecular gut content analysis to evaluate predator-prey abundances and consumption throughout the growing season. The field observation results indicated that ladybeetles occurred prior to the onset of rapid population growth of peanut aphids in the intercropping system, and the ladybeetles consumed peanut aphids in the intercropping system were detected in early season when abundances of peanut aphids were low. Maize can provide shelter or food for predators such as thrip insect pests, water droplets and an appropriate maize leaf microclimate. Our previous studies showed that the temperature of the semienclosed space comprising maize leaves was lower than that comprising cotton leaves, and the humidity was higher in the space comprising maize leaves than in that comprising cotton leaves (unpublished data). The shape of peanut leaves is the same as that of cotton leaves. The microclimate shelter composed of maize leaves could be considered better than that of peanut leaves. Maize can support natural enemies via shelter, nectar, alternative prey/hosts, and pollen (SNAP) and can provide benefits to predators to potentially enhance the biological control of insect pests in peanut. The SNAP components may be the reason for the relatively high density of predators on maize, and experiments such as those involving food traceability by metabarcoding or temperature and humidity testing have been conducted to determine the key factors involved. Therefore, maize in the intercropping system significantly increased the abundance of predators, and the peanut aphid population in the intercropping system was effectively suppressed during the early season.

Although it is generally recognized that increased predator abundance can subsequently reduce crop damage by reducing pest populations, this concept has rarely been demonstrated (Cahenzli et al., 2017; Shapira et al., 2018). In addition, aside from pest suppression, in-field pest control, which is the process of maintaining the density of pests to avoid causing crop injury or economic loss, is the ultimate aim of biological control (Schellhorn et al., 2015). Future studies on biological control services need to provide more data on crop yields and to assess the effects of these services on crop productivity (Gurr et al., 2017). In our study, compared with the monoculture systems, the strip intercropping system presented a significantly higher pod maturity index and peanut production. Regression analysis revealed that peanut aphid suppression contributed to the pod maturity index and peanut production, indicating that peanut/maize strip intercropping could achieve in-field pest control via natural enemies. This study provides a mechanism to construct a simple model for strip intercropping systems to estimate biological pest control in terms of yield advantage and pest control. In addition, strip intercropping is a promising way to transform traditional row intercropping systems into wide-strip intercropping systems that can be mechanized via existing machinery (Wang et al., 2015). Future research should focus on other pests, in both peanut and maize, in response to peanut/maize strip intercropping.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2019.106682.

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