Doctoral Dissertation (Shinshu University)

# A new trial on conservation biological control — utilization of indigenous predators enriched with the introduction of plant residue in organic field

# 生物保全に配慮した生物防除の試み—有機農業圃場への植物残渣導入による土着天敵利用の害虫防除

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# Chapter 1 Introduction

## 1.1 The intensive agriculture and its disadvantages

Modern intensive agriculture aims at obtaining high yield by using the modern techniques. Chemicals are used to stimulate rapid growth, increase size, reduce disease and eliminate pests. In recent years, although the modern intensive agricultural production has been supplying our current resources and demands (Martin, 2000), many modern tactics cause a series of problems to environment and food quality such as pesticides/chemical residue (Hill et al., 1995; Cabras and Angioni, 2000), water pollution (Crinnion, 2009, Chung and Chen, 2011) and biodiversity declines in farmland (Gardner, 1996; Donald, 2001).

Such deterioration of natural environment and agricultural products has become a great problem for human beings. Therefore, researches and agricultural producers have been trying to reconsider the intensive farming systems, seek better strategies to obtain safer food and protect natural environment as far as possible.

## 1.2 Environmentally friendly agricultural management

Environmentally friendly agricultural management which works in harmony with nature has been receiving increasing attention, such as integrated pest management (IPM). It was defined as ‘the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms’ (FAO, 2002). The goal of IPM is to control populations of pests below levels that result in economic damage. The strategies of IPM include physical, cultural, chemical and biological control. The concept of IPM can be used in both conventional and organic agriculture. Different from the conventional agriculture, organic agriculture excludes the use of synthetic chemicals while ecological practices including crop rotation, no-tillage, mulching and biological pest control are used together. In the organic farmland of the present study, besides various physical and ecological practices, biological pest control was paid attention to.

Biological pest control applies ecological theories to the pest control and focuses on the interaction among organisms including predation or parasitism. Three items are included:

1) classical biological control, in which exotic natural enemies are introduced to reduce a pest;

2) augmentative biological control, in which insectary reared natural enemies supplement indigenous populations.

Although the introduction of allochthonous natural enemies for pest control had been applied in many cases, the risk should not be ignored because the introduced natural enemy might become an invasive pest (Yano, 1999; Van Lenteren et al., 2003) and lead to the loss of biodiversity (Van Lenteren et al., 2006). And sometimes the augmentative release of large numbers of insectary reared natural enemies is not economical (Ehler, 1998; Collier and Van Steenwyk, 2004).

3) conservation biological control. It depends on naturally occurring predators/parasitoid that has been well adapting to the local farmland system, aims to enrich the abundance of them by conservation practices for pest control, which can reduce the risk of local ecological damage and economic cost (Barbosa, 1998). In the present study, I focused on such method in organic farmland.

In farmland, many generalist predators have the potential to be natural enemies such as wolf spiders (Riechert and Lockley 1984), predacious carabids (Suenaga and Hamamura 1998, Lang et al. 1999), frog (Hirai 2007), lady beetles (Koch 2003) and lacewings (Senior and McEwen 2001). However, sometimes because of insufficient diet resources, their density is relatively low and fails to control pest to the satisfactory level. During the growing period of crops, predators can prey on pests in crops; while during the period without pest, they have to search other alternative sources for their survival. Therefore, it is meaningful to provide living sources for these indigenous predators to increase their survival rate. Before the occurrence of pests in crops, whether natural enemies can reach a high density affects the success of biological pest control.

## 1.3 Tactics of conservation biological control – through vegetation diversity and plant residue mulch

**Vegetation diversity.** Many biological literatures have well confirmed that enhanced vegetation diversity or the establishment of semi-natural compensation areas could improve the habitat of natural enemies and contribute to their survival (Chamberlain, 1999; Browne, 2000, Weibull, 2000). One of cases is sown weed strips/attrahent plant/alternative host plant within farmland or around farmland boders. Studies have confirmed the effectiveness of such non-cropped habitat in providing refuge place and alternative diets/hosts for spiders (Haughton, 1999) and carabids (Holland, 2000) and parasitoids (Powell, 1986, Frank and Shrewsbury, 2004). However, sometimes if the utilization rate of farmland is rather high, there is little field margin large enough for non-crop planting; and sometimes it might not be accepted by many producers because of fertilizer input for non-crops and nutrients competition between non-crop plants and target crops.

**Plant residue mulch.** This method tries to utilize the energy of plant residue to enrich the abundance of alternative diets for indigenous natural enemies, and the enriched natural enemies populations are expected to control pests. This strategy is the theme of the present study.

## 1.4 Common views of plant residue mulch and our focus

The mulch of plant residue in field can exert a series of effects to the field. This mainly include: 1) increase the content of soil organic matter (Campbell et al., 1991; Wu et al., 2002); 2) enhance the activity of microorganism (Wang et al., 2011; Liu et al., 2012); 3) reduce soil moisture evaporation rate and improve water-use efficiency of crops (Unger, 1978; Amir and Sinclair, 1996); 4) improve the soil porosity permeability (Tangyuan et al 2009); 5) improve the soil nutrient (Lao et al., 2002).

The above items were mainly effects of plant residue on soil condition, however, in the present study, we try to focus another effect of plant residue enriching the abundance of soil fauna and predators. Such effect has been realizing by more and more researchers.

## 1.5 Enriching the alternative preys of indigenous predators by mulching plant residue in field

In the present study, we tried a more economic, easy-to-do and environment-safe method to provide alternative prey for indigenous predators: introduction of plant residue in the organic farmland. The reason of such adoption is stated as follows:

1) In farmland, many generalist predators belong to both grazing food web (herbivore-crop-predator) and detritus food web (plant residue-microorganism -detritivore-predator). The energy source of detrital food web is from the dead tissues of plant or soil animals, and 70-90% of all primary production eventually enters the detrital food web (Waring, 1979). The trophic categories of detrital food web consist of microbe, fungivores, bacteriovores, detritivores, herbivores, omnivores and predators (Moore et al., 1988).
2) Plant residue can be as the food resource for many primary decomposers such as bacteria, actinomycetes, fungi, algae, and protozoa (Neher, 1999). They release the majority of energy fixed in residues effectively in the entire decomposition food web (Moore, 1988, Tian et al., 1992) and promote biological activity (Figure 1.1).

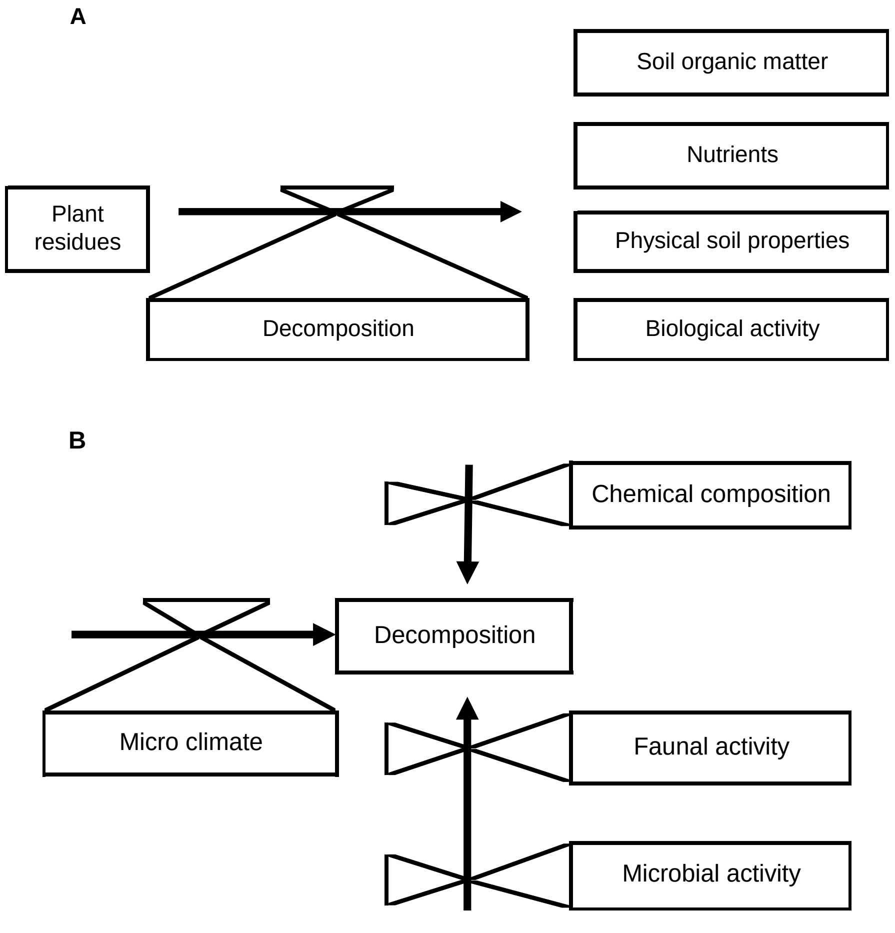


**Figure 1.1 A, a conceptual model illustrating the controlling role of decomposition in the effect**

**of plant residue on soil properties; B, conceptual model illustrating speed-driving variables on decomposition (Tian et al., 1992)**

3) Detritivorous arthropods including collembolan animals, dipteran animals, nematodes and the majority of mites, constitute the mainly fungivores and the majority of energy in their body was derived from microorganisms associated with detritus (Chen and wise, 1999; Ruess, 2000; Hättenschwiler, 2005;). Collembolan animals feed on different soil microbiota including bacteria, actinomycetes and algae with a preference for fungi (Chen and Wise, 1999). The densities of collembolan animals, dipteran animals and animals of Acarina could increase in response to enhancement of detritus in the litter layer (Cheng and Wise, 1997; Chen and Wise, 1999).
4) Part of abundance-enriched species can be as preys for indigenous predators. Evidences have proved that collembolan animals and dipteran animal are major prey in diet of wolf spiders (Edgar, 1969, Nyffeler, 1999; Chen, 1999; Wise et al., 1999); ground beetle species increased with increasing availability of isotomid prey (Birkhofer, 2008). Spiders also prey on carabid larvae and herbivores (Table 1.1).
5) Recently some studies have focused on impact of plant residue mulch on soil communities in farmland, urban landscape or mesocosm design (Table 1.2). These studies found that mulch of plant residues (wheat straw, rice straw, maize stover and weed straw) can help enrich the soil fauna abundance. And some studies have found that plant residue mulches may not only influence the abundance of a range of invertebrates, but also these invertebrates would contribute to pest control (Settle et al.,1996; Halaj et al., 2000; Thomson and Hoffmann, 2007) (Table 1.3).

### 1.6 Employing indigenous predators as pest control agent

1) Existing density of indigenous predators. Density of predators is the first limitation for pest control application. Kossou (2001) reported that *Rhabdepyris* sp., *Chelonus* sp. and *Evania* sp., was the natural enemies of cowpea pest, but their density was rare thus they could not contribute to pest control in the local farmland. In the present experiment plot, they are some native predators including lacewing and assassin bug, but their densities were rather low and individuals only occur sporadically. However, indigenous generalist predator such as wolf spiders can well adapt to the local environment and widespread distribute over the farmland, which

might due to the stable colonizing ability in the local area.

2) Temporary persistence of predators, especially in the absence of target pest. Riechert (1984) pointed out that if the density of polyphagous predators was maintained through the utilization of a complex assemblage of preys, they can be effective controller of their prey. As reported by Stern (1964) in California, when an entire field of alfalfa was mowed, the native lygus bug could not continue stayed in the alfalfa farmland, and they tried to migrate to a cotton field to search alternative preys. In the present study, the introduced plant residue in farmland is expected to enrich the alternative preys of the indigenous generalist predators to keep and enrich their persistence especially in the absence of target pest.
3) Response/predatory capacity of predators to target pest. A predator with poor response to target pest can not be chosen as a good pest control agent. The response can be at least tested in laboratory (Murdoch, 1972) and also be understood by analysis of predator's food menu using stable isotope analysis ($\delta^{13}$C and $\delta^{15}$N) in the present study.

### 1.7 Objective of the present study

The objective of this study was:

1) Examine the response of alternative preys, indigenous generalist predators and target pests in the plot with plant residue introduction;
2) In laboratory, test the functional response of predators to lepidopteran pest, and further estimate the actual predation capacity of predator;
3) Evaluate the response/efficacy of predator for pest suppression through the estimation of their food menu by stable isotope ($\delta^{13}$C and $\delta^{15}$N) analysis in the introduction plot;
4) Proposal a new trial of conservation biological control of pest by introduction of plant residue in organic farmland.

**Table 1.1 Feeding habits of predators based on literature or observation in the current study**

| Taxa | Diet | References |
|---|---|---|
| *Lycosidae* | Collembola (*Isotoma viridis* , *Proisotoma minuta* , *Lepidocyrtus violaceus* , *Orchesella ainslei* , *Tomocerus flavescens, Entombrya* sp., *Hypogastrura* sp.) | Aitchison, 1984 |
| *Pardosa pseudoannulata* | plant hopper (*Nilaparvata lugen* ) | Heong, 1990 |
| *Pardosa agrestis,*<br>*Pardosa amentata* | Isotomidae, Entomobryidae, Dolichopodidae, Drosophilidae, Muscidae | Nyffeler and Benz, 1988,<br>Nyffeler, 1999 |
| *Pardosa ramulosa* | Leaf hopper (*Macrosteles fascifrons* ), Chironomidae | Oraze et al., 1989 |
| *Wolf spider* | Chironomidae (Chironomus sp.), Collembola | Settle et al., 1996 |
| *Pardosa agrestis* | Chironomidae (*Chironomus* sp.),<br>Bibionidae adult (*Penthetria japonica* ),<br>Cricket nymph (*Teleogryllus emma* ),<br>Leafhopper (*Bothrogonia ferruginea)* ,<br>Grasshopper (*Tetrix japonica* ),<br>Collembola (*Proisotoma* sp., *Hypogastrura* sp.),<br>Coleopteran larvae (mainly *Harpalus* spp.)<br>Lepidopteran pest larvae, | Observed in current study |
| Predacious carabids | Lepidopteran pest larvae, Coleopteran larvae | Observed in current study |
|  | Diptera, earth worm | Symondson et al., 2006 |
|  | Grasshopper, slug, housefly | Fawki and Toft, 2005 |
| *Hyla japonica* | Lepidopteran pest larvae | Observed in current study |
|  | Formicidae, Diptera, Coleoptera,<br>Collembola, Diplopoda, Araneae, Chilopoda | Hirai, 2007 |

## Table 1.2 Literatures on the application of plant residue mulch increasing fauna abundance

| Mulch types/species | Method | Conclusion | Study site | References |
|---|---|---|---|---|
| Rice straw,<br>*Gliricidia* prunings,<br>maize stover,<br>Acioa prunings,<br>Leucaena prunings | Mulching in field | Mulching with plant residues has a clear effect on soil fauna populations (Millipedes, ant, termite, Earthworm), and the effects vary with chemical composition of plant residues added. Apart from the nutritional quality of plant residues, mulching effects on soil microclimate may have major effects on soil fauna populations. | Ibadan, Nigeria | Tian, 1993 |
| Weed Residue | Residues left in situ where weeds are uprooted;<br>or placed in small piles throughout the field | Spider, Staphylinid beetle , Carabid beetles, density was greater when residue was piled or used as mulch than in the no-residue plots;<br>Ants were not affected by weed residue placement;<br>Among pests, only Chaetocnema sp., was significantly greater in plots with strips | | Afun, et al., 1999 |
| Wheat straw | Introduction of wheat straw insoil using a mesocosm design | Earthworm and Collembola abundance increased significantly | Wageningen, Netherland | Gestel and Kruidenier, 2003 |
| *Brachiaria ruziziensis* ,<br>*Crotalaria retusa* ,<br>*Mucuna pruriens* . | Mulching in field | Detritivores (Formicidae,Termitidae,Lumbricidae), herbivores, and predators were more abundant in plot covered with grass mulch than in no-mulch plot. | Cotton-growing region of Cameroon | Bre´vault, et al., 2007 |
| Organic mulch (Hardwood, recycled wood, pine bark) | Mulching in field | Saprophyte (Millipedes, Segmented worms, Isopods) and predators (Centipedes, Spiders, Ants, Beetles) were significantly higher in the organic mulches than in non-mulched plot | Ohio, Amrica | Jordan and Jones, 2007 |

## Table 1.3 Literatures on the application of plant residue mulch for pest control

| Mulch types/species | Method | Conclusion | Study site | References |
|---|---|---|---|---|
| Crop straw | Mulch in vineyards | Surface mulches may also influence the abundance of a range of invertebrates. Potentially, an increase in natural enemies will contribute to pest control. | Victoria, Australia | Thomson and Hoffmann, 2007 |
| Compost cow manure | Add to rice field | By increasing organic matter in test plots they could boost populations of detritivores and plankton feeders, and in turn significantly boost the abundance of general predators; reducing early-season predator populations with insecticide applications caused pest populations to resurge later in the season. | Java, Indonesia | Settle et al., 1996 |
| Wheat straw | Made modular habitat refugia by using wheat straw mulched on the surface of field | Refugia held 5-36 times the spider density compared with open field. Almost 60% more spider species were found in refugia than in open field.Abundance of harvestmen, carabids, and staphylinid beetles also significantly increased in habitat refugia;<br>Soybean seedlings grown within1mof habitat refugia suffered 33% less insect damage compared withplants at control location;<br>Increased habitat cover and provision of alternative prey in habitat refugia may have caused this dramatic predator increase. | Oxford | Halaj, et al., 2000 |

# Chapter 2 Effect of plant residue introduction on alternative preys in organic field

## 2.1 Introduction

In Chapter 2 and 3 I tried to examine the effect of plant residue to population densities of soil fauna (including alternative preys), indigenous predators and pests. Two types of food webs in field were concerned, grazing food web (including herbivores/pest, crops and predators) and detritus food web (including plant residue, decomposers, detritivores and predators). In detritus food web, plant residue provides nutrient for decomposers that can serve as food resource for small invertebrates, and some invertebrates can serve as food source for predators. A substantial fraction of the energy source of detritus food web is from organic materials such as dead plant residue (Chen and Wise, 1999). Some generalist predators such as spiders, predacious carabid beetles and frogs belong to both two types of food webs because they consume both detritivores and herbivores. For the persistence of generalist predators, some species of decomposers and detritivores are important alternative diets for them. Many soil fauna species are not alternative preys for predators but they are involved in degrading of organic matter, mineralizing of nutrients, improving soil condition for the sustainable development of communities (Crossley et al., 1989).

We hypothesize that enhancing the energy base of detritus food web can enrich various soil fauna including alternative preys of predators, then increase the abundance of predators; and eventually the predator play a greater role in grazing food web-preying on pest. The abundance of alternative preys is crucial to the abundance of predators.

In this chapter, a new trial of introducing plant residue in organic field was tried to enrich the alternative preys of indigenous generalist predators. The aim was to understand the effect of plant residue mulch on soil fauna (including alternative preys) in field. The types, population densities and biomass of alternative preys in the plot with introduction of plant residue mulch was investigated and analyzed.

## 2.2 Materials and methods

### 2.2.1 Experimental site

Experiment was conducted in the organic field at International Natural Farming Research Center, located in the Matsumoto Plateau area (N36°/E137°, altitude 700 m), Nagano, Japan. The locality has a medium climate with mean air temperature 18～26°C in warm season (April－October) and -1－(＋8)°C in cold season (November－March), and mean annual precipitation 1031 mm (rain and snow) over the previous 30 years. The seasonal precipitation is almost even. The experiment was performed in both greenhouse and open field.

The greenhouse and open field have been managed in organic ways without chemical fertilizers and pesticides for 20 years. The greenhouse is bordered by a road in the east, an organic farmland in the north (0.2 ha.), an organic farmland (0.5 ha.) in the west and an organic farmland (0.2 ha.) in the south. Organisms can migrate between the adjacent farmlands. The open field is bordered by an organic farmland in the east (0.1 ha), a wild lawn in the north (0.1 ha.), an organic farmland (0.5 ha.) in the west and an organic farmland (0.5 ha.) in the south. Organisms can migrate between the adjacent farmlands.

### 2.2.2 Design of experiment

**Design in greenhouse.** A rainout shelter greenhouse with 36 m long and 6 m wide was separated into two parts as two experimental plots and three greenhouses were used for experimental replications. Spinach was sown in October of previous year and harvested in mid April of the second year. After that, seedlings of cabbage (*Brassica oleracea* (L.) var. capitata L. cv. Ajiboshi) were transplanted into field on June 20. Treatments were designed in each house as follows (Figure 2.1):

1) Introduction plot: from October 2009, along both side walls of the greenhouse, plant residue (mixture of wheat straw and cereal weeds (mainly *Eleusine indica* and *Digitaria ciliaris*), with around 1/2 and 1/2 of total dry weight, respectively) were mulched with the pile height as 5 - 10 cm on the ground surface and eventual dry weight as around 1500 - 2500 g m$^{-2}$, and this rate was kept by supplementing plant residue once a month;

2) No-introduction plot: the space along side walls was left clear without plant residue mulching.

**Design in open field.** The open field is 300 m away from the greenhouse with the size as 60 × 30 m. Wheat was sown in the field in October of previous year and harvested in mid June of the second year. Seedlings of cabbage (*Brassica oleracea* (L.) var. capitata L. cv. Ajiboshi) were transplanted into field on June 20 of the second year. The experiment was designed as follows (Figure 2.2):

1) Introduction plot: from October 2009, strips of plant residue mulching were made along a longwise direction of the field, the width of each strip was 50 cm and the distance between every two strips was around 2 m. Plant residue with the same composition as in greenhouse were mulched with the pile height of 5 - 10 cm above the ground and eventual dry weight of around 2500 g $m^{-2}$; this rate was kept by supplementing plant residue once a month;
2) No-introduction plot: the space of field was left clear without plant residue mulching.

### 2.2.3 Soil fauna investigation

**Density investigation**. According to the climate of the local area (Figure 2.3), soil samples were collected in Spring (April 1-10, 2010/2011), Summer (July 10 - 20), Autumn (October 25 - November 5) and winter (January 20 - February 5). Each time three to five samples were taken randomly from each experiment plot and eventually nine to 15 samples were taken from introduction and no-introduction plots respectively. According to the study of Fujita (1990) in the local area, soil fauna in the soil of 0 - 10 cm depth accounted for more than 80% abundance of total soil fauna of 0 – 30 cm. Hence, in this study, the quadrat soil sample with 50 cm long, 50 cm wide and 10 cm deep was collected for investigation. In plant residue mulching area, the half-decomposed plant residue and the soil under it was included in one sample, and in the no-introduction plot only soil was collected. Macro fauna were picked out by hand and counted, meso fauna were separated by Tullgren funnel with diameter of 30 cm and mesh opening of 2 mm. A 60 watt bulb and reflector on top of funnel served as the heat light source to drive the animals downward into a glass cup containing water. Soil sample was left on the funnel until it became dry (48-72 hours). After the extraction period, the contents of the glass cup were transferred to a petri dish with the diameter of 15 cm for species identification and number counting. The counting was performed under a binocular dissecting microscope. For herbivores including cricket, grasshopper and leafhopper; the density per 50 × 50 cm on the surface of field ground was counted.

**Biomass of soil fauna**. Measuring the biomass of soil animals is a way to consider the soil ecosystems from the point of energy. The biomass of organisms was expressed by measuring their dried body weight. For the groups of collembolan animals and oribatids, the total dried weight of 500 individuals was measured and then a single individual was calculated. For the large-bodied such as earthworm, 50 or 10 individuals were selected to check the biomass.

### 2.2.4 Date analysis

Soil fauna density differences between the introduction and no-introduction plots were analyzed using general linear models (GLMs). Repeated measures ANOVA of the density of pests in the treatment (introduction and no-introduction plot) plots were performed over two years of 2010 and 2011. Factors of plant residue treatments (plant residue introduction and no-introduction) and experiment site (greenhouse and open field) was treated as between-subjects factors, and measures in 2010 and 2011 were treated as within-subjects. All statistical analyses were performed using the SPSS statistical package (Statistical Product and Service Solutions, version 16.0).

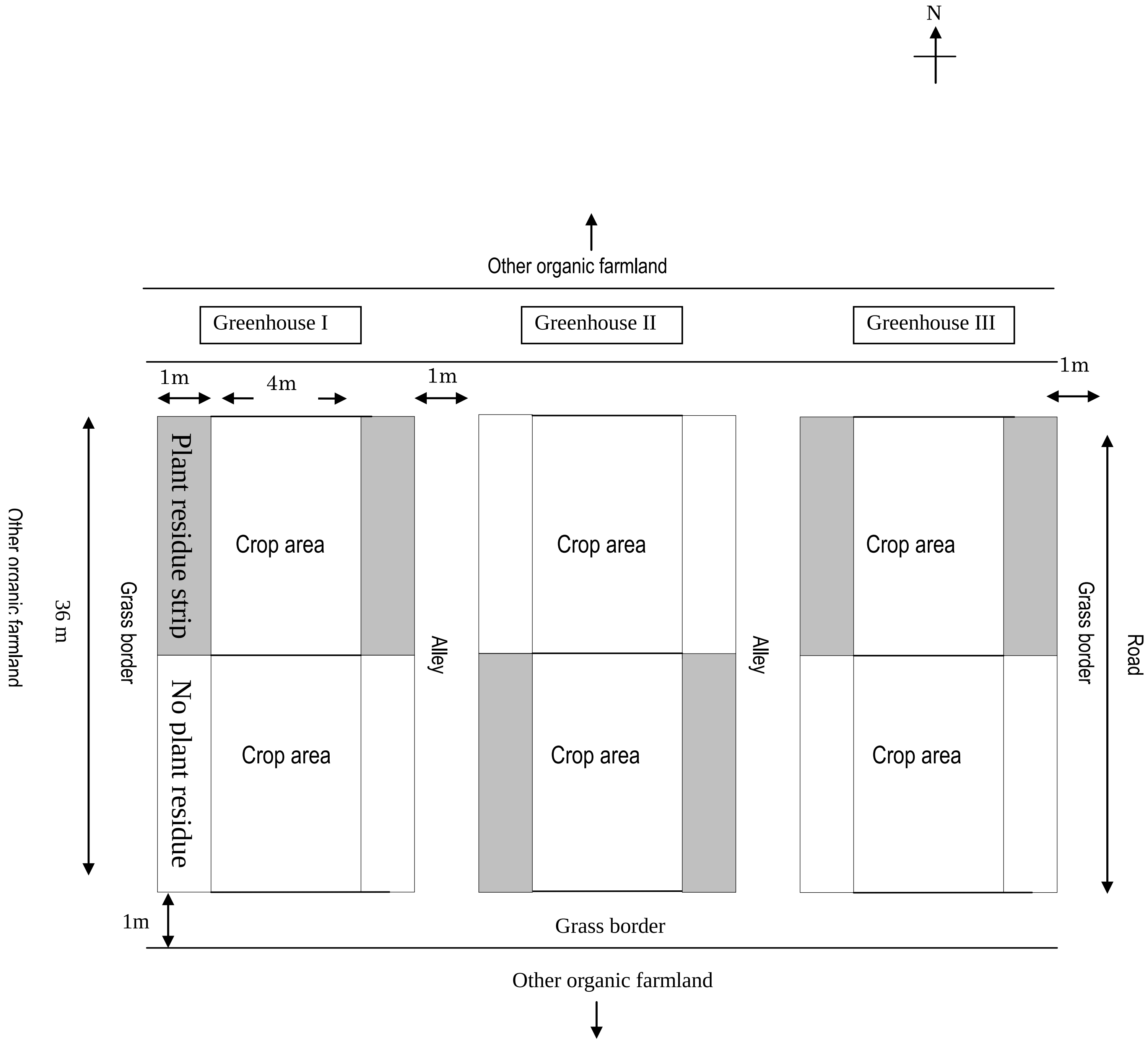


**Figure 2.1 Plot design in the greenhouse**

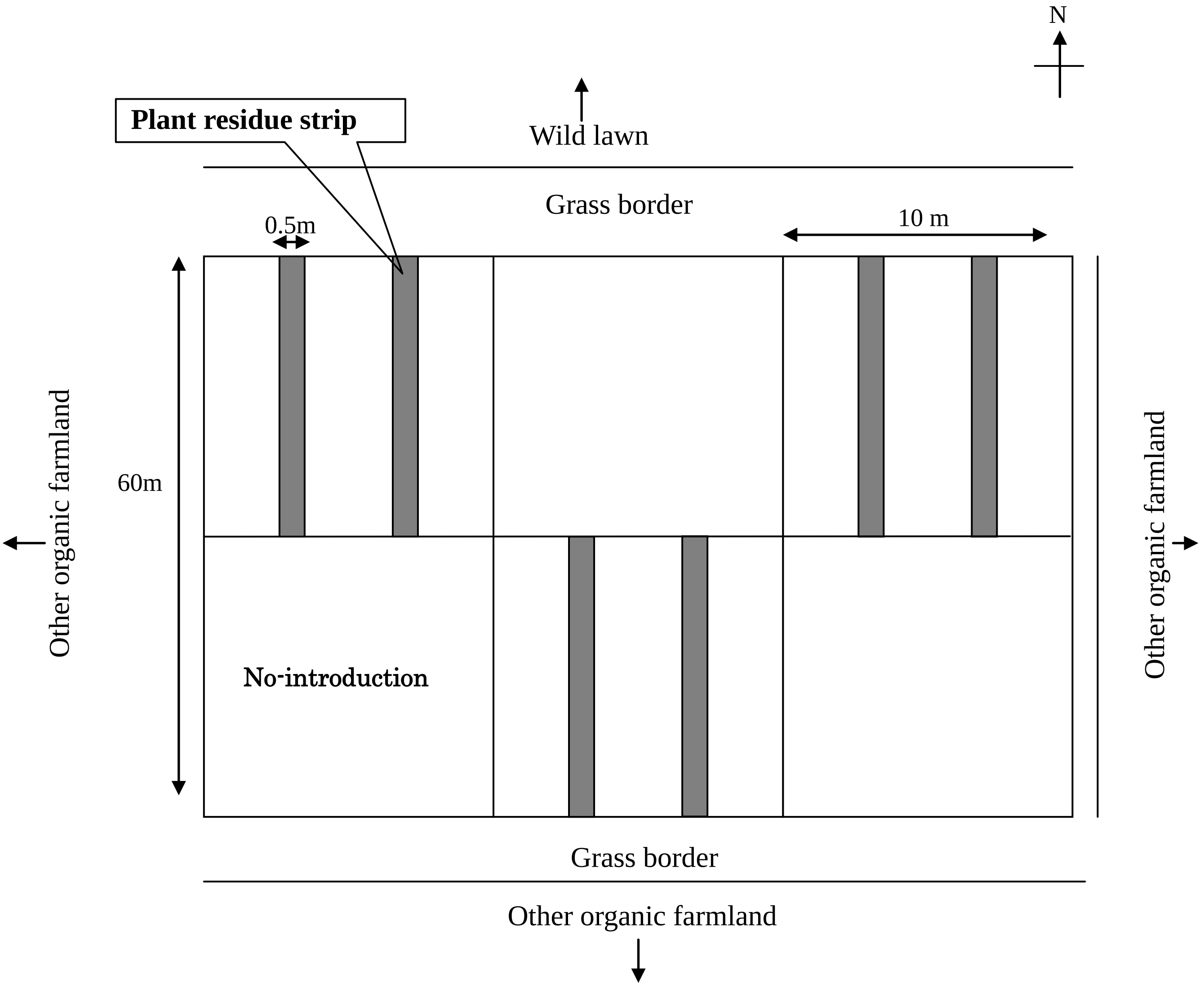
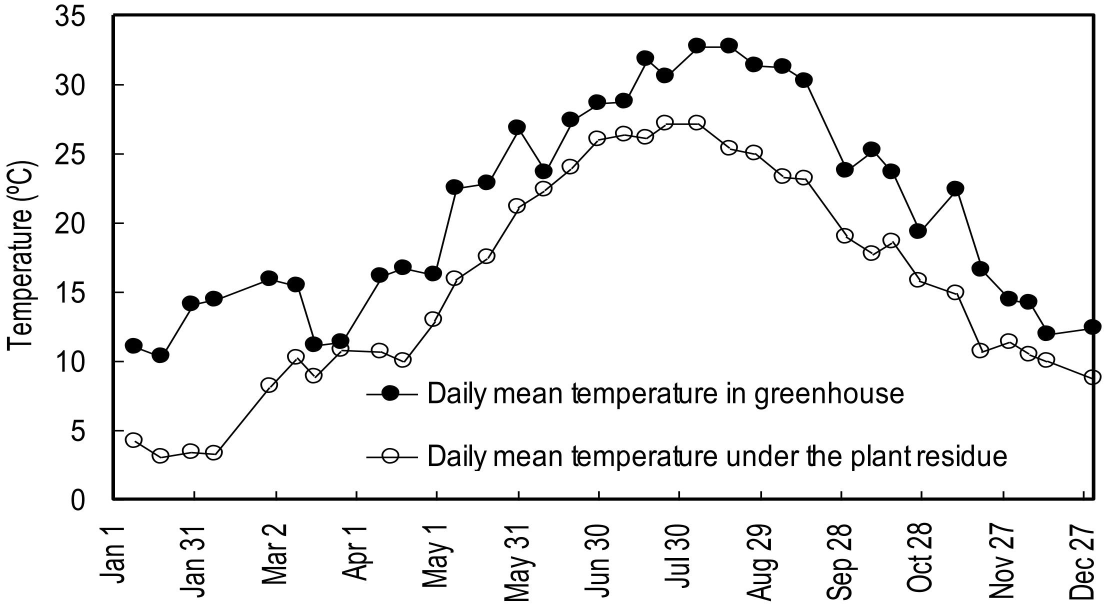
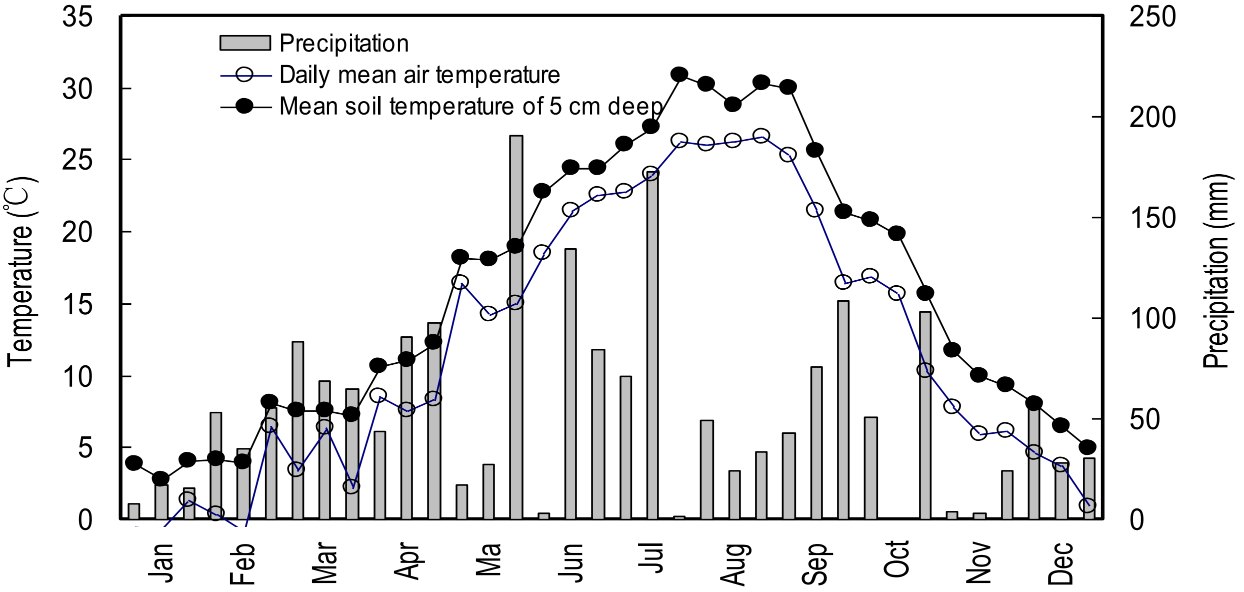


Figure 2.2 Plot design in the open field





## 2.3 Result

### 2.3.1 Identification of alternative preys

Most soil fauna were identified to family, genus or species and the main groups of fauna is shown in Table 2.1. 26 groups soil fauna was identified, the introduction and no-introduction plots showed almost the same species richness. Based on some literatures (Table 1.1) and our own observation, the alternative preys included in the present experiment plot were identified. They mainly included: collembolan animals (mainly included *Proisotoma* sp. and *Hypogastrura* sp.); dipteran larvae (*Chironomus* sp., *Coenomyia* sp. and *Penthetria japonica, and Chironomus* sp. accounted for more than 70%); coleopteran larvae (*Harpalus* larvae accounted for more than 50%); herbivores (mainly *Tetrix japonica*, *Bothrogonia ferruginea* and *Teleogryllus emma*); diplopods (mainly *Oxidus* spp.)

### 2.3.2 The introduction plot showed higher abundance of alternative preys

The population density and biomass of alternative preys of four seasons in 2010 and 2011 was showed. By Repeated measures ANOVA analysis (Table 2.2), generally speaking, density and biomass of the alternative preys were significantly higher in the introduction plot than in no-introduction plot in most seasons (Figure 2.4 and Figure 2.5). There was no significant difference of density and biomass of fauna between different sites (greenhouse and open field) and years (2010 and 2011). The interaction of treatment×sites, treatment×year and site×year was not significant except for diplopods.

Compared with the no-introduction plot, in the introduction plot, the density/biomass of collembolan animals showed 2.4 – 10.0 times higher, dipteran larvae showed 4.0 – 6.0 times higher, herbivores showed 1.6 – 3.3 times higher, coleopteran larvae showed 2.0 – 6.3 times higher and diplopods showed 1.4 -6.8 times higher respectively. Such significant density difference of soil fauna between two plots indicated that those fauna were dependent on the introduction of plant residue at different degree.

Over four seasons, density of collembolan animals was abundant in spring, summer and autumn and decreased in winter. Dipteran animals density increased from spring, reached the maximum in autumn and decreased in winter. Herbivores were mainly abundant in summer and autumn and scarce in winter. The coleopteran larvae were abundant through the year and especially active in summer and autumn.

In addition, some other soil fauna including at least 15 groups showed significant higher density in the introduction plot, such as earthworm, oribatids and staphylinids (Figure 2.4 and Figure 2.5). These soil fauna may not serve as alternative preys for indigenous predators, but they can help improve the condition of soil.

The investigation was performed eight months after the establishment of plant residue introduction, and the residue was continuously supplemented into strips. Generally, there was no significant difference between investigation results of the year 2010 and 2011, although some species showed some fluctuations through two years.

Collembolan animal, dipteran animal, coleopteran larvae and earthworm showed relatively stable density dynamics, while the diplopods showed significant higher density in 2011.

Table 2.2 Repeated measures ANOVA of alternative prey density (A) and biomass (B) in the introduction and no-introduction plots over the two years

**A**

| Taxa | **Treatment** | Site | Year | Treatment×site | Treatment×year | Site×year | Treatment×site×year |
|---|---|---|---|---|---|---|---|
| Collembola | 0.002 | 0.750 | 0.349 | 0.812 | 0.411 | 0.955 | 0.407 |
| Diptera | < 0.001 | 0.081 | 0.830 | 0.149 | 0.871 | 0.753 | 0.927 |
| Coleoptera | < 0.001 | 0.130 | 0.013 | 0.702 | 0.014 | 0.091 | 0.257 |
| Herbivore | 0.050 | 0.185 | 0.937 | 0.418 | 0.896 | 0.708 | 0.330 |
| Diplopoda | 0.009 | 0.001 | 0.001 | 0.022 | 0.005 | 0.064 | 0.064 |
| Earthworm | 0.006 | 0.673 | 0.752 | 0.583 | 0.523 | 0.350 | 0.293 |
| Oribatida | < 0.001 | 0.166 | 0.014 | 0.145 | 0.042 | 0.132 | 0.407 |
| Staphylinid | 0.044 | 0.194 | 0.519 | 0.397 | 0.463 | 0.302 | 0.260 |

**B**

| Taxa | **Treatment** | Site | Year | Treatment×site | Treatment×year | Site×year | Treatment×site×year |
|---|---|---|---|---|---|---|---|
| Collembola | 0.002 | 0.075 | 0.353 | 0.811 | 0.411 | 0.961 | 0.406 |
| Diptera | 0.003 | 0.612 | 0.321 | 0.526 | 0.290 | 0.329 | 0.310 |
| Coleoptera | <0.001 | 0.130 | 0.013 | 0.702 | 0.014 | 0.090 | 0.256 |
| Herbivore | 0.060 | 0.114 | 0.710 | 0.371 | 0.903 | 0.997 | 0.368 |
| Diplopoda | 0.009 | 0.001 | 0.002 | 0.021 | 0.008 | 0.059 | 0.059 |
| Earthworm | 0.008 | 0.737 | 0.651 | 0.645 | 0.466 | 0.327 | 0.279 |
| Oribatida | <0.001 | 0.165 | 0.014 | 0.145 | 0.041 | 0.132 | 0.402 |
| Staphylinid | 0.046 | 0.202 | 0.499 | 0.383 | 0.484 | 0.316 | 0.248 |

Table 2.1 Taxonomic list of investigated soil fauna in the present study

| Order | Family | Genus/species |
|---|---|---|
| Collembola | | |
| | Isotomidae | |
| | | *Proisotoma* sp. |
| | Hypogastruridae | |
| | | *Hypogastrura* sp. |
| Hymenoptera | | |
| | Formicidae | |
| Orthoptera | | |
| | Gryllidae | |
| | | *Teleogryllus emma* |
| | Tetrigidae | |
| | | *Tetrix japonica* |
| Homoptera | | |
| | Cicadellidae | |
| | | *Bothrogonia ferruginea* |
| Diptera | | |
| | Chironomidae | |
| | | *Chironomus* sp. |
| | Tabanidae | |
| | | *Coenomyia* sp. |
| | Bibionidae | |
| | | *Penthetria japonica* |
| | | *Bibio japonica* |
| Oribatida | | |
| Pseudoscorpiones | | |
| | Neobisiidae | |
| | | *Microcreagris japonica* |
| Araneae | | |
| | Linyphiidae | |
| | | *Erigone atra* |
| | Thomisidae | |
| | | *Xysticus* sp. |
| Isopoda | | |
| | Armadillidiidae | |
| | | *Armadillidium vulgare* |
| | Olibrinidae | |
| | | *Porcellio scaber* |
| Haplotaxida | | |
| | Megascolecidae | |
| Tubificida | | |
| | Enchytraeidae | |
| Coleoptera | | |
| | Staphylinidae | |
| | | *Paederus* sp. |
| | Carabidae | |
| | | *Harpalus* sp. |
| | Aphodiinae | |
| | | *Aphodius* sp. |
| Hemiptera | | |
| | Cydnidae | |
| | | *Aethus nigritus* |
| Symphyla | | |
| | Scutigerellidae | |
| | | *Scutigerella* sp. |
| Geophilomorpha | | |
| | Geophilidae | |
| | | *Geophilids* sp. |
| Lithobiomorpha | | |
| | Lithobiidae | |
| | | *Lithobius* sp. |
| Diplura | | |
| | Japygidae | |
| | | *Occasjapyx* sp. |
| Polydesmida | | |
| | Paradoxosomatidae | |
| | | *Oxidus* sp. |

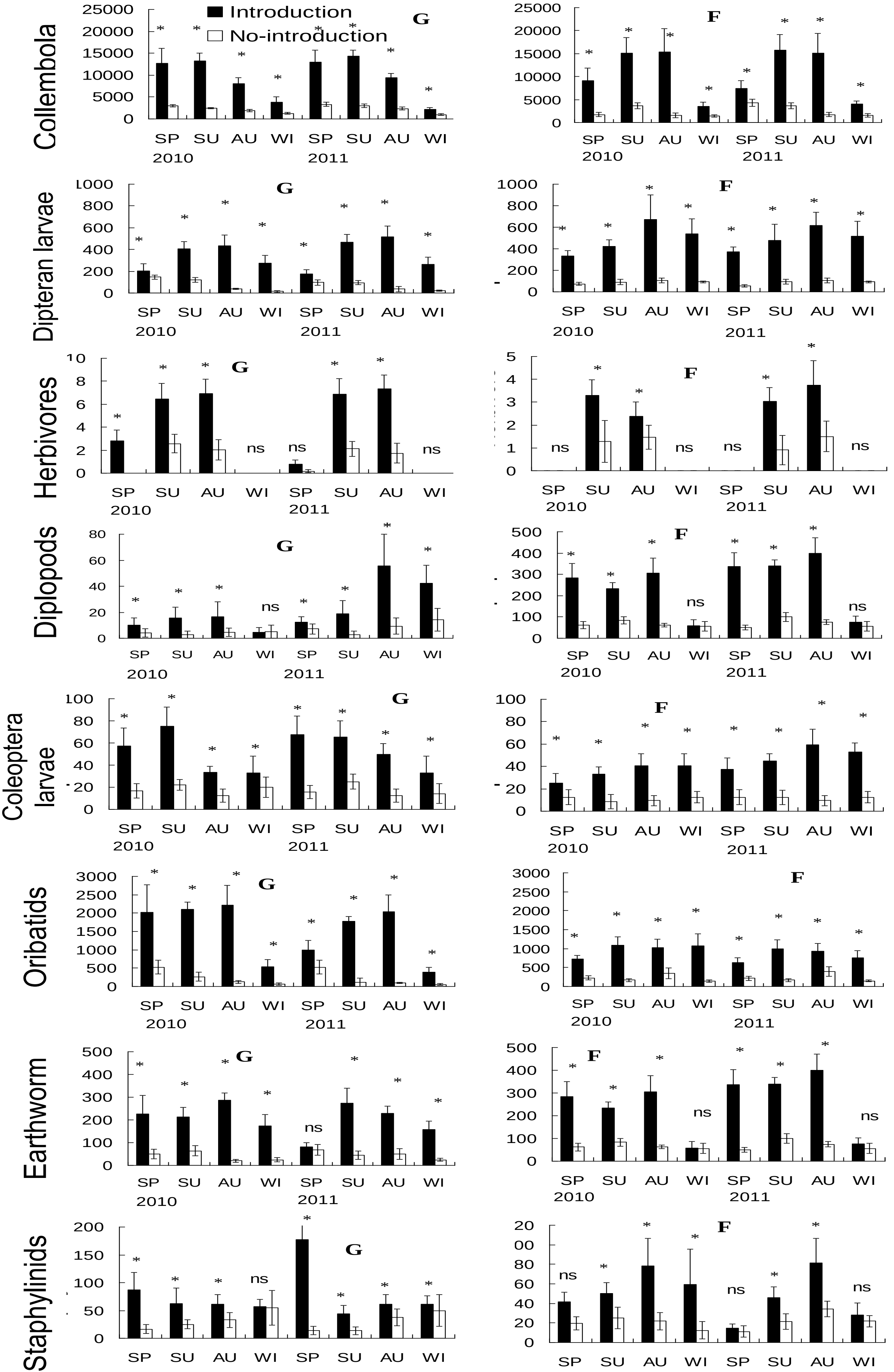


**Figure 2.4 Density of alternative preys and some other soil fauna (individuals per 50×50×10 cm of soil) in 2010 and 2011. SP, spring, SU, summer, AU, autumn, WI, winter, G, greenhouse, F, open field. The symbols of * means significant difference at 5% level and ns means no significant difference based on the repeated measures ANOVA.**

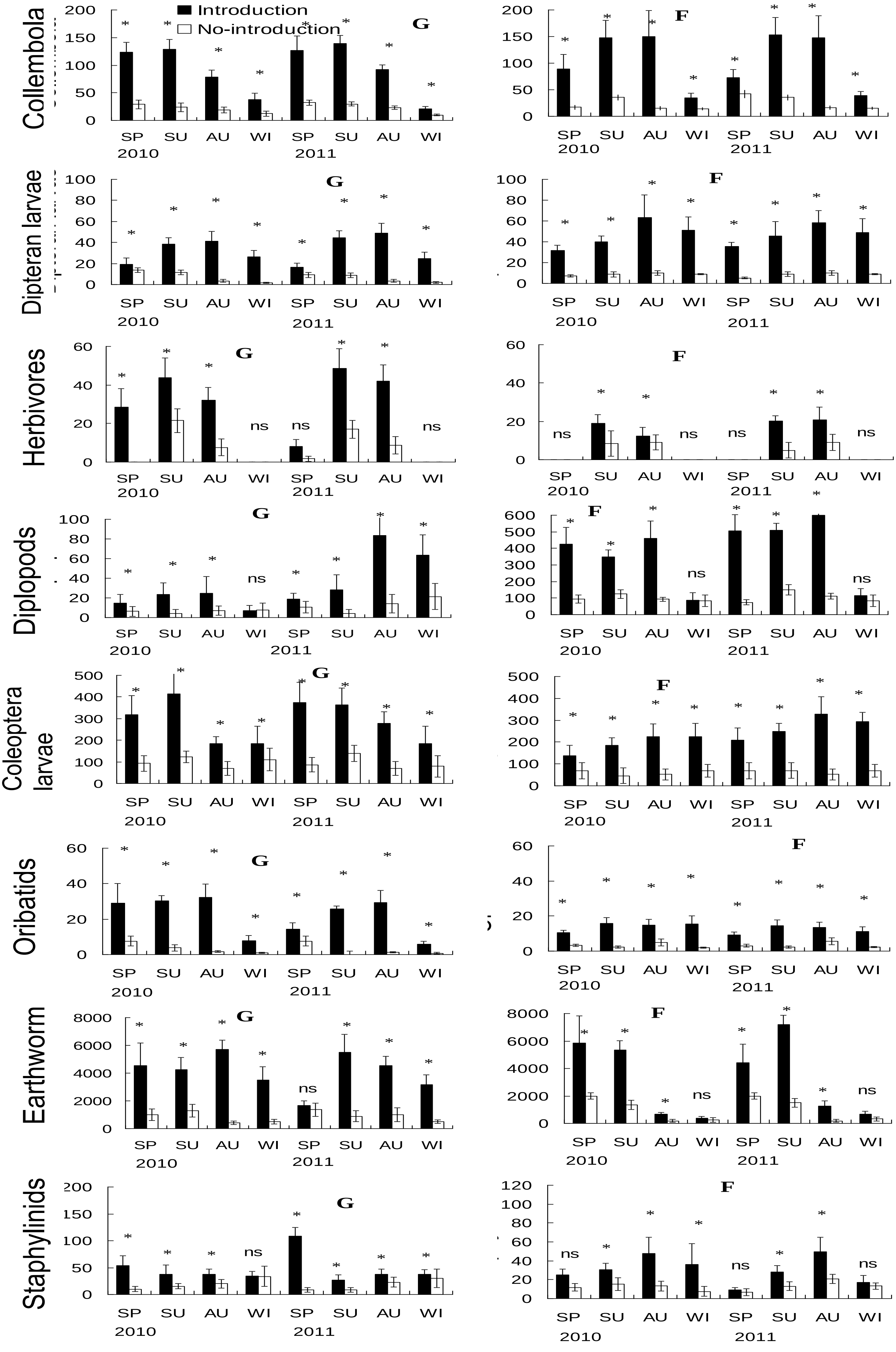


**Figure 2.5 Biomass of alternative preys and some other soil fauna (individuals per 50×50×10 cm of soil) in 2010 and 2011. SP, spring, SU, summer, AU, autumn, WI, winter, G, greenhouse, F, open field. The symbols of * means significant difference at 5% level and ns means no significant difference based on the repeated measures ANOVA.**

## 2.4 Discussion

The abundance of some soil fauna and alternative preys of predators was significantly higher in the plant residue introduction plot through two years shown in Figure 2.4 and 2.5. This result was consistent with those of Settle et al. (1996), Halaj et al. (2000) and Thomson (2007) in providing evidence of a positive contribution of the mulch of plant residue to the abundance of soil fauna. This result indicated that the plant residue mulch in the field can significantly enrich the abundance of some soil animals, which can provide recommendations regarding plant residue introduction for enriching alternative diet for some indigenous generalist predators.

As a kind of energy carrier, plant residue can be decomposed by many decomposers such bacteria, fungi, and protozoa (Neher, 1999). These decomposers can be as the diet of collembolan animals, dipteran animals (Chen and wise, 1999; Ruess, 2000). Herbivores including cricket, grasshopper and leafhopper do not feed on decomposed plant residue, while they tend to be active in place with plant residue mulching (Stinner and House, 1990; Brévault et al., 2007). Such increase also benefits the target of enhancing indigenous predators as it is well known these herbivores are preys of some predators such as wolf spiders. Numerous coleopteran larvae feed on fresh or decomposing plant material on or in the soil (Egert et al., 2005). Earthworms feed on plant residue and are important initial decomposer in soil ecosystem. Oribatids feed on a wide variety of material including living and dead plant, fungal material, or break down organic material in the soil in a similar manner to earthworms (Li, 1988).

The degree of density increase among soil animal species from the no-introduction to introduction plot was different (Table 2.3). The dipteran and collembolan animals showed the largest density ratio of introduction : no-introduction (5.5 and 4.9); the next was oribatids (4.0), coleopteran larvae (3.7), diplopods (3.7) and earthworm (3.3), the smallest was herbivore (2.4). Such results indicated that among these fauna, the dipteran and collembolan animals were much dependent on the introduction of plant residue and the plant residue could provide good diet for them as shown before. For herbivores, plant residue may not be a good food source for them. Such difference may affect the consumption by predators such as wolf spiders, and this will continue explored by stable isotope analysis in Chapter 5.

Last, it is important to notice that whether some enriched detritivores may become pests (Stinner and House 1990). For example, millipedes prefer to dwell in soil covered with mulch and may cause severe damage to cotton seedlings (Brévault et al., 2007), and black beetle (*Heteronychus* spp.) was reported to have caused significant damage to rice grown with mulch at the beginning of a growing season in Madagascar (Ratnadass et al., 2006). However, Wilson-Rummenie et al. (1999) reported that the conservation practices did not increase the incidence of insect pests on emerging seedlings in Australia. In the present plot, some pest insect such as scarabaeid larvae and bibionid larvae sometimes increased in introduction plot, but they had not become threat pests. The explanation for the process of the increase of alternative preys may be not enough, thus the improvement for this is required in future.

**Table 2.3 Ratio of soil fauna densities in the introduction and no-introduction plot**

| Taxa | Introduction/No-introduction | |
|---|---|---|
| | Range | Average |
| Collembolan animal | 2.0-9.9 | 4.9 |
| Dipteran animal | 4.4-6.8 | 5.5 |
| Coleopteran larvae | 2.0-6.3 | 3.7 |
| Herbivores | 1.6-3.3 | 2.4 |
| Diplopods | 1.4-6.8 | 3.7 |
| Earthworm | 1.5-6.7 | 3.3 |
| Oribatids | 2.4-7.5 | 4.0 |

# Chapter 3 Effect of plant residue introduction on indigenous predators and cabbage pests

## 3.1 Introduction

In many cases, it might be a challenge to build a rather high biodiversity in farmland and the high level of biodiversity does not necessarily benefit the development of natural enemies (Rosenheim, 1993; Baggen, 1998). However, it is promising to specially enrich the abundance of one or several species of natural enemies by artificial practices (Emden, 1974; Andow, 1985; Wratten, 1998). Nowadays, the generalist predators serving as potential pest control agent have been receiving increasing attention (Symondson et al., 2002). One of important advantages of generalist predators is the broad diet spectrum of them, which can enhance the persistence of their populations. Through a year, the period of vegetable growth and the relevant pest occurrence was relatively short, i.e., two – three months for cabbage growth and cabbage pest occurrence. Therefore, the alternative preys played an essential role in the persistence of indigenous generalist predators when pests do not occur. In this chapter, plant residue was introduced into cabbage field to find out whether and to what extent the indigenous predator abundance can be enriched for pest suppression.

In Chapter 2, it has been confirmed that the introduction of plant residue could enrich the alternative preys of predators. In this Chapter, it was expected that the enriched alternative preys can increase the abundance of the predators, and eventually contribute the pest suppression. It is important that whether the density of indigenous predators can reach a high level before the emergency of pests.

The experiment was performed in greenhouse and open field and designed as in Chapter 2. The objective was to examine the population densities of predators and pests in the plot with plant residue introduction and compare the interaction between them.

## 3.2 Materials and methods

### 3.2.1 Selection of pest control agents in the present organic farmland

During the investigation of fauna in experiment plot we found many predators such as lacwing (Hemerobiidae), rove beetle (Staphylinidae), ladybeetle (Coccinella), assassin bug (Reduviidae), wolf spiders (*Pardosa agrestis*), predacious carabids (*Dolichus halensis, Chlaenius. pallipes* and *Harpalus* spp.) and Japanese tree frogs (*Hyla japonica*) (Table 3.1). However, according to preliminary observation and testing, mainly predators including wolf spiders, some predacious carabids and tree frog show predatory capacity to lepidopteran pest effectively, well adapt in the local area and can persist through the year. Therefore, these three groups of predators were preliminary selected as potential pest control agent for further investigation and testing.

**Table 3.1 Indigenous predators in the present experimental plot**

| Predators | Prey | Feeding habits/Density in summer |
|---|---|---|
| Lacewing (Hemerobiidae) | Aphid (Zhu, 2005),<br>Delphacidae (Rioja, 2006),<br>Spider mite (Oatman, 1985), etc. | Seldom feed on lepidopteran pest and usually more used by releasing into farmland to suppress aphids and mites (Senior and McEwen, 2001); its density can be enhanced by providing attracants rather than plant residues (New, 1975) |
| Rove beetle (Staphylinidae) | Aphid (Dennis, 1991),<br>Delphacidae (Salim and Heinrichs, 1986),<br>Cicadellidae (Manley, 1977),<br>Red spider mite (Perumalsamy, et al., 2009),<br>Banana weevil (Koppenhöfer, 1994), etc. | Response of rove beetle to *Pieris rapae* is low (Moreno, 2006) |
| Ladybeetle (Coccinella) | Aphid (Blackman, 1967),<br>Delphacidae (Gupta,1989; Koch, 2003), etc. | Response of ladybeetle to *Pieris rapae* is low, uaually is used for homopteran pest control |
| Assassin bug (Reduviidae) | *Helicoverpa armigera*<br>(Grundy and Maelzer, 2000),<br>Lepidopteran pest,<br>aphid (Cao, et al., 2007), etc. | Reduviidae is more used by releasing inundatively (Grundy and Maelzer, 2000);<br>The main habitat is forest other than farmland in Japan (http://www.insects.jp/konbunhankame.htm) |
| Webbing spider | Nematocera, Homoptera,<br>Brachycera, Coleoptera and<br>Hymenoptera (Nentwig, 1983) | Seldom prey on Lepidopteran pest larvae |
| Wolf spider | Lepidopteran pest (Schmaedick and Shelton, 2000),<br>aphid (Nyffeler and Benz, 1988),<br>alternative preys (soil fauna, herbivores, etc.) | Widespread in local area, persist through the year and show larger predatory capacity to lepidopteran pests. |
| Predatory carabid (*D.halensis* , *C.pallipes* , *H.griseus* ) | Lepidopteran pest, aphid,<br>alternative preys (soil fauna, herbivores, etc.) | Widespread in local area, persist through the year and show larger predatory capacity to lepidopteran pests. |
| Japanese tree frog | Lepidopteran pest, aphid (Hirai, 2007),<br>alternative preys (soil fauna, herbivores, etc.) | Widespread in local area, persist through the year and show larger predatory capacity to lepidopteran pests. |

### 3.2.2 Investigation content in the plot with and without plant residue introduction

**Investigation of indigenous predators.** For wolf spiders *Pardosa* spp. (*Pardosa agrestis* accounted for > 70% of all species), investigation was carried out during the period from 10:00 to 16:00 of day time when the spider individuals are most active. The density was examined by a quadrat of one square meter on the ground once a week throughout the year. For each investigation, the quadrat was repeated for ten times.

For frogs, investigation was performed in late afternoon by ocular number estimation every ten days. Holding a pole in hand, randomly selected a point in cabbage plot and stood there, gently flapped the surrounding ten square meters of the cabbage plants to let all frogs emerge and accounted their number. For each investigation, repeated for ten times.

Among predacious carabids species, *Dolichus halensis, Chlaenius pallipes* and *Harpalus* spp. are well known as predators of lepidopteran pests and distribute commonly in the local area. In the present plot, *Harpalus* mainly include *Harpalus tridens*, *Harpalus griseus* and *Harpalus sinicus*. Four pitfall traps in each plot with the diameter of 10 cm and depth of 15 cm without bait and liquid were sunk into field diagonally at intervals of three meters with the mouth flushed with the ground surface and a plastic cover was placed 10 cm above each trap to protect it from rainfall. For each investigation, the setting was repeated three times. Pitfall traps were kept for 48 hours and then numbers of captured carabids were recorded. After recording, carabids individuals were released in the same field. The investigation was performed once in every 10-14 days throughout the year.

**Pest investigation.** In the present plot, pests appearing in cabbage include four types of Lepidoptera, cabbage butterfly (*Pieris rapae* (L.)) [Lepidoptera: Pieridae], diamondback moth (*Plutella xylostella* (L.)) [Lepidoptera: Plutellidae], *Argyrogramma agnate* (S.) [Lepidoptera: Noctuidae] and *Spodoptera litura* (F.) [Lepidoptera: Noctuidae], of which the most abundant is *P. rapae*. Density of lepidopteran larvae in cabbage plants was investigated in the morning when pest larvae are most active on the leaves. Ten to twenty plants were selected randomly and the number of pest larvae from 2nd to 5th instars in each plant was recorded once a week and the density of pests was expressed as number of larvae per 10 square meters per day including 20 plants. The investigation was performed from the beginning of July to late September during which the infestation was occurred by the pests. The 1st larvae of lepidopteran pests are susceptible to environment changes and subjected to a high risk of mortality, hence in the density investigation and functional response testing, the 1st instar larvae were not taken into account.

**Calculation of damage index.** Damage degree of cabbage was divided into six ratings, shown numerically as 0 to 5. A leaf with no damage was defined as 0, those with 1-20%, 21-40%, 41-60%, 61- 80% and 81-100% of the leaf area damaged were defined as 1, 2, 3, 4 and 5, respectively. The pest damage index (PDI) was calculated as PDI=Σ (Number of damaged leaves of a certain rating × Rating value) × 100/(Total leaf number × Highest rating value).

### 3.2.3 Date analysis

Refer to Chapter 2.

### 3.2.4 Abundance of alternative preys

In order to compare the population dynamics of predators, alternative preys and pests, the total biomass of potential alternative preys including collembolan animals, dipteran larvae, coleopteran larvae, herbivores and diplopods was used. The population dynamics of the three groups of organisms in one experiment plot was depicted in one statistic plot. In order to clearly check the time lags among different population dynamics, the individual number per 10 square meters of predators and pest, and alternative preys biomass per (50×50×10 cm)/2 of soil was used to compare.

## 3.3 Result

### 3.3.1 Population dynamics of wolf spiders

Density of wolf spiders of *Pardosa* spp. (*Pardosa agrestis* accounted for > 70% of all species) was investigated. Wolf spiders in this area have one generation a year and overlapping generations were obvious during through the year. In both greenhouse and open field, in the cold season of January, the spider density in field ground was near to zero but in fact many individuals stayed under the plant residue mulching to overwinter. From early March, these individuals began to come out for capturing alternative diets or mating activities, and population density began to increase with fluctuations.

Generally, over the year of 2010 and 2011, wolf spiders density was significantly higher in the introduction plot than in no-introduction plot (repeated measures ANOVA for two years: $F_{1,\ 132}=117.5$, $P < 0.001$, Table 3.2 and Figure 3.1). There was no significant difference of wolf spiders density between different sites (greenhouse and open field) and years (2010 and 2011). Population dynamics were generally similar between the year 2010 and 2011.

In the greenhouse, in cold season from January to the end of May, the average adult spider density was around eight times higher in introduction than in no-introduction plot (repeated measures ANOVA, $P < 0.001$). From early March, the population density decreased gradually with fluctuations until early June. From mid June, the adult spider density increased with fluctuations and reached a peak period in October with the value as 50-60 individuals/10m$^2$ in introduction plot and 10-20 individuals/10m$^2$ in the no-introduction plot. In the second half year, the average seasonal density was around 4.4 times higher in introduction than in no-introduction plot (repeated measures ANOVA, $P < 0.01$). After late October, the density in both introduction and no-introduction plot began to decrease. However, many individuals hid themselves in overwintering sites under the plant residue strips when the whether got cold in the introduction plot. When temperature rose, many adult individuals came out and lingered in and near the plant residue strips.

In the open field, the population trend of dynamics was similar in greenhouse. In winter season, different from in greenhouse, adult wolf spiders density in the open field was low but in fact many individuals stayed under the plant residue mulching to overwinter. From early February, these individuals began to come out in sunny days for capturing alternative diets or performing mating activities. Population density began to increase from early April, developed with several fluctuations in May, June, July and August, and reached a peak period in the late September-early October. The average seasonal adult wolf spiders density in the introduction plot was 4.0 times higher than that in no-introduction plot through a year (repeated measures ANOVA, $P < 0.01$) (Figure 3.1).

**Table 3.2 Repeated measures ANOVA (P value) of population density of predators in the introduction and no-introduction plot over the two years**

| Predators | **Treatment** | Site | Year | Treatment×site | Treatment×year | Site×year | Treatment×site×year |
|---|---|---|---|---|---|---|---|
| Wolf spider | **<0.001** | 0.437 | 0.927 | 0.921 | 0.452 | 0.350 | 0.847 |
| *Harpalus* spp. | **<0.001** | 0.524 | 0.738 | 0.744 | 0.966 | 0.593 | 0.700 |
| *D.halensis* | **0.001** | <.001 | 0.242 | 0.032 | 0.675 | 0.369 | 0.743 |
| *C.pallipes* | **0.01** | 0.621 | 0.301 | 0.915 | 0.288 | 0.089 | 0.955 |
| Frog | **<0.001** | 0.241 | 0.022 | 0.846 | 0.028 | 0.166 | 0.129 |

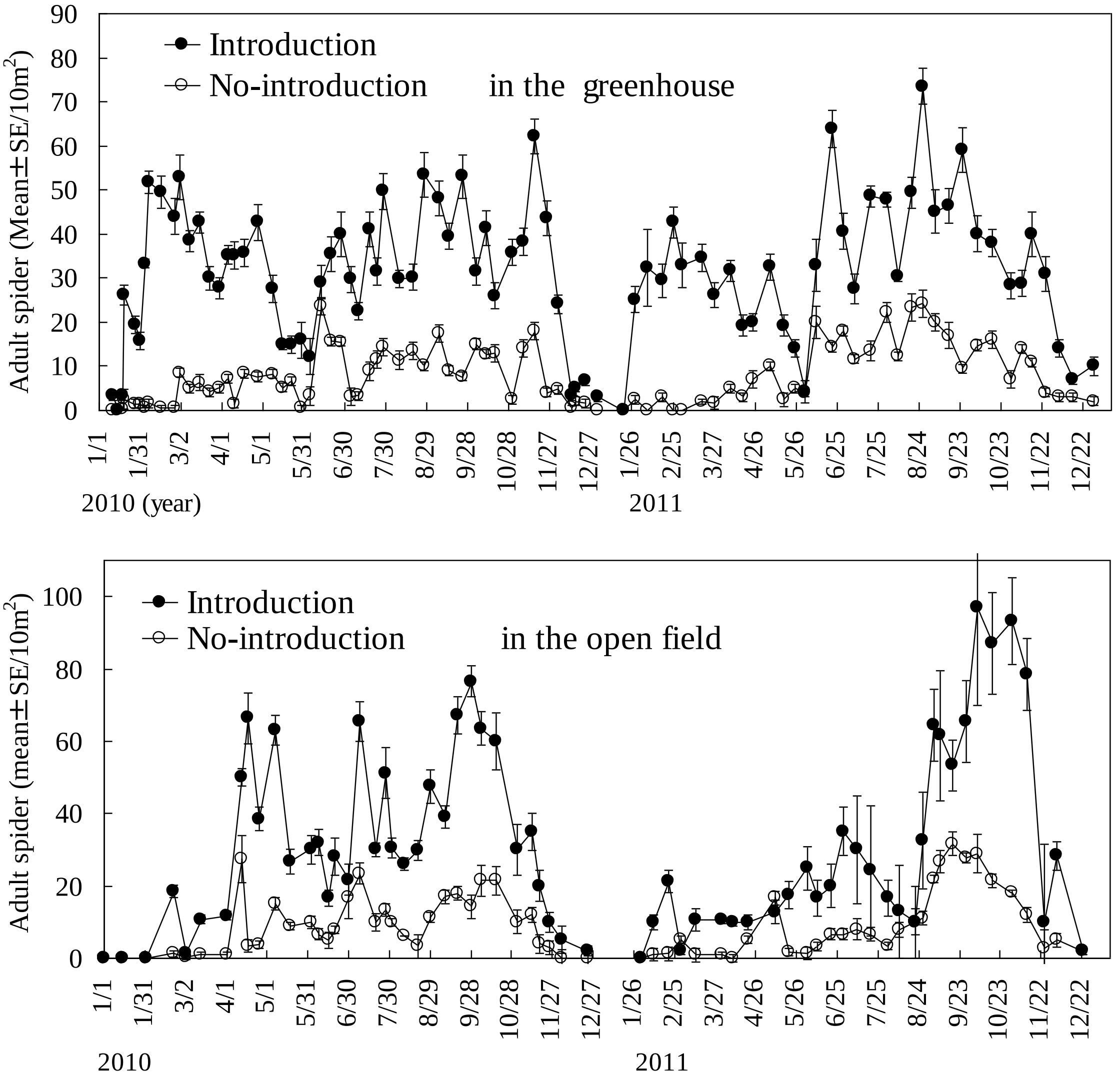


**Figure 3.1 Population dynamics of adult wolf spiders (mean±SE/10m$^2$) in the greenhouse and open field, the error bars were at the 95% confidence level**

### 3.3.2 Population dynamics of predacious carabids

Before June, predacious carabids activity-density was at a relatively low level, and from the beginning of June, it began to increase rapidly and reached peak in the period of August – September. Significant difference of predacious carabids was found between introduction and no-introduction plot was found (repeated measures ANOVA, $P < 0.01$, Table 3.2 and Figure 3.2-3.4). The average density of these predacious carabids was 2.3 – 3.5 times higher in the introduction plot than in no-introduction plot. Generally, there was no significant difference of carabids density between different sites (greenhouse and open field) and years (2010 and 2011) but the *D. halensis* showed significantly higher density in the greenhouse (repeated measures ANOVA, $P < 0.001$).

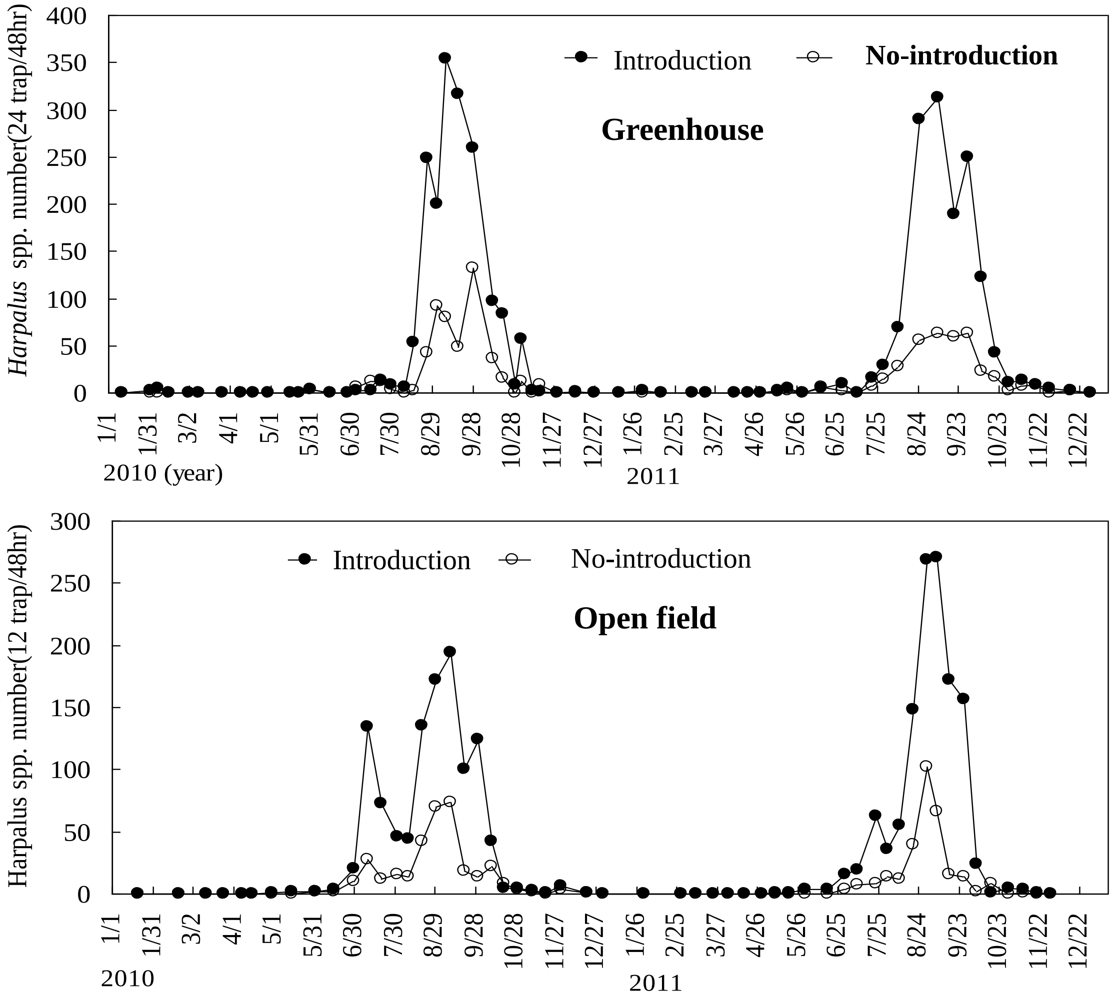


**Figure 3.2 Dynamics of *Harpalus* spp. population in the greenhouse and open field**

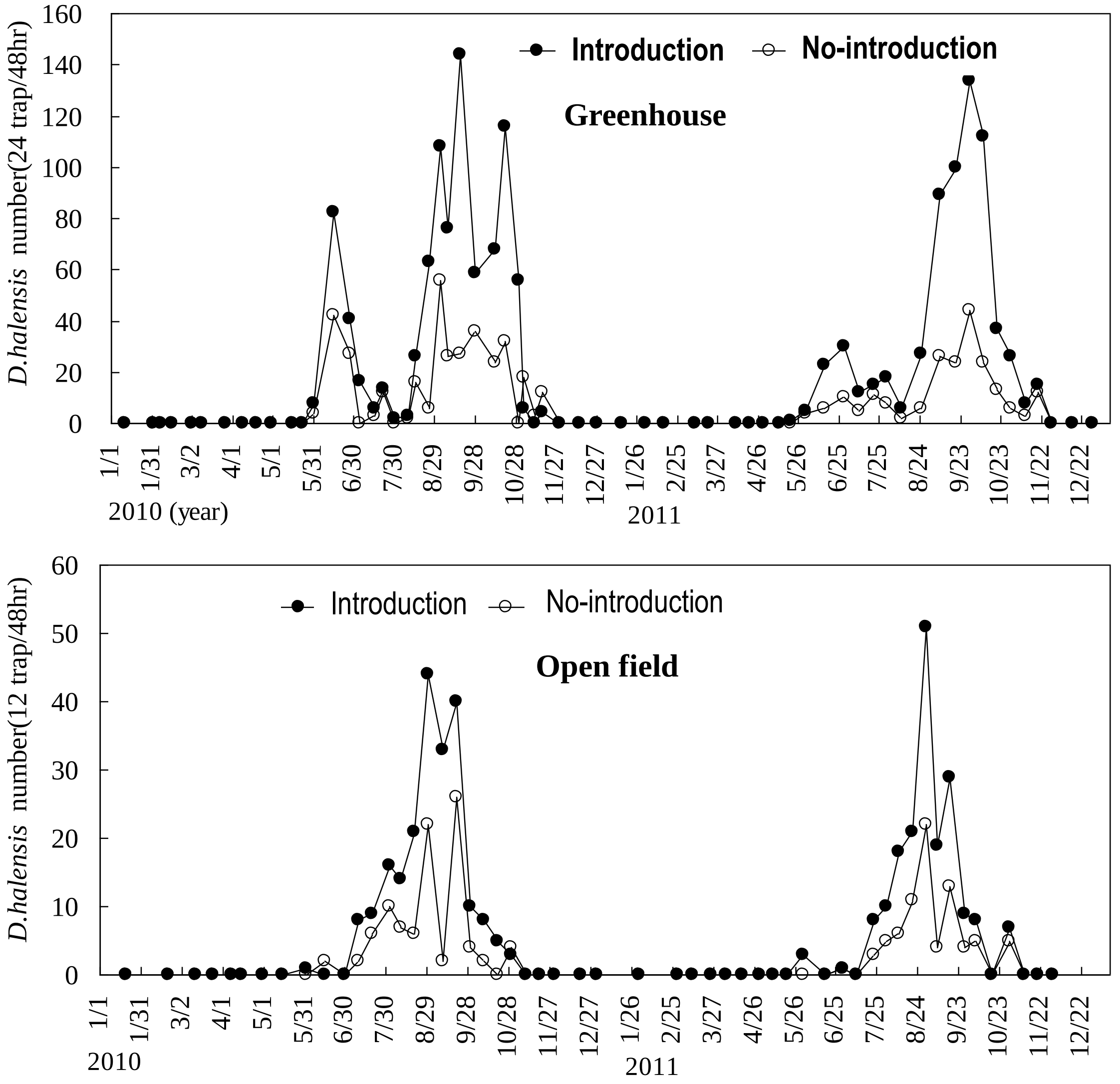


**Figure 3.3 Dynamics of *D. halensis* population in the greenhouse and open field**

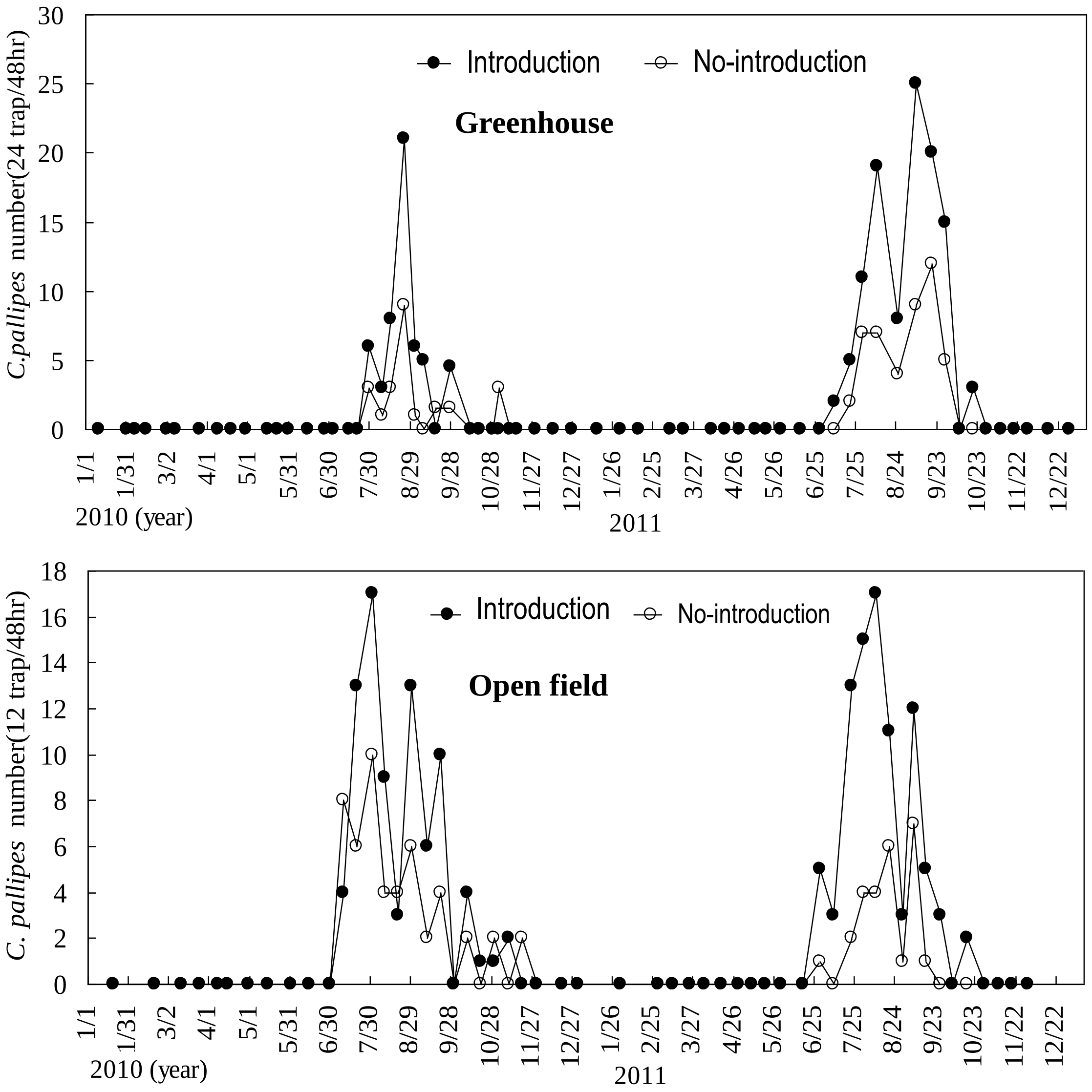


**Figure 3.4 Dynamics of *C.pallipes* population in the greenhouse and open field**

### 3.3.3 Population dynamics of Japanese tree frogs

Japanese tree frog (*Hyla japonica*) was the main frog species with its body size as about 1 × 3 cm. Adult frogs become active from the beginning of June until November. From the beginning of July, frog density began to increase rapidly and reached a high level (10 – 15 individuals/10m$^2$) and kept until later September.

Significant difference of tree frogs was found between introduction and no-introduction plot (repeated measures ANOVA, $P < 0.01$, Table 3.2 and Figure 3.5). The average density of frogs in introduction plot was around 3.0 times over the no-introduction plot. Similar result of frog was obtained in two years.

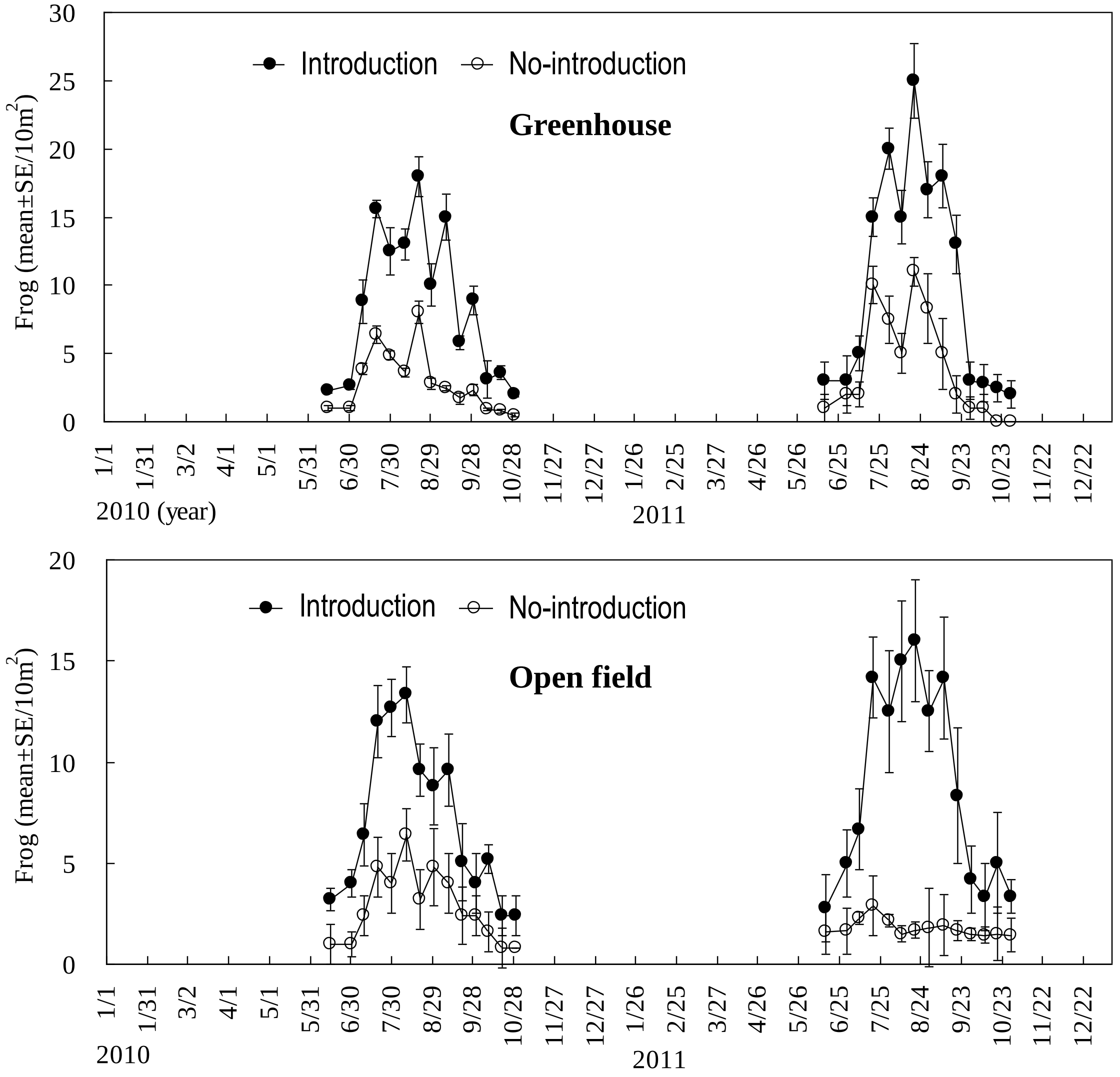


**Figure 3.5 Population dynamics of Japanese tree frogs in the and open field, the error bars were at the 95% confidence level**

### 3.3.4 Population dynamics of pests

The dynamics of pest population were examined based on the larva density of lepidopteran pest (including *P. rapae*, *P. xylostella*, *A. agnate* and *S. litura* and density of *P.rapae* accounted for > 60% of all pest species) from early July to later September. In order to well understand the development of pest population, the larvae was divided into three groups as young instar (2nd instar), mid instar (3rd instar) and old instar (4-5th instar). As the body size of *P. xylostella* was relatively small, the total number of all instars was recorded.

Generally, pest population began to increase from early July with fluctuations and reached an abundant period from mid August to mid September, and decreased after later September.

Generally, over the year of 2010 and 2011, population densities of pests were significantly or marginally significantly lower in the introduction plot than in no-introduction plot (repeated measures ANOVA for two years, Table 3.3 and Figure 3.6-3.9). The pest densities in no-introduction plot were 1.8 – 3.0 times higher than in introduction plot (pest population density decreased by 45-67%). There was no significant difference between two years, and the interaction of treatment × sites, treatment × year and site × year was not significant (Table 3.3). Among several pest species, *P. rapae* showed the largest density difference between the introduction and no-introduction plot (P = 0.002). *P. rapae* and *P. xylostella* showed significantly higher density in the greenhouse than in the open field (Table 3.3).

**Table 3.3 Repeated measures ANOVA (P value) of population density of pests in the introduction and no-introduction plot over the two years of 2010 and 2011.**

| Pests | | **Treatment** | Site | Year | Treatment×site | Treatment×Year | Site×year | Treatment×site×year |
|---|---|---|---|---|---|---|---|---|
| *P.rapae* | young | **0.007** | <0.001 | 0.067 | 0.087 | 0.534 | 0.644 | 0.969 |
| | mid | **0.040** | 0.020 | 0.274 | 0.316 | 0.670 | 0.758 | 0.855 |
| | old | **0.001** | 0.002 | 0.220 | 0.097 | 0.419 | 0.207 | 0.322 |
| | total | **0.002** | <0.001 | 0.150 | 0.098 | 0.573 | 0.235 | 0.902 |
| *P.xylostella* | | **0.009** | 0.038 | 0.040 | 0.558 | 0.316 | 0.525 | 0.892 |
| *A.agnata* | young | **0.009** | 0.054 | 0.072 | 0.333 | 0.926 | 0.045 | 0.666 |
| | mid | **0.008** | 0.106 | 0.302 | 0.403 | 0.734 | 0.804 | 0.957 |
| | old | **0.085** | 0.885 | 0.110 | 0.870 | 0.710 | 0.118 | 0.683 |
| | total | **0.010** | 0.388 | 0.036 | 0.772 | 0.905 | 0.018 | 0.596 |
| *S.litura* | young | **0.061** | 0.090 | 0.084 | 0.733 | 0.707 | 0.346 | 0.867 |
| | mid | **0.046** | 0.779 | 0.885 | 0.915 | 0.924 | 0.800 | 0.110 |
| | old | **0.058** | 0.236 | 0.747 | 0.481 | 0.992 | 0.414 | 0.956 |
| | total | **0.031** | 0.405 | 0.141 | 0.932 | 0.492 | 0.167 | 0.161 |

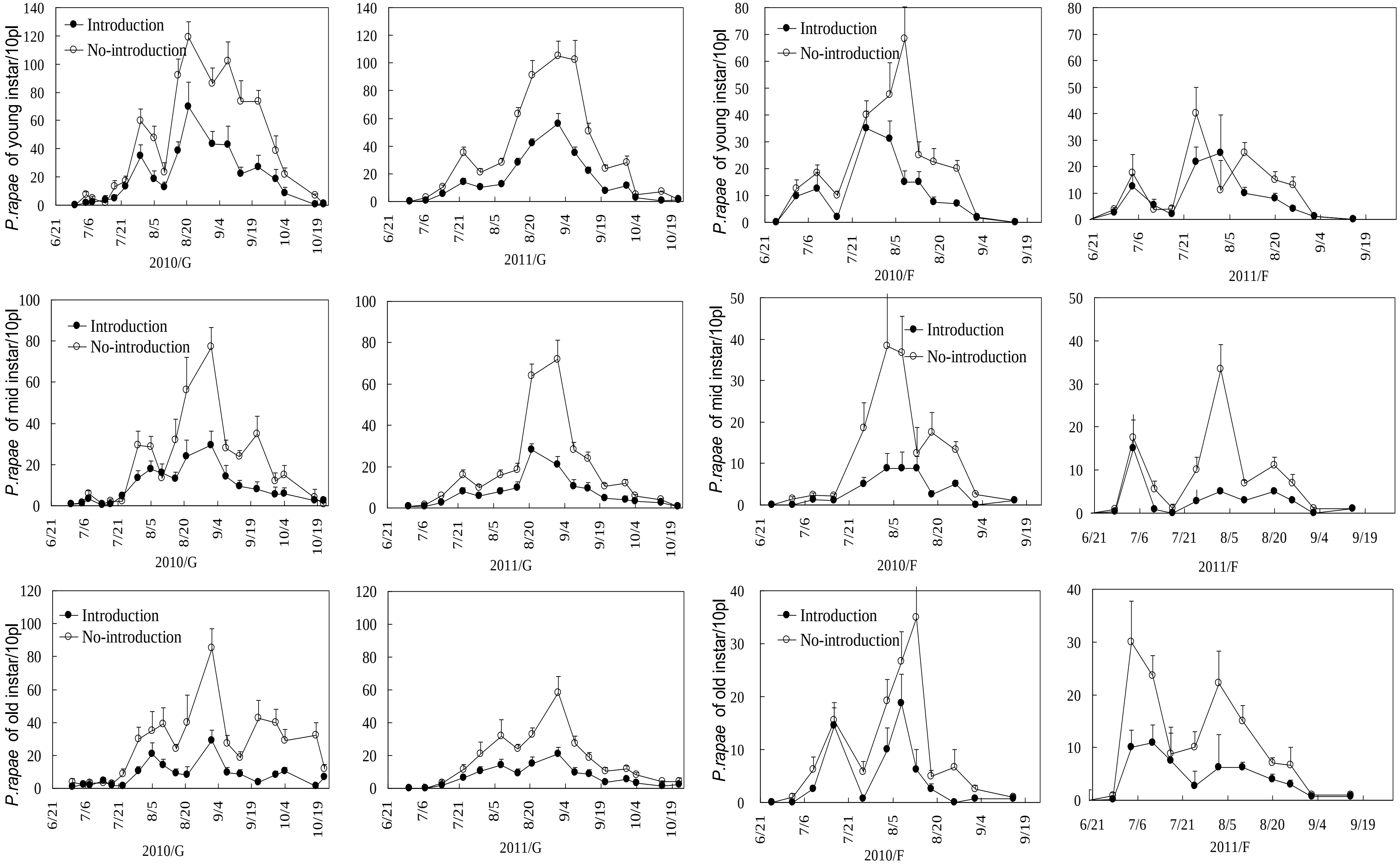


**Figure 3.6 Population dynamics (mean±SE/10m$^2$, 95% confidence) of *P. rapae* larvae in greenhouse (G) and open field (F), from upper: young instar (2nd instar), mid instar (3rd instar) and old instar (4-5th instar)**

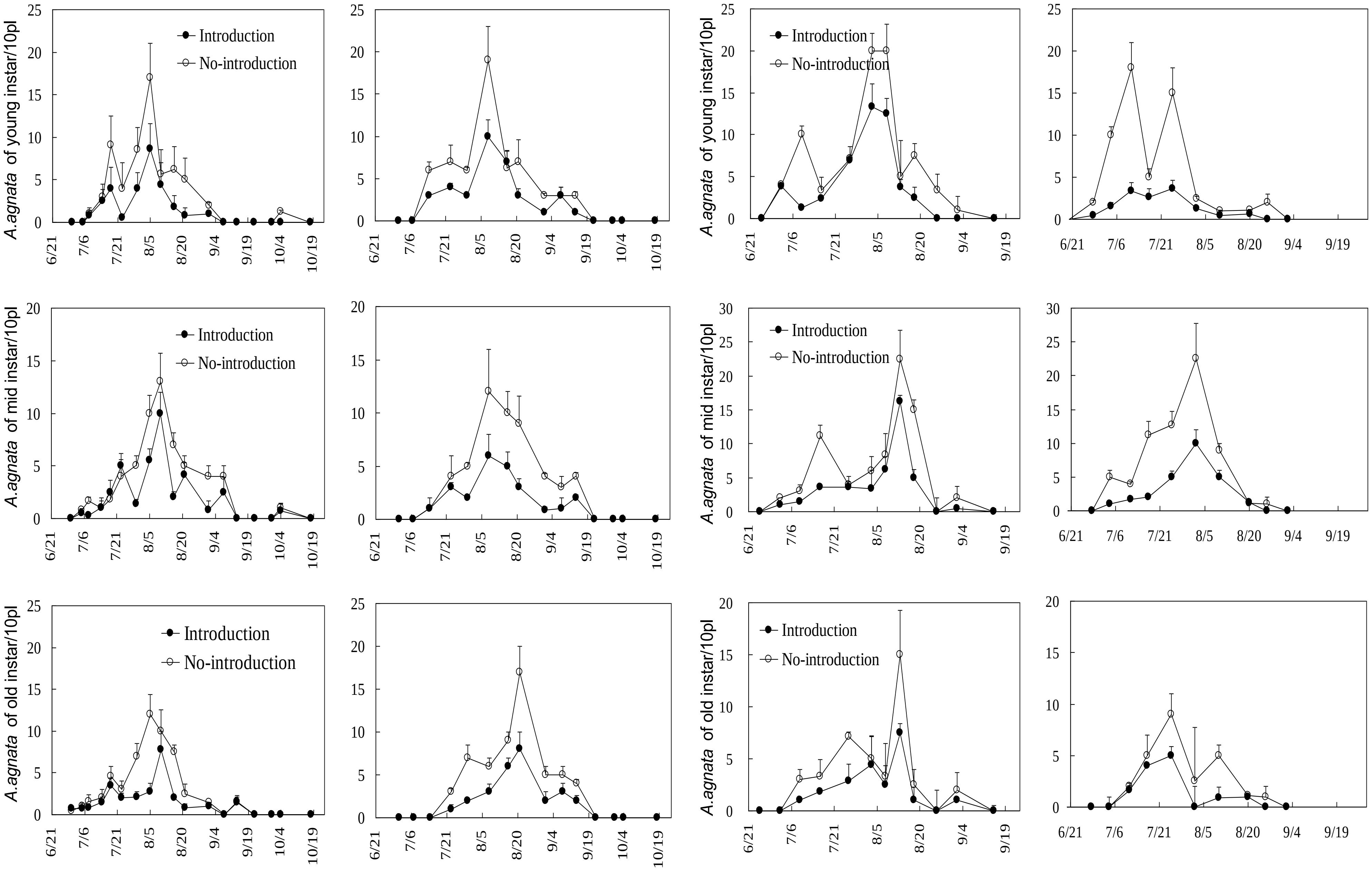


**Figure 3.7 Population dynamics (mean±SE/10m$^2$, 95% confidence) of *A.agnata* larvae in greenhouse (G) and open field (F), from upper: young instar (2nd instar), mid instar (3rd instar) and old instar (4-5th instar)**

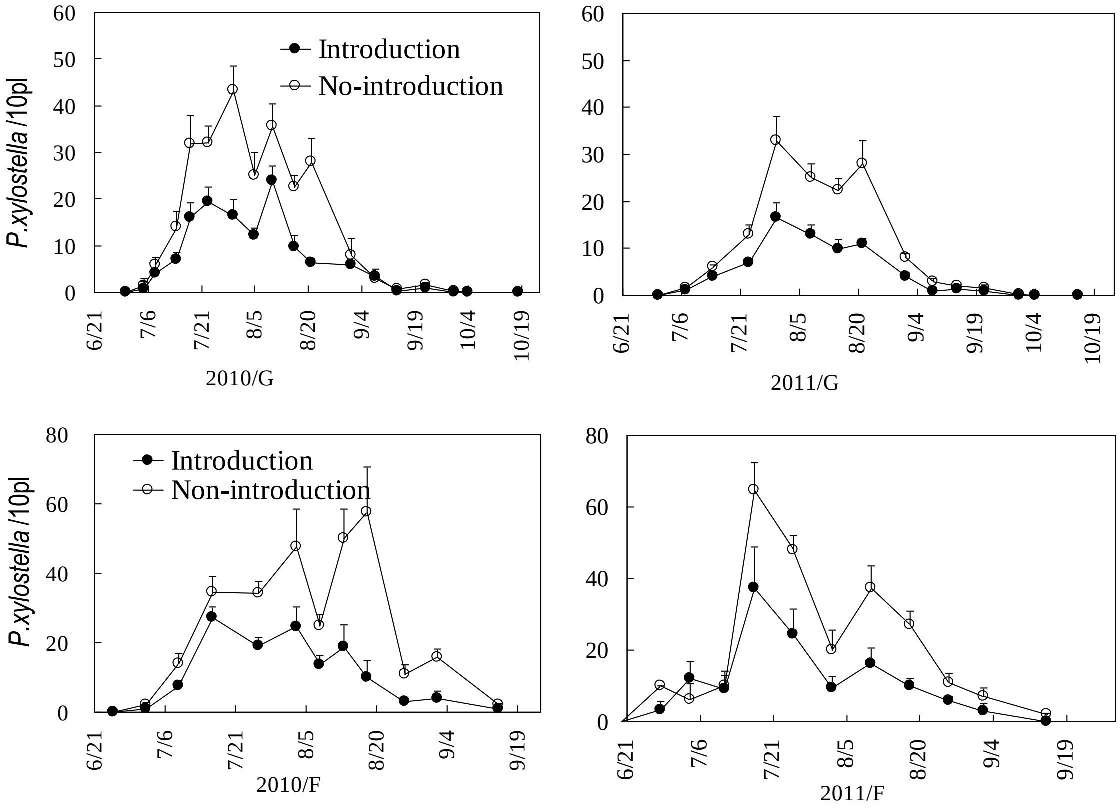


**Figure 3.8 Population dynamics (mean±SE/10m$^2$, 95% confidence) of *P. xylostella* larvae in greenhouse (G) and open field (F)**

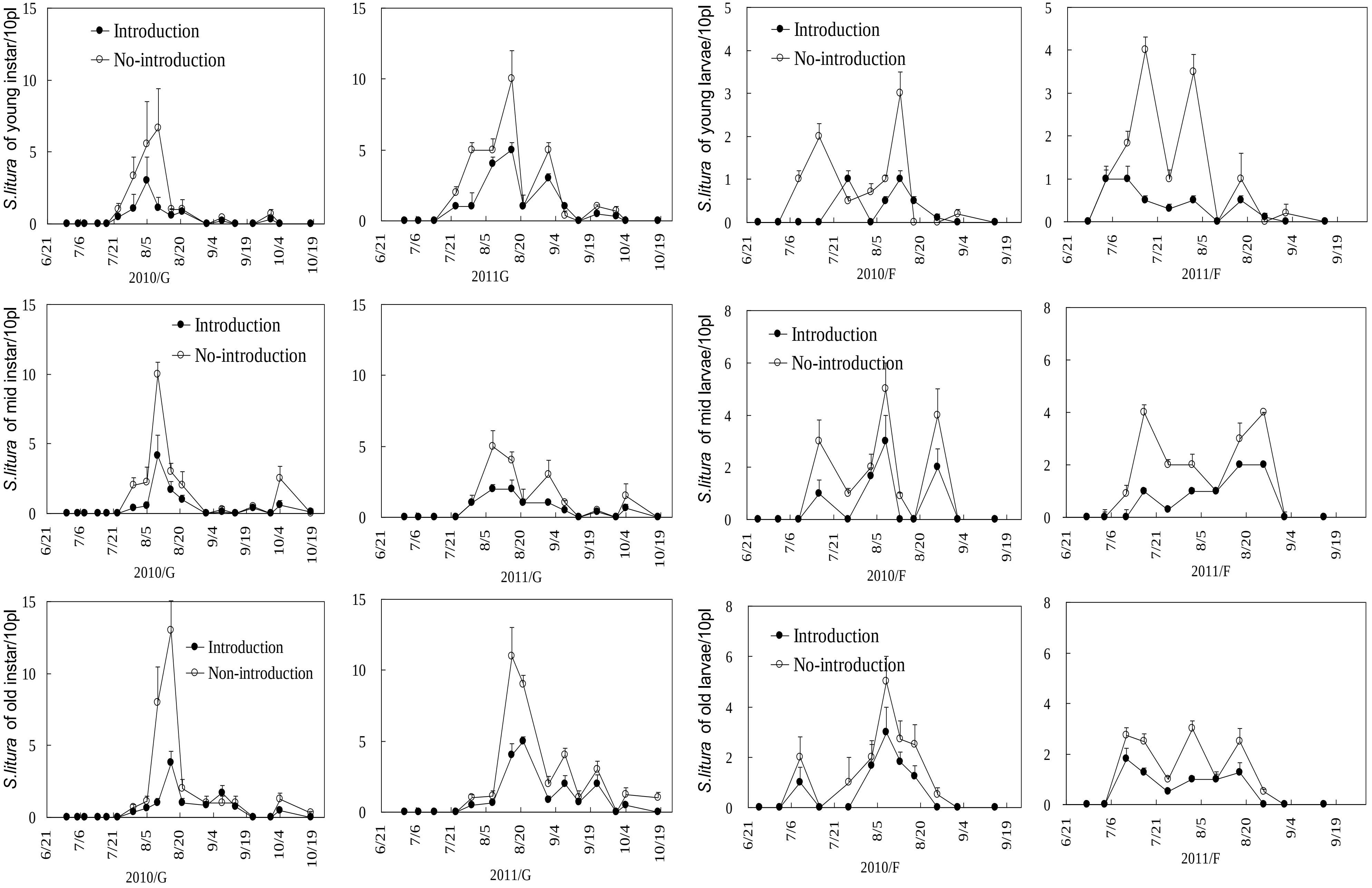


**Figure 3.9 Population dynamics (mean±SE/10m$^2$, 95% confidence) of *S. litura* larvae in greenhouse (G) and open field (F), from upper: young instar (2nd instar), mid instar (3rd instar) and old instar (4-5th instar)**

### 3.3.5 Relations between the population dynamics of predators, alternative preys and pest

From the data through two years, three points was found. First, before the rapid increasing of pest population (around mid July), population density of wolf spiders had already entered a rapid-increase stage and reached a relatively high level. The high density of wolf spiders encountered the rapid-increase of pest, which may contribute to the pest suppression (Figure 3.10).

Second, in the introduction plot, wolf spider population increased with pest population and soon the latter obviously decreased, and one of peaks of wolf spiders followed the peak of pest (time lag as 10 – 30 days). Combined with the obvious reduction of pest in the introduction plot, it was indicated that the wolf spiders could suppress the pests in the introduction plot.

Third, before the occurrence of target pest, the alternative prey population was at a increasing stage. The introduction plot showed higher wolf spider density and higher alternative prey density than the no-introduction plot, which indicated that the high density of wolf spiders in the introduction plot might be supported by alternative preys.

In some cases, before the rapid-increase of pest population, the density of predacious carabids such as *Harpalus* spp. and *C. pallipes* had not reached a higher level, but the density peaks of them usually followed the peak of pest (time lag as 10 – 30 days) (Figure 3.11). Therefore, pest-control power of carabids might be limited.

In the introduction plot, when pests started appearing, population density of frog had already begun to increase rapidly (Figure 3.12 and 3.13). These frogs with high density encountered with the increasing pests density and could voraciously feed on and reduce them. In contrast, in no-introduction plot, when pest population began increasing, density of frogs was much lower than that in introduction plot, hence pests increase rapidly and reached higher density. It is obvious that the suppression power of natural predators must be limited in the no-introduction plot.

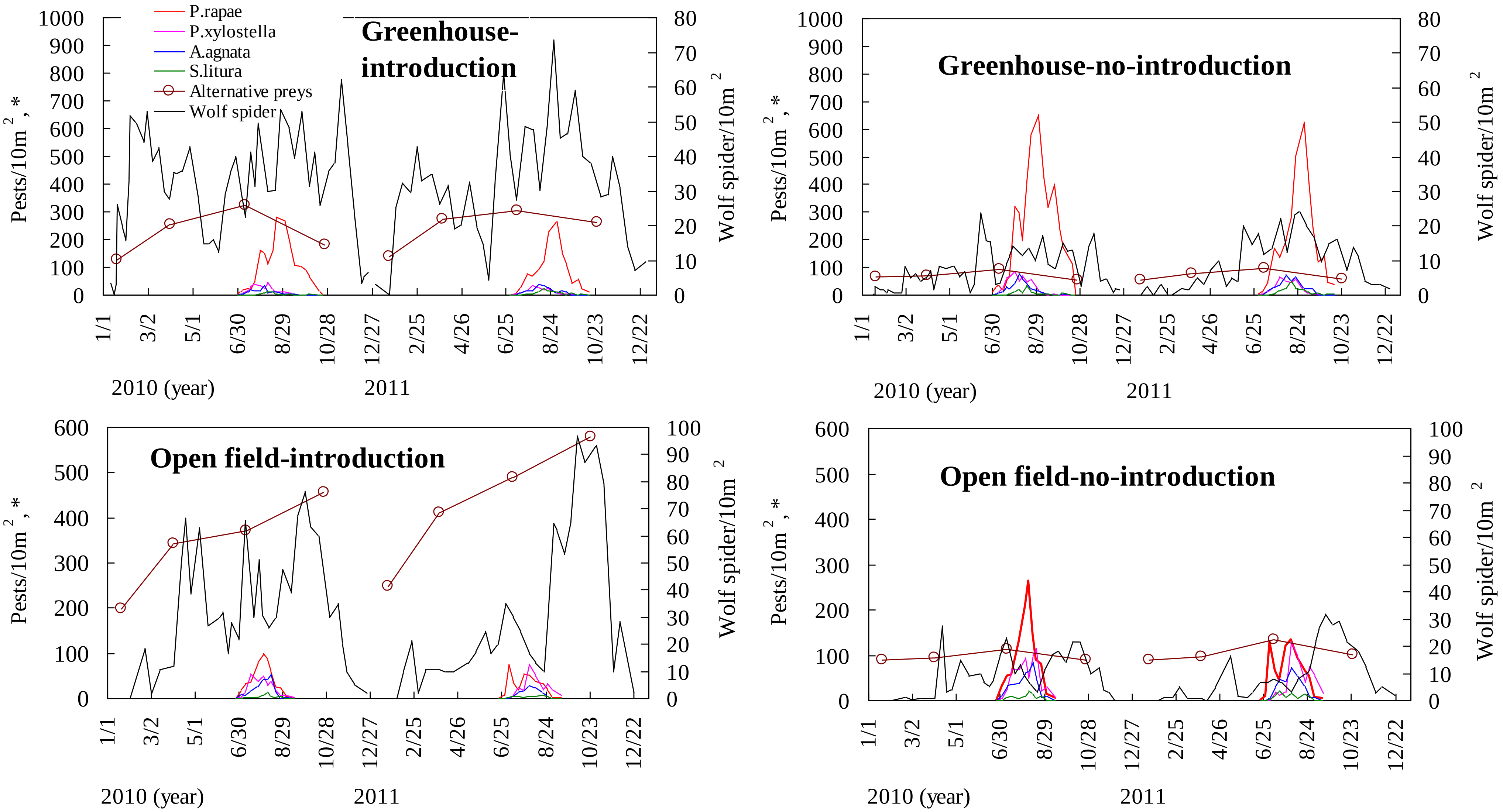


**Figure 3.10 Comparison among population dynamics of wolf spiders, alternative preys and target pests**

For predators, adults were investigated, for pests, total population density of *P. rapae*, *P. xylostella*, *A. agnate* and *S. litura* were included. The alternative preys included groups of Collembola, dipteran larvae, herbivores, coleopteran larvae and diplopods, and their total biomass was expressed by Y axis (* means biomass (mg) of alternative preys per 0.5 ×50×50×10 cm of soil showed by Y axis).

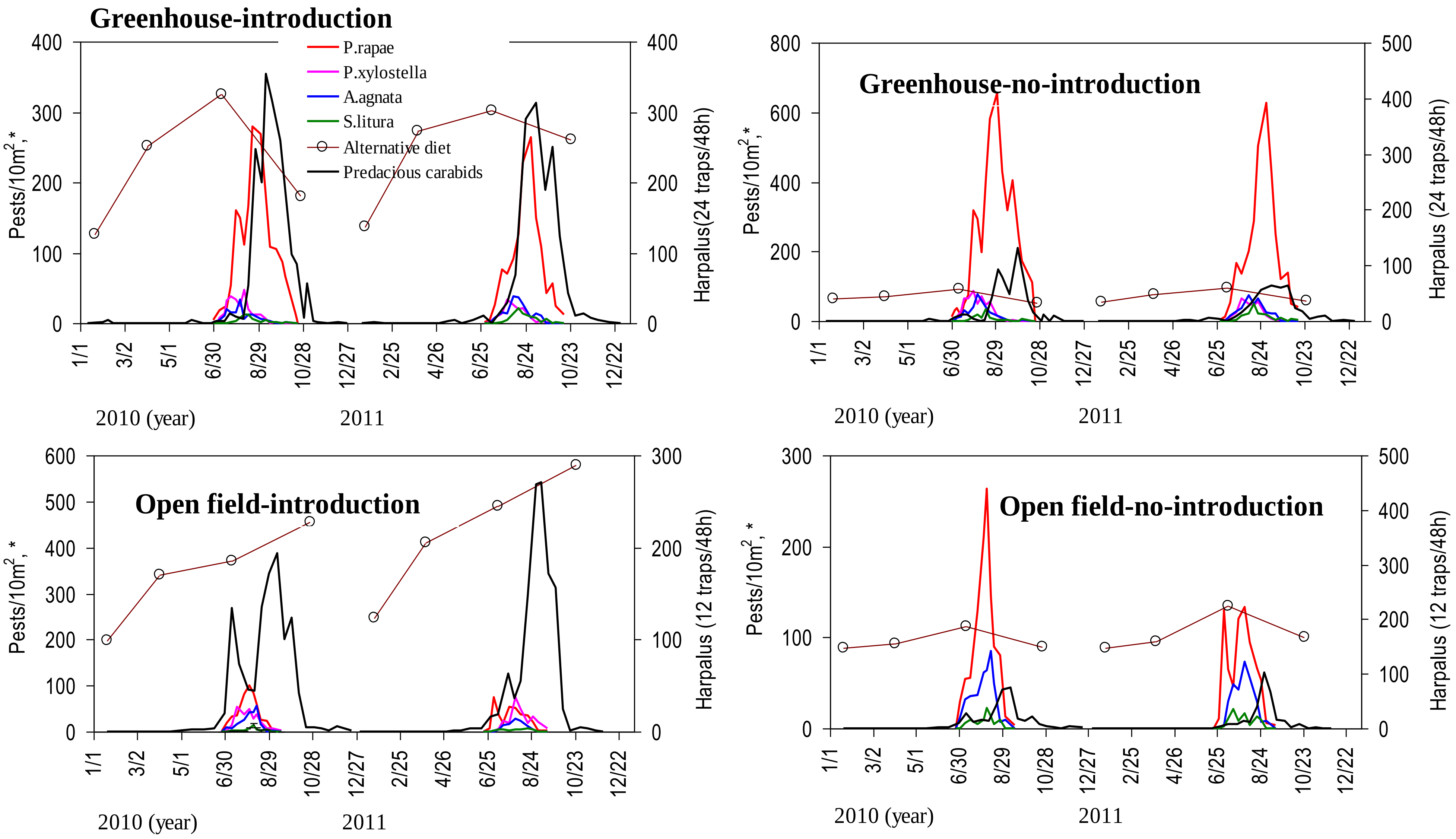


**Figure 3.11-a Comparison among population dynamics of carabid of *Harpalus* spp., alternative preys and target pests**
(For explanation refer to Fig. 3.10).

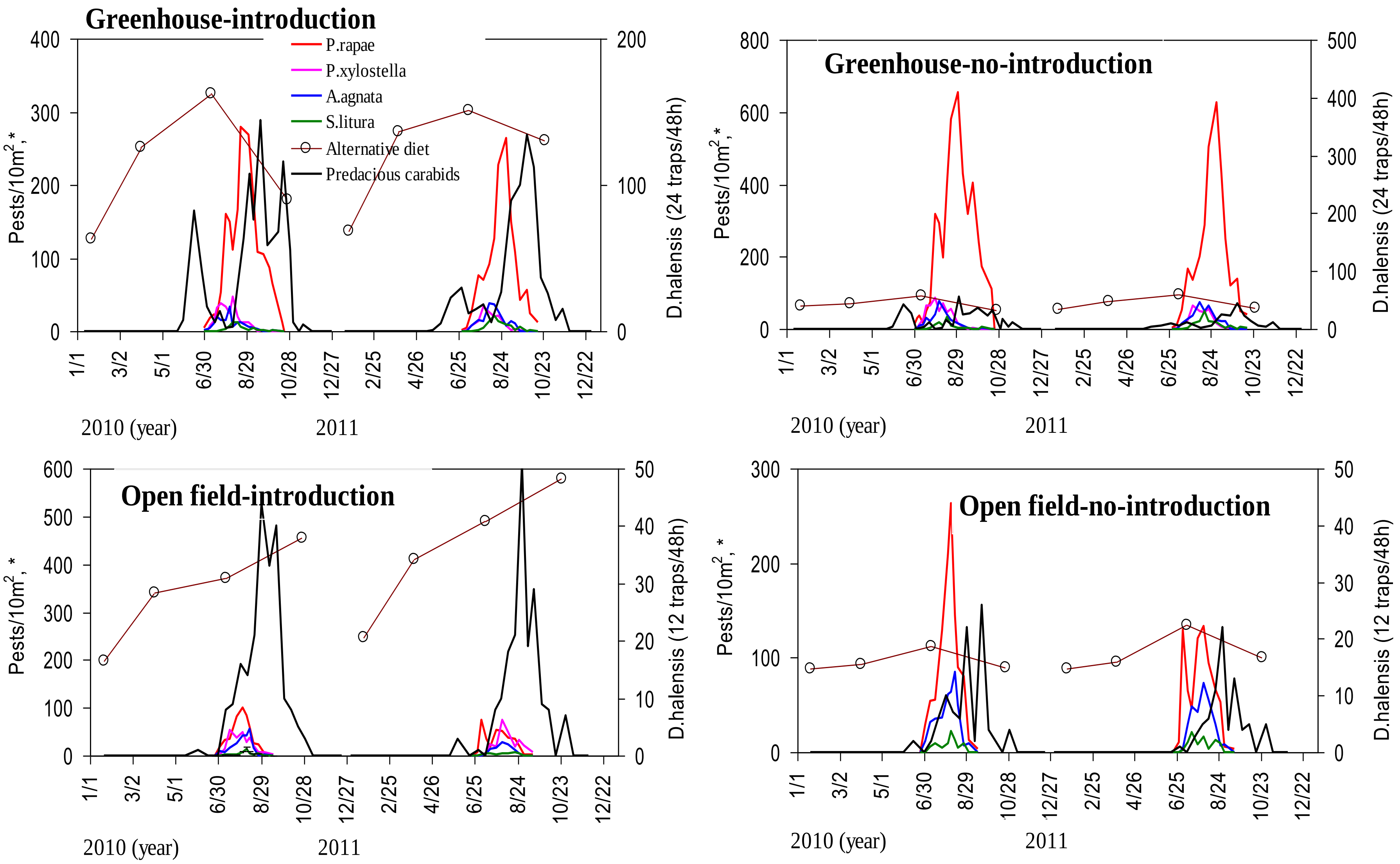


**Figure 3.11-b Comparison among population dynamics of carabids of *D. halensis,* alternative preys and target pests** (For explanation refer to Fig. 3.10).

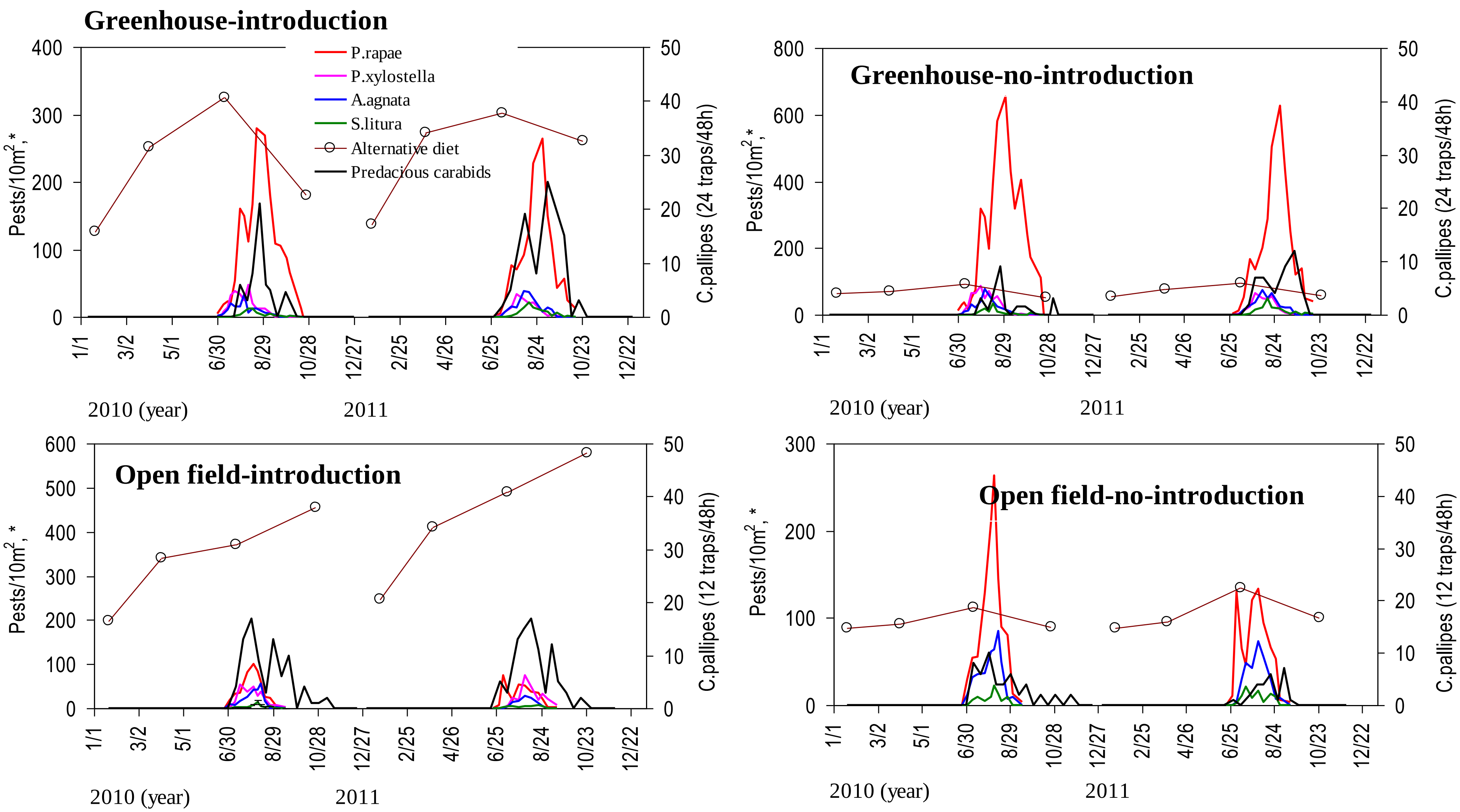


**Figure 3.11-c Comparison among population dynamics of carabids of *C. pallipes*, alternative preys and target pests** (For explanation refer to Fig. 3.10).

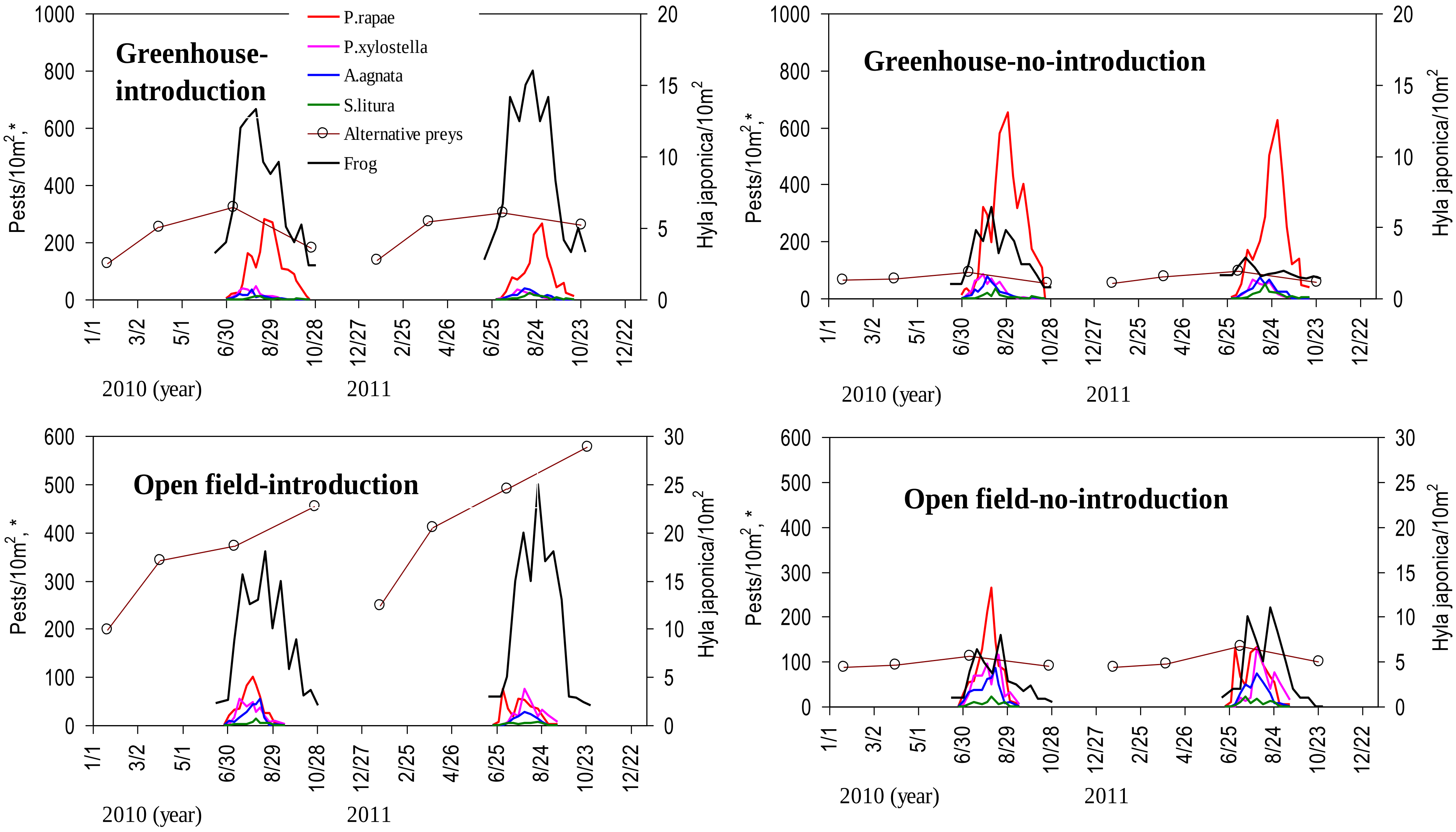


**Figure 3.12 Comparison among population dynamics of tree frog, alternative preys and target pests** (For explanation refer to Fig. 3.10).

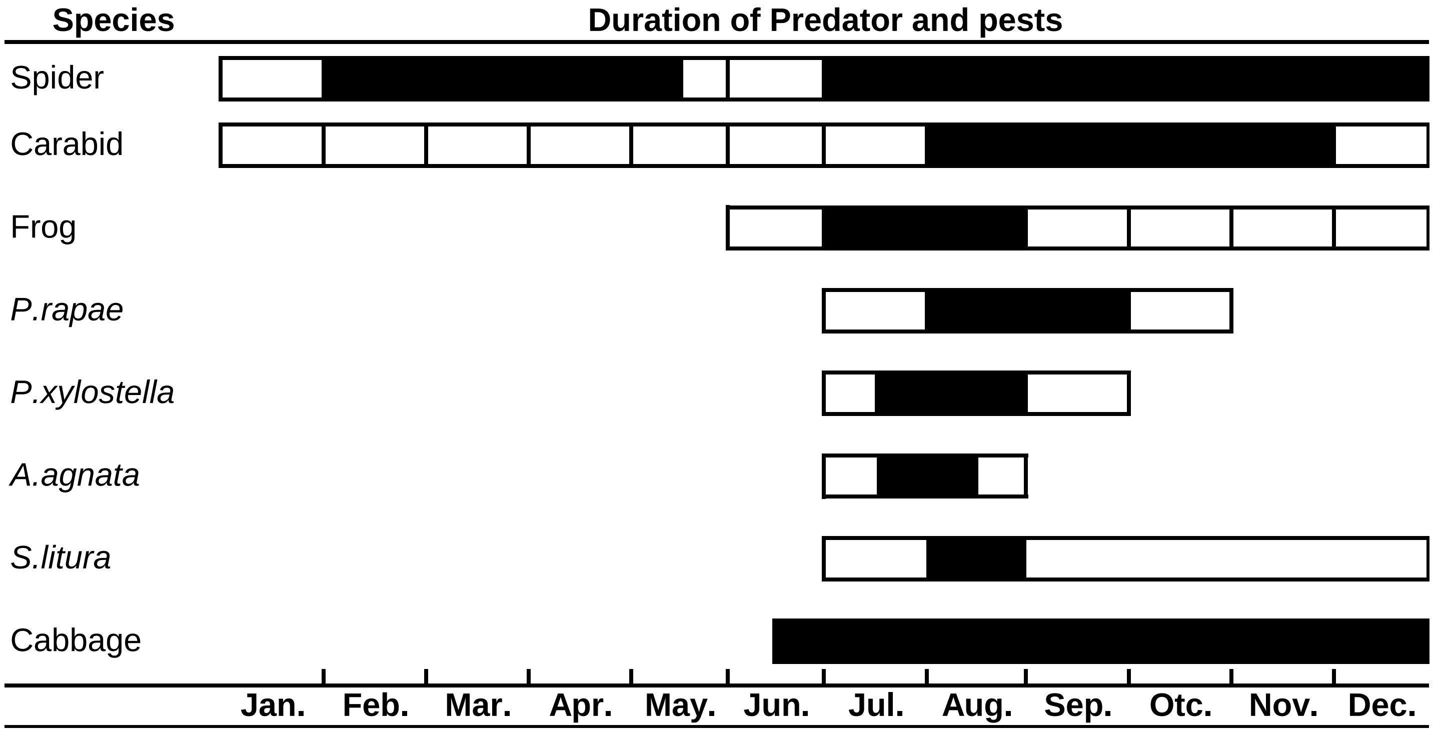


**Figure 3.13 Comparison of occurrence period of predators and pests and the cabbage growth period. The time line was from January to December of the year. Rectangle bars indicate the occurrence period of predators and pests and the black part of rectangle indicates the abundant periods of them.**

### 3.3.6 Damage degree of cabbage

The pest damage degree of cabbage was expressed using the damage index based on the proportion of eaten area of expanded leaves near the ball. During the growth period of cabbage, the pest damage index (PDI) of cabbage increased with the increasing of pest density, and when pest population decreased, the rate of PDI rising become slow and reached a stable level at harvest period of cabbage. The eventual PDI value in the no-introduction plot was 1.8-2.2 times over the introduction plot either in greenhouse or open field (Figure 3.14).

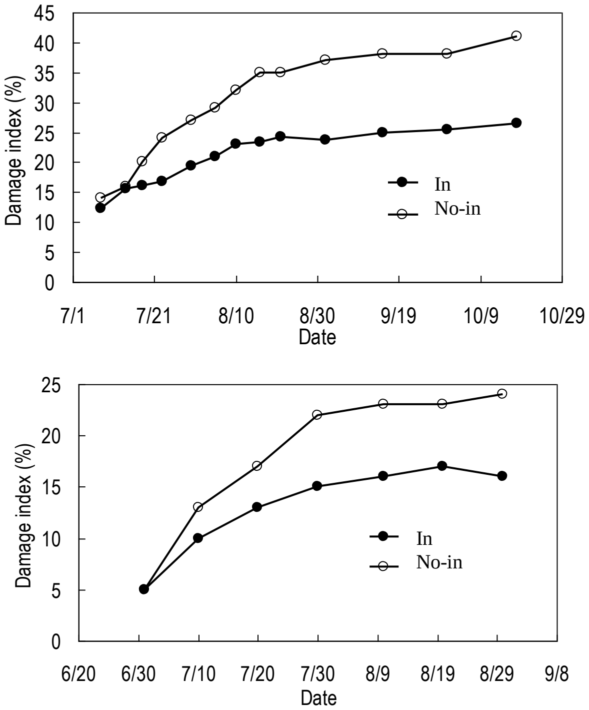


**Figure 3.14 Damage degree of cabbage in greenhouse (upper) and open field (bottom), in, introduction, no-in, no-introduction plot**

## 3.4 Discussion

### 3.4.1 Summary of Chapter 2 and Chapter 3

Until this Chapter, a series of observational and experimental studies were presented. Several points were obtained:

① By preliminary observation, three groups of well-distributed indigenous generalist predators was selected as potential pest control agent;

② The introduction of plant residue into organic farmland could boost alternative preys of generalist predators, and consequently contributed to the pest control of cabbage;

③ Before the rapid increase of pest, population density of predators such as wolf spiders and tree frogs had already reached a high level, which indicated that the enriched alternative preys gave the predator population a ‘head start’ on later-occurring pest population, and eventually the enriched predators could strongly suppress pest density.

However, the evidence of predator-prey relations was not enough and the mechanism of the potential generalist predators is needed to be completed. Therefore, a serial of experiments was designed and performed in Chapter 4 and Chapter 5.

### 3.4.2 Relations between alternative preys and predators

From the previous literature, we can get sufficient information about the alternative preys and indigenous predators. It was proved that wolf spiders can potentially prey on collembolan animals (Aitchison, 1984; Møller Marcussen et al., 1999; Agustí et al., 2003; Lawrence and Wise, 2004; Shultz et al., 2006), dipteran animals (Aitchison 1984), chironomids (Mellbrand et al., 2009; Raikow et al., 2011), coleopteran larvae (Palokangas and Neuvonen, 1992) and herbivores (Heong, 1990; Oraze et al., 1989). Further, in the present plot, it was observed that wolf spiders can feed on larvae or adult of collembolan animals, *Harpalus* larvae, chironomids, drosophilids, muscids and some herbivores (Table 1.1). In plot with plant residue, high density of herbivores may help to attract predators (Janssen, 1999). Predacious carabids were found to prey on dipteran animals, earth worm (Symondson et al., 2006), grasshopper, slug, housefly (Fawki and Toft, 2005). It was observed that predacious carabids including *D. halensis* and *C. pallipes* showed predatory capacity to some phytophagous carabids larvae in the present plot (Table 1.1). As reported by Hirai (2007), Japanese tree frogs can prey on animals of

Diptera, Coleoptera, Collembola, Formicidae, Diplopoda, Araneae, Chilopoda (Table 1.1). Therefore, due to such predator-prey relations, the enriched alternative preys would provide ample food sources for indigenous generalist predators and contribute to the enrichment of them.

### 3.4.3 Meaning of high density of predators in plant residue mulch in winter season

In winter season from December to January, most of daytime, wolf spiders and tree frogs hide themselves under the mulch of plant residue and spiders occasionally come out on some warm sunny days (Figure 3.15). It was indicated that such overwintering wolf spiders in plant residue mulch contributed to the population increase in spring, and then contributed to the formation of 'head start' before the occurrence of pests in summer season (Table 3.1). Such process in detail is needed to be more cleared in future work.

### 3.4.4 Different influences of plant residue to various predators

As shown in Table 3.4, compared to the no-introduction plot, in the introduction plot, the average increase degree of densities of various predators was different. The wolf spiders showed the largest density ratio of introduction : no-introduction (3.4 - 4.0), the next was *Harpalus* spp. (3.5), tree frogs (3.1), *D. halensis* (2.8) and *C. pallipes* (2.3). Such difference indicated that wolf spiders were more dependent on plant residue system. This may because wolf spiders with high activity could easy get sufficient alternative preys in the introduction plot. It was reported that ground beetles was sensitive to environmental disturbance (Rainio and Niemelä, 2003), thus the adaptability of *D.halensis* and *C.pallipes* in the present study was weaker than other predators. The optimum utilization of these predators for pest control need continuous studied in future.

### 3.4.5 Other effects on the pest density

Some studies confirmed that the change of microclimate of field could affect the distribution and emergency of pest (Stigter 1984; Gethi et al., 1993). In the present study, according to our observation, there was no difference of microclimate around the expanding cabbage leaves including temperature and humidity between the introduction and no-introduction plot. Adults of the four species of pests usually lay eggs on expanding leaves, thus the influence of microclimate to pest development was similar between the plots. Preliminarily we have made the same design of plant residue introduction in a conventional farmland, the microclimate there was similar in the organic field with the same plant residue treatment, but the density of predators was much smaller than in organic field, and eventually, we found that the pest damage degree in the conventional field was much higher than in organic field. Therefore, in the present study, it was believed that the pest suppression was mainly caused by predators in the plot with plant residue introduction in organic field. And it is still important to check other influence of plant residue to the pest population in future studies.

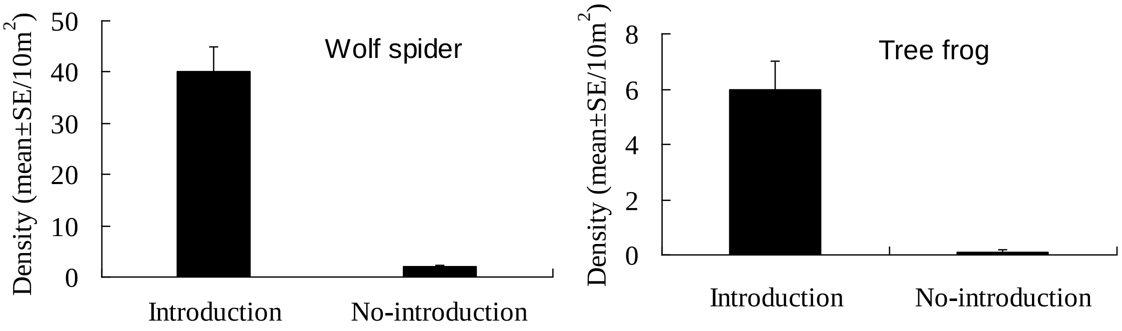


**Figure 3.15 Overwintering wolf spiders and tree frogs in introduction and no-introduction plot in December – January, the error bars were at the 95% confidence level**

**Table 3.4 Ratio of predators in the introduction and no-introduction plot**

| Taxa | Introduction/No-introduction |
|---|---|
| Wolf spider | 3.4-4.0 |
| Predacious carabids | |
| *D.halensis* | 2.8 |
| *C.pallipes* | 2.3 |
| *Harpalus spp.* | 3.5 |
| Japanese tree frog | 3.1 |

# Chapter 4 Evaluation of the predatory capacity of indigenous predators to target pests

## 4.1 Introduction

Usually in farmland, there are various species of predators but many of them may not be the effective pest control agents. This might because: 1) some predators seldom select pests as their diet or their predatory capacity on pests is poor; 2) even though they have strong predatory capacity on pests, their population density is too low and hence the predation power is weak; and 3) during period without pest, predators can not well find alternative resources for the persistence of their populations. In my previous study, it was found that the indigenous predators including wolf spiders, predacious carabids and Japanese tree frogs can avoid the disadvantages mentioned above in (2) and (3). Now, it is meaningful to understand the predatory capacity (functional response) of these predators to pests to examine whether they can be appropriate pest control agent in farmland.

Functional response in ecology is the change of consumption rate of a consumer as a function of diet density. Three basic types of responses had been identified: type I in which the consumption rate of a consumer increases linearly with prey density and then reaches a constant value when the consumer is satiated, type II in which the attack rate increases at a decreasing rate with prey density until it becomes constant at satiation, type III in which the attack rate accelerates at first and then decelerates towards satiation (Holling, 1959).

In this chapter, the functional response of predators including wolf spiders, predacious carabids and Japanese tree frogs on lepidopteran pest larvae was tested to understand the theoretical predatory capacity of these indigenous predators to pests. And combining the result of functional response and the observation of predators' behavior in field, the actual predation capacity of indigenous predators in field was estimated.

## 4.2 Materials and methods

**Source of pests and predators.** Larvae of *P. rapae, P. xylostella*, *A. agnata* and *S.litura* were collected from cabbage plant in the field. Adult predators including wolf spiders (*P. agrestis*), *D. halensis*, *C. pallipes* and *H. griseus* were collected from the cabbage field.

**Apparatus design.** The experiment was performed in laboratory with the temperature as 25℃. Before beginning, for spider and carabids, a piece of wet filter was placed into a petri dish (d = 8.7 cm) and a piece of cabbage leaf (d = 5 cm) was placed on the wet filter. For frog, wet filter and cabbage leaf were placed into a plastic pot with the basal diameter of 10 cm and height of 10cm.

**Predatory capacity of predators on different larvae of pest.** Body size of pest larvae affect feeding amount of predators. Before the testing of functional response, the predatory capacity on different ages of pest was tested. Individuals of *P. rapae* larvae of different age (young, mid and old) and one adult predator were placed into container mentioned above. The setting was lasted for 24 hours and the number of remaining larvae was recorded. The number of prey consumed was then calculated as the initial abundance minus the number of remaining prey. Each treatment was replicated three to five times.

**Functional response.** One predator individual and *P.rapae* larvae (2nd instar) with different density gradient were introduced into the container. The density gradient of pests larvae was 0, 3, 5, 9, 15, 20, 25 individuals per container. Before the beginning of experiment, the predators were subjected to starvation for 12 hours. After the predator was placed into container, the treatment lasted for 24 hours and the number of remaining larvae was recorded. The number of prey consumed was calculated as the initial abundance minus the number of remaining prey. Each treatment was replicated three to four times. Each feeding event was conducted with a unique individual predator and unique set of prey.

Holling type II functional response was used to fit the experimental data (Holling, 1959) by using software of Excel and SPSS 16.0. The equation is: $Na=aNT/(1+T_h aN)$, where $N_a$ is consume rate of predators, $N$ is food density, $a$ is rate at which the consumer encounters food items per unit of food density and is called the attack rate, $T_h$ is handling time, the average time spent on processing a food item.

**The estimation of predation ability of predators in field.** Because of the different condition between farmland and laboratory, the predation capacity of predators on pest in the field would be smaller than that in laboratory. Therefore, based on the result of functional response, a formula was presented to estimate the predation ability of predators in field.

## 4.3 Result

### 4.3.1 Predatory capacity of predators on *P. rapae* larvae of different instars

Predators showed larger feeding number on 2nd *P. rapae* instar than on old instar (Table 4.1). Predacious carabids and Japanese tree frogs could prey on both young and old larvae while wolf spiders usually only fed on young larvae. Thus, the 2nd instar was used for functional response test.

**Table 4.1 Predatory capacity of predators on *P.rapae* larvae of different age**

| Age of larvae | Body length (mm) | No. of larva for preying | | Predators | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Wolf spider | *D. halensis* | *C. pallipes* | *H. griseus* | *H. japonica* |
| 2nd instar | 8~12 | 25 | Eaten number | 12~15 | 15~20 | 16~20 | 8~10 | 15~21 |
| | | | Fresh weight of the eaten (mg) | 180-250 | 150~300 | 160-300 | 80-150 | 150~300 |
| 3rd | 13~15 | 25 | Eaten number of *P.rapae* | 0 | 5~8 | 5~8 | 0-2 | 5~10 |
| | | | Fresh weight of the eaten (mg) | 0 | 200~400 | 200~400 | 0-100 | 200-500 |
| 4-5 instar | 22~29 | 25 | Eaten number of *P.rapae* | 0 | 2~4 | 3~5 | 0 | 4~6 |
| | | | Fresh weight of the eaten (mg) | 0 | 200-500 | 300-500 | 0 | 400-800 |

### 4.3.2 Functional response of predators on pests

As shown in Table 4.2, wolf spiders, predacious carabids and frogs showed strong predation ability on the 2nd larvae of *P. rapae, A. agnata*, *P. xylostella* and *S. litura*. The predatory capacity of *D. halensis*, *C. pallipes* and *H. japonica* on pest larvae was stronger than spiders and *H. griseus*. For one predator individual, its consumption rate decreased with the density rise of given prey, and then become stable. Functional response of all predators in present experiment fitted the equation of Holling type II as shown in Table 4.3 and Figure 4.1-4.5.

The attack rate (*a*), also called instantaneous preying rate, is a rate at which the consumer encounters food items per unit of food density, it defines how steeply the curve approaches the upper asymptote. *D. halensis, C. pallipes* and *H. japonica* showed the similar attack rate to pests, thus, they had similar abilities to search lepidopteran larvae, and *H. griseus* showed lower attack rate. A certain predator showed similar attack rate to four species of pests.

Handling time ($T_h$) is the average time spent on processing a food item and the reciprocal of $T_h$ is the theoretical maximal preying quantity. Of four species of pests, the wolf spider showed the highest $T_h$ to *P. xylostella* (54.7min) and the lowest to *A. agnata* (46.9 min). *D. halensis* showed the highest $T_h$ to *P.xylostella* (23.0min) and lowest $T_h$ to *A. agnata* (15.8min). *C. pallipes* showed the highest $T_h$ to *P. xylostella* (25.9min) and lowest $T_h$ to *S. litura* (15.8min). *H. griseus* showed the highest $T_h$ to *P.xylostella* (82.1 min) and lowest $T_h$ to *A. agnata* (63.4 min). *H. japonica* showed the highest $T_h$ to *P. xylostella* (33.1 min) and lowest to *A. agnata* (17.3 min) (Table 4.2). As the reciprocal of $T_h$ (max$N_a$) is the maximal preying number of pests, the lower value of $T_h$ means the higher theoretical number of pests captured by predators per day.

**Table 4.2 Parameters estimated by Holling type II response**

| Predators | Parameters | Species of prey | | | |
|---|---|---|---|---|---|
| | | *P.rapae* | *P.xylostella* | *A. agnate* | *S.litura* |
| Wolf spider | a | 1.195 | 1.336 | 1.489 | 0.916 |
| | $T_h$ (day) | 0.036 | 0.043 | 0.039 | 0.037 |
| | max*Na* | 27.778 | 23.256 | 25.641 | 27.027 |
| | $R^2$ | 0.989 | 0.993 | 0.969 | 0.997 |
| *D. halensis* | a | 1.082 | 1.157 | 1.143 | 1.122 |
| | $T_h$ (day) | 0.013 | 0.017 | 0.011 | 0.014 |
| | max*Na* | 76.923 | 58.824 | 90.909 | 71.429 |
| | $R^2$ | 0.999 | 0.997 | 0.995 | 0.990 |
| *C. pallipes* | a | 0.900 | 0.900 | 1.133 | 0.820 |
| | $T_h$ (day) | 0.011 | 0.018 | 0.014 | 0.011 |
| | max*Na* | 90.909 | 55.556 | 71.429 | 90.909 |
| | $R^2$ | 0.999 | 0.997 | 0.975 | 0.980 |
| *H. griseus* | a | 0.661 | 0.700 | 0.800 | 0.860 |
| | $T_h$ (day) | 0.052 | 0.057 | 0.044 | 0.047 |
| | max*Na* | 19.231 | 17.544 | 22.727 | 21.277 |
| | $R^2$ | 0.973 | 0.990 | 0.999 | 0.960 |
| *H. japonica* | a | 1.080 | 1.116 | 1.010 | 1.156 |
| | $T_h$ (day) | 0.022 | 0.023 | 0.012 | 0.022 |
| | max*Na* | 45.455 | 43.478 | 83.333 | 45.455 |
| | $R^2$ | 0.966 | 0.990 | 0.970 | 0.981 |

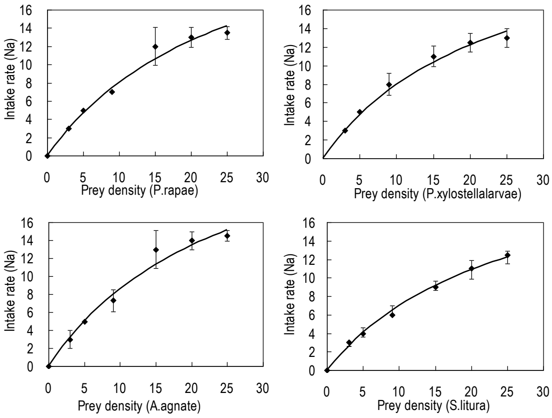


**Figure 4.1 Response curve of wolf spider with different densities of pests**

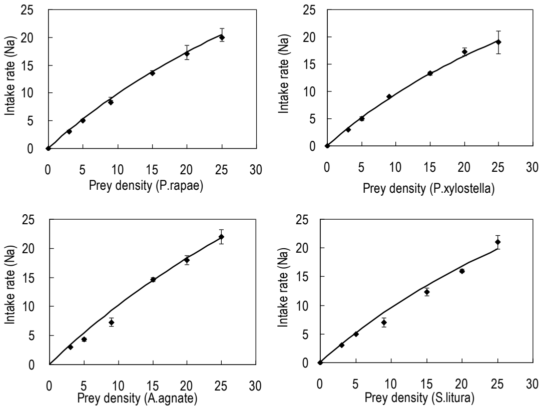


**Figure 4.2 Response curve of *D. halensis* with different densities of pests**

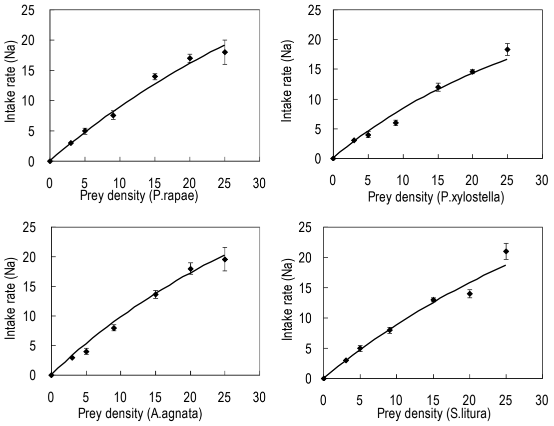


**Figure 4.3 Response curve of *C. pallipes* with different densities of pests**

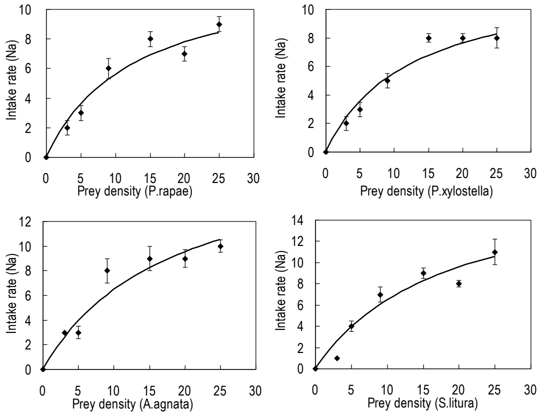


**Figure 4.4 Response curve of *H. griseus* with different densities of pests**

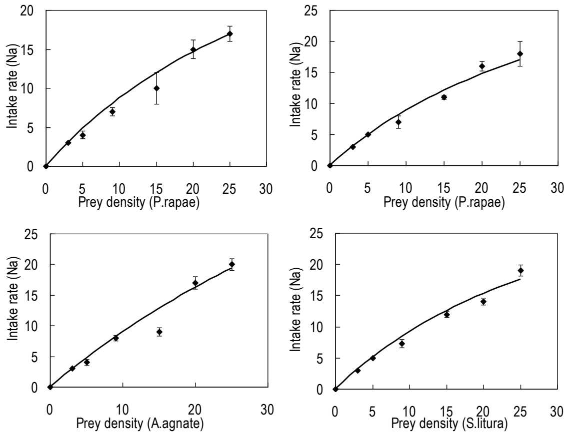


**Figure 4.5 Response curve of *H. japonica* with different densities of pests**

### 4.3.3 Estimation of actual predatory capacity of predators in field

#### 4.3.3.1 Presentation of formula

By performing the functional response, $1/T_h$ can reflect the predation potential of predators, however, it is difficult for predators to exert such great influence to pests, because there are much differences of heterogeneity between laboratory condition and actual farmland and interference among predators affects the predation efficiency. Therefore, the result of laboratory testing can not well reflect the reality of farmland. Hence it is need to further analyze the predation ability of natural enemies on the base of result in laboratory testing. A formula was presented to estimate the predation capacity of predators:

$$C_n = \frac{\mathbf{T_{daily}}}{24} \times \frac{1}{T_h} \times F_{preying} \times N$$ (*number of consumed preys/predators in certain area/day*), where $C_n$ is the predation capacity of predators (natural enemies), $T_{daily}$ is time available for predation of a predator per day (hour), $T_h$ is the handling time obtained from the functional response testing, $\frac{1}{T_h}$ is number of preys consumed by a predator per day, $F_{preying}$ is the frequency of a predator preying on pests on cabbage leaves, $N$ is the density of predators. $\frac{\mathbf{T_{daily}}}{24} \times \frac{1}{T_h} \times F_{preying}$ means the number of consumed preys by one predator per day.

The proportion of decrement of pest caused by increment of predators in introduction plot was estimated by:

$$E = \frac{\text{Difference of } C_n \text{ between two plot}}{\text{Difference of pest density between two plots}}$$

#### 4.3.3.2 Determining parameters

***$T_{daily}$* of predators**. The daily active time ($T_{daily}$) of indigenous predators during the cabbage growth period was observed in plots. For wolf spiders, it was observed that during the cabbage growth period from July to October, the daily activity time was usually from 6:00 in the morning to 20:00 at night, as around 14 hours per day.

For predacious carabids, it was observed that adult carabids usually hide in shelter place in day time and come out at night. During the period from July to October, individuals come out of shelter places from around 16:00 of the day, and become active from 18:00 to 24:00, and hide again before 8:00 in the morning, and during the day time of forenoon and noontime the activities of individuals was low. Therefore, the average daily active time was around 14 hours.

For frogs, it was observed that in summer season, frogs become active after 15:00 and until around 10:00, and from early morning about 5:00 individuals become active again until 10:00, and become inactive during the noontime. Therefore, the daily active time of frogs was around 12 hours.

**Handling time ($T_h$) of indigenous predators.** As shown in Table 4.3, the actual handling time of one predator was observed in both laboratory and farmland condition. There was no significant difference between calculated handling time and the actually observed handling time ($P > 0.30$). Thus, the calculated handling time based on functional response testing can be used to estimate the actual predatory capacity of indigenous predators. Assumed that a predator wandering on cabbage leaves was always preying on pests, hence the reciprocal of $T_h$ (max$N_a$) is the maximal preying number of pests. Although the value of max$N_a$ was much greater than the actual predatory capacity of predators, $T_h$ value is useful to estimate the actual predatory capacity of natural enemies.

**Table 4.3 The calculated handling time and the actually observed handling time**

| Predator | Calculated handling time from equation (min) | Actually observe handling time in laboratory (min) |
|---|---|---|
| Wolf spider | 52 ± 2 | 40 ± 3 |
| *D. halensis* | 20 ± 1 | 20 ± 3 |
| *C. pallipes* | 19 ± 2 | 16 ± 4 |
| *H. griseus* | 72 ± 4 | 60 ± 10 |
| *H. japonica* | 28 ± 3 | 25 ± 8 |

**$F_{preying}$ of the predators**. As generalist predators, indigenous predators, only part of their time was spent on preying on pests, or only part of individuals in population preying on pests at some time. Edgar (1969) held that it is assumed that the proportion of spiders feeding in the samples is representative of the proportion feeding in the whole population. In the current study, during the cabbage growth period, this proportion of three types of indigenous predators was investigated and estimated (Table 4.4). For example, according to the observation in the present cabbage plots, during the occurrence of pests, around 10% of adult spiders were always active on the cabbage leaves. It was consumed that this proportion is the frequency that individuals of spider population always present on cabbage leaves where pests parasitize. Hence ‘10%’ can be considered as the mean proportion of spiders likely to be preying on pests at any time.

#### 4.3.3.3 Calculation

The value of $T_{daily}$, $F_{preying}$ and average density of indigenous predators ($N$) during the occurrence of pests was shown in Table 4.4.

For instance, during period from mid July to mid September, in introduction plot, the predation capacity of predators (number of consumed preys/10 $m^2$/day) was:

Wolf spiders: $C_n=\frac{14}{24}\times\frac{1}{0.0364}\times0.1125\times(40～45)=70～80$,

*Harpalus* beetles: $C_n=\frac{10}{24}\times\frac{1}{0.0500}\times0.05\times(30～40)=12～16$,

*D.halensis*: $C_n=\frac{10}{24}\times\frac{1}{0.0136}\times0.05\times(10～20)=15～30$,

*C.pallipes*: $C_n=\frac{10}{24}\times\frac{1}{0.0135}\times0.05\times(5～20)=7～30$,

Japanese tree frogs: $C_n=\frac{10}{24}\times\frac{1}{0.0198}\times0.175\times(10～15)=50～75$,

The sum in introduction plot: $C_{n\text{-}in}=150～230$;

And, in the no-introduction plot:

Wolf spiders: $C_n=\frac{14}{24}\times\frac{1}{0.0364}\times0.1125\times(10～15)=18～27$

*Harpalus*: $C_n=\frac{10}{24}\times\frac{1}{0.0500}\times0.05\times(10～15)=4～6$,

*D.halensis*: $C_n=\frac{10}{24}\times\frac{1}{0.0136}\times0.05\times(3～10)=5～15$,

*C.pallipes*: $C_n=\frac{10}{24}\times\frac{1}{0.0135}\times0.05\times(2～5)=3～7$,

Japanese tree frogs: $C_n=\frac{10}{24}\times\frac{1}{0.0198}\times0.175\times(3～6)=15～30$, The sum in the no-introduction plot: $C_{n\text{-}no\text{-}in}$=45～85.

Compared to no-introduction plot, in introduction plot the increment of $C_n$ is: $C_{n\text{-}in}$ - $C_{n\text{-}no\text{-}in}$ =115～145 (mean=130)

According to investigation (Chapter 3), during the same period, the difference of average density (No./10m$^2$) of pest in two plots was: in greenhouse: 450-190=260; in open field: 330-150=180.

Then, $E_{greenhouse}$=130/260=0.50, $E_{open\ field}$=130/180=0.72.

Therefore, 50% ～70% of pest decrement in introduction plot was caused by the predation of increment of predators including wolf spiders, predacious carabids and frog.

**Table 4.4 Value of $T_{daily}$, $F_{preying}$ and average density (*N*) investigated in the field**

| Predators | | $T_{daily}$ | Predators density (*N*) (10m$^{-2}$) | | Proportion (frequency) of predators always presenting on cabbage leaves ($F_{preying}$) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Introduction | No-introduction | July | August | September | October | Average |
| Wolf spider | | 14 | 42～45 | 10～13 | 1/20 | 1/5 | 1/10 | 1/10 | 0.1125 |
| Predacious carabids | *Harpalus* | *10* | 30～40 | 10～15 | 1/20 | 1/20 | 1/20 | 1/20 | 0.0500 |
| | *D.halensis* | *10* | 10～20 | 3～10 | 1/20 | 1/20 | 1/20 | 1/20 | 0.0500 |
| | *C.pallipes* | *10* | 5～20 | 2～5 | 1/20 | 1/20 | 1/20 | 1/20 | 0.0500 |
| Frogs | | 14 | 10～15 | 3～6 | 1/5 | 1/5 | 1/5 | 1/10 | 0.1750 |

## 4.4 Discussion

Compared with the previous study of functional response of spider to pest (Table 4.5), the selected predators in this study showed relatively strong potential predation capacity to cabbage pest. The laboratory results presented in this study suggest that the selected three predators have a potential to be conservation biological control agent of cabbage pest in organic field. For all predator-prey relationships analyzed, the type II functional response could be applied to the data well.

It was also found that wolf spiders mainly capture young pest larvae (around 10 mm), while *D. halensis*, *C. pallipes* and Japanese frog can consume both young and old larvae but the preference has not been clear. Compare to carabids and frogs, wolf spiders can concentrate much on the young larvae. Spiders usually can capture and kill more prey than they consume (Maloney et al., 2003), they may kill as many as 50 times the number of prey it consumes (Riechert and Lockley, 1984). Such ‘superfluous killing’ may benefit the influence of spider to pests (Riechert, 1999). Therefore, the concentration on pest and ‘superfluous killing’ behavior may augment the predation power of spiders and make them more effective than other predators.

However, the present testing was performed in petri dish in room, which was rather different from field condition and should be interpreted with care. Although some predators show high predatory capacity to pests, in field they were not as effective as in room (Suenaga and Hamamura, 1998). Under field conditions, factors such as spatial complexity (Kareiva, 1990) and temperature (Zamani et al., 2006) may adversely influence the effectiveness of natural enemies (Gitonga et al. 2002). Generalist predators usually have a wide spectrum of preys and the pest is only one of preys for them. It was observed that only parts of individuals of a predator population were active on cabbage leaves searching pests in a certain period. Therefore, we directly investigated the proportion (preying frequency) of predator individuals that was active on cabbage leaves. This proportion was considered as the individuals those can effectively capture pests in farmland.

Another important factor is handling time of predators. We think that the calculated handling time of predators from the Holling equation can reflect the actually handling time, because there was no significance difference between calculated and observed handling time.

From the formula $C_n = \frac{\mathbf{T_{daily}}}{24} \times \frac{1}{T_h} \times F_{preying} \times N$, the estimated actual predatory capacity was much smaller than theoretical value from Holling equation, indicating the influence of variable environment condition. It is important for people to maintain a high predator density ($N$ value) so that the number of available predators preying on pests would increase. However, due to environment capacity, there must be limitation of increase of predator population, i.e., according to our observation for several years, the average density of adult wolf spiders in their most abundant season was 50-80 individuals/10m$^2$ in the present plot, and it was difficult to exceed this range much more. Therefore, the value of $C_n$ in the formula had a limitation, which meant that indigenous predators in farmland have a limitation of suppression power on pests. This is might one of reasons that sometimes indigenous predators can not well suppress pests density to an acceptable low level. Because of the variations of the field, the value of $C_n$ varied with different plots with various environment characteristics.

Because of variation of environmental factors in the farmland, it is not easy to ensure the accuracy of the estimation of predators' actual ability. Parameters such as $F_{preying}$ and $T_{daily}$ were roughly investigated, thus the estimation result may not well fit to the truth in the field. However, this study presented an idea combining the laboratory experiment and field investigation to check the efficacy of pest control agents. Such estimation method still has some flaws, further improvement is needed in the future work.

**Table 4.5 Previous studies on the predation capacity of several species of predators**

| Parameters | *Predator-prey* | | | | |
|---|---|---|---|---|---|
| | *Pirata subpiraticus-S.litura* | *Erigonidium graminicola-S.litura* | *Propytaea japomica-P.litura* | *Orius agilis-P.litura* | *Pardosa pseudannulata-P.litura* |
| Attack rate ($a$) | 0.8079 | 0.9594 | 0.4424 | 0.5031 | 1.3503 |
| Handling time ($T_h$) | 0.0386 | 0.1633 | 0.00848 | 0.02683 | 0.014 |
| Maximum intake rate (max$N_a$) | 25.9 | 6.12 | 118.0 | 37.3 | 71.4 |
| References | Jiang, 2001 | Jiang, 2001 | Ge, 2009 | Ge, 2009 | Tian, 2011 |

# Chapter 5 Exploring predation mechanism of indigenous predators in the plot with plant residue by stable isotope analysis

## 5.1 Introduction

According to our previous study, the abundance of natural enemies was enriched by the plant residue introduction into field, and the cabbage pests were controlled to a low level. One attribute of generalist predators that may contribute to their effectiveness as biological agents is their broad diet, which confers the ability to sustain populations on alternative energy sources when pests are scare in the grazing food chain as ‘crop-pest-predators’. Therefore, it is meaningful to understand the energy flow, trophic characteristics and feeding behavior of soil fauna and indigenous predators from the perspective of food chain by stable isotope ($^{13}C$ and $^{15}N$) analysis. In previous studies, the feeding behavior of some generalist predators in farmland has been estimated by gut analysis and by feeding experiments. Gut analysis sometimes may be misleading if assimilation is not considered. As the element such as carbon and nitrogen compositions of animals reflect their diets, trophic characteristic and diet contribution of animals can be derived from chemical analysis. Dietary data highlight the importance of opportunistic diets and show differences in the diets of species between distinct trophic areas. A synthesis of the references for these data and the observations of predators’ feeding behavior was provided in Table 1.1.

Stable isotope analysis is increasingly being used as a tool to study food-web interactions. The ratio of naturally occurring stable isotopes in the consumer tissue reflects the entire feeding history of the organism. Stable isotopes of the same element participate in chemical reactions at different rates, which result in a preferential net incorporation of heavier isotopes in the consumer’s body, a process termed “fractionation”. Thus, the isotopic ratio changes as isotopes move through the food chain. It was expected that the introduced plant residue would affected the energy and trophic structure of food web in field. We tried to analyze the trophic relationships and food preference of predators in introduction plot by using stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). $^{15}N/^{14}N$ of consumers could enrich stepwise relative to their foods and therefore reflect the trophic position of consumers. $^{13}C/^{12}C$ change little with energy transfer between trophic levels and is used to identify compartmentalization in food webs (Coat et al., 2009; Tiunov, 2007).

The main objectives was to understand the feeding characteristic/response of a predator (using wolf spiders) to target pest in the introduction plot by stable isotope analysis, and to evaluate the meaning of the method of stable isotope analysis in conservation biological control.

## 5.2 Material and method

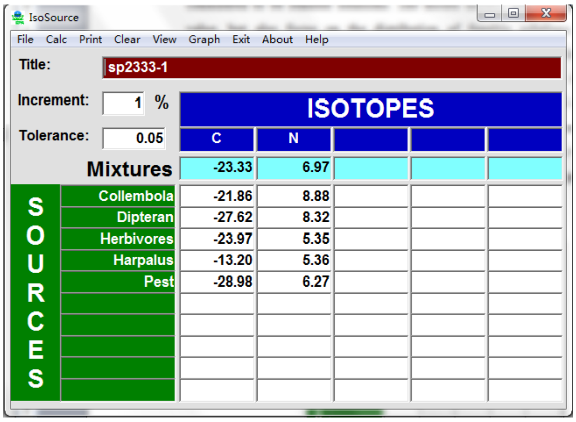
**Sample preparation**. Samples for isotope analysis were collected around two months after the appearing of pests (September) so that predators had enough time to digest and absorb the pests. Plant residue including wheat straw, weed straw and cabbage leaves were sampled. Fauna samples included: collembolan animals (*Proisotoma* sp. and *Hypogastrura* sp.), soil mites (oribatids), earthworm (Megascolecidae), dipteran animals (*Chironomus* sp.), herbivores (*Tetrix japonica*, *Bothrogonia ferruginea* and *Teleogryllus emma*), wolf spiders (*P. agrestis*), jumping spider (*Evarcha albaria*), predacious carabid beetles (*D. halensis*, *C. pallipes* and *Harpalus* spp.); frogs (*H. japonica*); lepidopteran pests (*P. rapae*, *P. xylostella*, *A. agnate* and *P. litura*) and some other common soil animals.

Plant samples were washed by distilled water. Fauna samples were collected by a combination of visual searching and pitfall trapping. For mesofauna and macrofauna, two or more individuals of each species were combined for one sample; small-bodied species required more individuals in order to provide an adequate amount of tissue for analysis (e.g at least 300 individuals per sample for collembolan animals and oribatids). Samples were kept alive in room condition for 2 days to allow their digestive tracts to clear in order to reduce contamination of the stable isotope sample.

**Stable isotope analysis.** Plant and animal tissues were oven dried at 60°C for three days and then finely ground in a ball mill. Between 1 and 2 mg of animal and plant tissue were placed in standard 8×5mm tin capsules and analyzed using an isotope-ratio mass spectrometer. The stable isotopic composition of element δ (δX) is expressed as a difference in ratios, in parts per thousand, from a standard: $\delta X = [(R_{sample}/R_{standard}) - 1] * 1000$, where $R_{sample}$ is the absolute isotopic ratio (heavy/light) of the sample and

$R_{standard}$ is the respective ratio of the standard.

**Analysis of predator’s menu.** The identified diet sources of predatorss were cross referenced with an extensive literature (mainly based on gut content analysis and feeding experiment) and observed directly in the present study (Table 1.1). One of aims of isotope analysis is to quantitatively determine the proportional contribution of several sources to a predator. As the number of potential food sources sometimes exceeds three, linear mixing models based on mass balance equations was adopted to solve the question. In this method, all possible combinations of each source contribution (0–100%) are examined in small increments (e.g., 1%). Combinations that sum to the observed mixture isotopic signatures within a small tolerance (e.g., ±0.1‰) are considered to be feasible solutions. The model usually not only gives a single mean value, but also focus on the distribution of feasible solutions. For instance, by calculation we can understand the distribution of feasible contribution of each prey and the frequency of each contribution (Figure 5.1). Because of the difference of stable isotope values of predators, the contributions of preys varied significantly. Such method was designed into a Microsoft Visual Basic program, Isosource version 1.3, by Phillips and Gregg (2003). In the present study, minor sources (contribution <5%) were omitted, while major ones (contribution >5%) were considered in the description of the animals' diet.



Title: sp2333-1

Increment: 1 %

Tolerance: 0.05

| | C | N | | | |
|---|---|---|---|---|---|
| Mixtures | -23.33 | 6.97 | | | |
| Collembola | -21.86 | 8.88 | | | |
| Dipteran | -27.62 | 8.32 | | | |
| Herbivores | -23.97 | 5.35 | | | |
| Harpalus | -13.20 | 5.36 | | | |
| Pest | -28.98 | 6.27 | | | |

**Figure 5.1 Multiple source linear mixing model (IsoSource 1.3) (one example)**

## 5.3 Result

### 5.3.1 Basic characteristic of food web components

Most samples measured were collected from open field and only parts of samples were also collected from greenhouse. From the result, there was little difference of stable isotope values of organism between the open field and greenhouse, therefore, the following statement was based on the data from open field. Wheat straws, C3 weeds, C4 weeds, cabbage leaves, soil and 26 groups of consumers were identified and their isotopic values are shown in Table 5.1.

The wheat straw (-28.18‰), C3 weed (-29.86‰) showed the lowest $\delta^{13}$C value, which were clearly distinguished from C4 weeds showing the highest $\delta^{13}$C value (-12～-14‰). According to $\delta^{13}$C value, three groups of soil fauna were clarified: C3-based group mainly depending on C3 resource (such as *Chironomus* sp.), C4-based group mainly depending on C4 resource (such as *Harpalus* spp., *Teleogryllus emma,* oribatids) and intermediate group combining C3 and C4 resource (such as collembolan animals of *Proisotoma* sp. and *Hypogastrura* sp., *Pterostichus microcephalus*, wolf spiders, *D. halensis*, frogs) (Table 5.1 and Figure 5.2). According to $\delta^{15}$N values, three groups was clarified: plant group (wheat, weeds, cabbage, less than 4.5‰), detritivores and herbivores group (*Harpalus* spp., herbivores of *Tetrix japonica*, *B. ferruginea* and *T. emma,* pests of *P. rapae*, *P. xylostella*, *A. agnate* and *S. litura,* 4‰ to 7‰), and predators (wolf spiders, *D. halensis,* oribatids, 7‰ to 13‰).

### 5.3.2 Comparison between introduction and no-introduction plot

For $\delta^{13}$C value, among 26 groups of organisms, 13 groups (50%) was similar between introduction and no-introduction plot, three groups (12%) (cricket, *C. pallipes* and jumping spider) showed slightly higher $\delta^{13}$C value in no-introduction plot but difference was not significant ($P > 0.1$), while 10 groups (38%) showed higher $\delta^{13}$C value in introduction than in no-introduction plot, but the difference was not significant ($P > 0.1$) (Figure 5.2 and 5.4). The alternative preys including dipteran larvae (*Chironomus* sp.), *T. japonica*, *B. ferruginea*, collembolan animal (*Hypogastrura* sp.) and *Harpalus* larvae and predator of wolf spiders showed higher $\delta^{13}$C value in introduction than in no-introduction plot, with the difference as 0.90‰, 2.26‰, 2.05‰, 3.42‰, 0.5‰, 1.01‰ respectively, and the difference was not significant ($P > 0.1$), however, such difference was still meaningful because it reflected the influence of plant residue to soil fauna and predators.

For $\delta^{15}N$, groups of *Harpalus, P.microcephalus* and oribatids showed 1.0 – 2.0‰ lower $\delta^{15}N$ value in introduction plot, groups of *D. halensis,* formicid, megascolecid showed 0.5 – 1.5‰ slightly higher $\delta^{15}N$ in introduction plot, and for other organisms, similar $\delta^{15}N$ value was found between the introduction and no-introduction plot (Figure 5.3 and 5.4). Generally speaking, plant residue lead to little changes of the trophic level of communities, and the trophic structure kept stable in both plots.

**Table 5.1 Signatures of $\delta^{13}C$ and $\delta^{15}N$ of components in field**

| Items | Introduction | | | | No-introduction | | | |
|---|---|---|---|---|---|---|---|---|
| | $\delta^{13}C$ | $\delta^{15}N$ | $\delta^{13}C$-SE | $\delta^{15}N$-SE | $\delta^{13}C$ | $\delta^{15}N$ | $\delta^{13}C$-SE | $\delta^{15}N$-SE |
| C3 weed | -29.86 | 4.23 | 0.09 | 0.05 | -29.86 | 4.23 | 0.09 | 0.05 |
| Wheat straw | -28.18 | 5.90 | 0.41 | 0.95 | 0.00 | 0.00 | 0.00 | 0.00 |
| Cabbage leave | -28.67 | 6.58 | 0.71 | 0.17 | -28.88 | 6.65 | 0.25 | 0.51 |
| **Pests** | | | | | | | | |
| *P. rapae* | -29.27 | 4.81 | 0.54 | 0.20 | -28.92 | 4.59 | 0.23 | 0.28 |
| *A. agnata* | -28.69 | 7.54 | 0.75 | 0.17 | -29.82 | 8.02 | 0.08 | 0.22 |
| *P. xylostella* | -28.64 | 4.32 | 0.50 | 0.30 | -29.24 | 7.90 | 0.02 | 0.01 |
| *S.litura* | -29.30 | 7.91 | 0.42 | 0.04 | -29.50 | 8.01 | 0.34 | 0.27 |
| **Diptera** | | | | | | | | |
| *Chironomus* spp. | -27.62 | 8.32 | 0.10 | 0.10 | -28.52 | 8.50 | 0.20 | 0.30 |
| **Herbivore** | | | | | | | | |
| *Tetrix japonica* | -23.67 | 4.60 | 0.45 | 0.12 | -25.92 | 4.64 | 0.92 | 0.86 |
| *Bothrogonia ferruginea* | -23.25 | 4.68 | 0.21 | 0.69 | -25.30 | 4.56 | 0.95 | 0.56 |
| *Teleogryllus emma* | -24.99 | 6.78 | 0.56 | 0.35 | -24.52 | 6.41 | 1.21 | 0.44 |
| **Wolf spider** | | | | | | | | |
| *P. agrestis* | -23.33 | 10.37 | 0.73 | 0.28 | -24.34 | 10.50 | 0.32 | 0.33 |
| **Collembola** | | | | | | | | |
| *Proisotoma* spp. | -22.34 | 10.76 | 0.38 | 0.30 | -25.02 | 11.25 | 0.00 | 0.00 |
| *Hypogastrura* spp. | -21.38 | 6.99 | 0.97 | 0.32 | -25.53 | 6.58 | 0.07 | 0.02 |
| **Predacious carabids** | | | | | | | | |
| *D.halensis* | -23.43 | 7.84 | 0.53 | 0.14 | -23.26 | 7.18 | 0.48 | 0.35 |
| *C.pallipes* | -24.94 | 8.33 | 0.33 | 0.23 | -22.62 | 8.95 | 0.80 | 0.39 |
| **Frog** | | | | | | | | |
| *Hyla japonica* | -16.56 | 4.65 | 0.29 | 0.15 | -16.96 | 4.50 | 0.00 | 0.00 |
| *Hyla japonica* | -23.33 | 4.97 | 0.55 | 0.90 | -23.42 | 4.96 | 0.51 | 0.87 |
| **Harpalus spp.** | -21.04 | 4.90 | 0.45 | 1.96 | -25.73 | 5.89 | 0.84 | 0.29 |
| **Harpalus spp.** | -15.58 | 4.37 | 0.26 | 0.00 | -16.23 | 4.93 | 0.60 | 0.06 |
| Oribatid | -24.89 | 13.04 | 0.24 | 0.34 | -25.25 | 13.88 | 0.03 | 0.72 |
| Enchytraeid | -24.10 | 10.76 | 0.06 | 0.07 | -24.45 | 9.18 | 0.51 | 0.68 |
| Sowbug (*Armadillidium* | -24.00 | 7.86 | 0.34 | 0.28 | -25.54 | 8.12 | 1.14 | 0.95 |
| Formicid | -22.69 | 9.03 | 0.77 | 0.23 | -23.09 | 8.18 | 0.68 | 0.70 |
| Jumping spider (*Evarcha albaria*) | -21.90 | 9.87 | 0.06 | 0.04 | -20.37 | 9.71 | 0.52 | 0.33 |
| Diplopod (*Oxidus* spp.) | -21.90 | 9.98 | 0.13 | 0.47 | -22.21 | 9.39 | 0.30 | 0.30 |
| *Anisodactylus* spp. | -19.79 | 6.15 | 0.89 | 0.41 | -23.84 | 6.60 | 0.59 | 0.59 |
| *Pterostichus microcephalus* | -24.29 | 6.47 | 0.29 | 0.07 | -24.56 | 8.71 | 0.16 | 0.30 |
| Soil | -24.43 | 4.55 | 0.23 | 0.26 | -24.29 | 4.65 | 0.24 | 0.32 |
| C4 weed | -13.50 | 2.98 | 0.31 | 0.83 | -13.17 | 3.78 | 0.42 | 0.96 |
| ***Harpalus* larvae** | -13.20 | 5.36 | 0.15 | 0.03 | -13.70 | 5.69 | 0.01 | 0.07 |

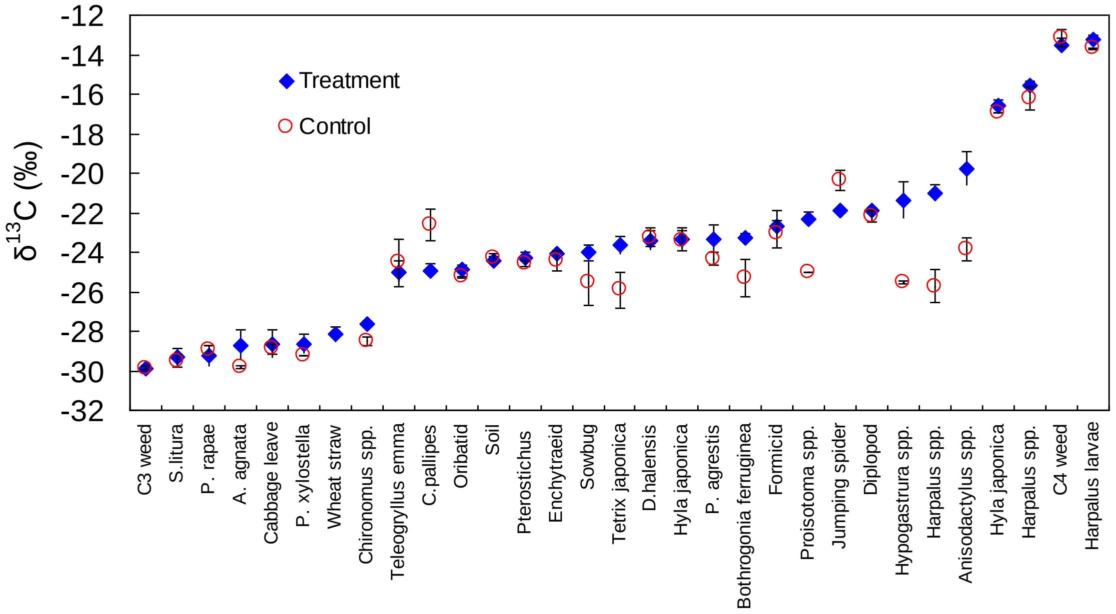


**Figure 5.2 Mean δ$^{13}$C values of components in experiment plot**

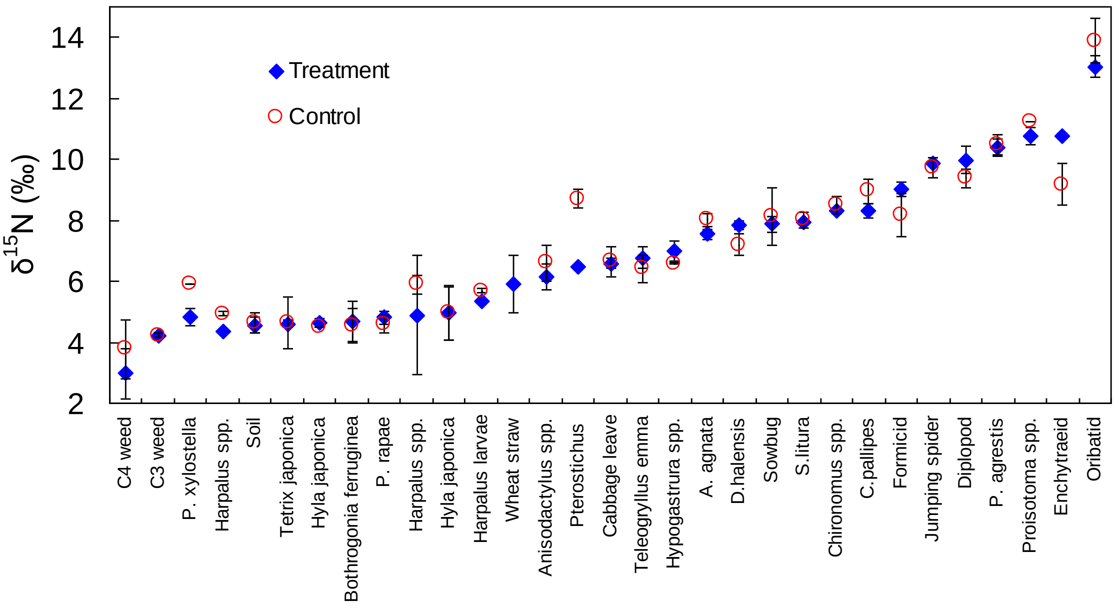


**Figure 5.3 Mean δ$^{15}$N values of components in experiment plot**

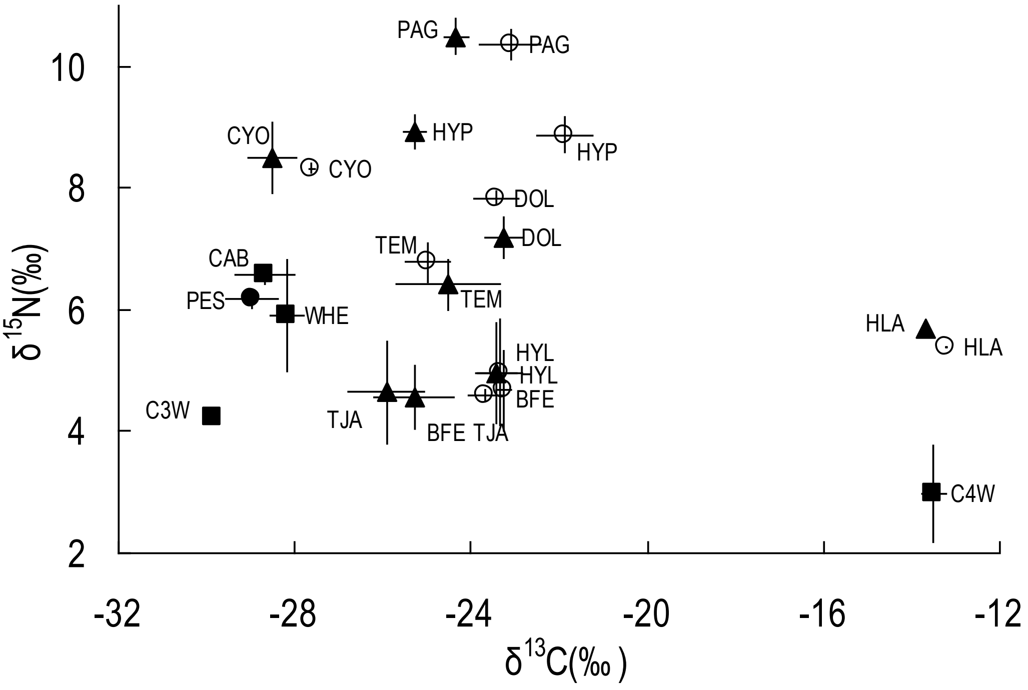


**Figure 5.4 Mean ± SE of stable isotope signature of $\delta^{13}C$ and $\delta^{15}N$ of plant residue (■), pest (●), potential alternative diet and predators in treatment (○) and control plot (▲). PAG, *P. agrestis*, HYP, *Hypogastrura* sp., CYO, *Chironomus* sp., TEM, *T. emma*, CAB, cabbage leaf, PES, Pest (*P. rapae*, *P. xylostella*, *A. agnate* and *S. litura*), WHE, Wheat straw, HYL, *H. japonica*, DOL, *D. halensis*, HLA, *Harpalus* larvae, TJA, *T. japonica*, BFE, *B. ferruginea*, C3W, C3 weeds, C4W, C4 weeds. Bars show standard error.**

### 5.3.3 Analysis of wolf spider's food menu

In the current study, as the potential diet of wolf spiders was well understand in previous literatures and observed in the present study (Table 3.6), the food sources contribution to wolf spiders was estimated based on the $\delta^{13}C$ value. The same work on predacious carabids and frogs is in progress.

Collembolan animal *(Hypogastrura* sp.), dipteran larvae (*Chironomus* sp.), herbivores (*T. emma*, *T. japonica* and *B. ferruginea*), *Harpalus* larvae and lepidopteran pests larvae were identified as the potential preys for wolf spiders, thus the proportional contribution of these diets to a spider was estimated based on the $\delta^{13}C$ values of their body. As the herbivore of *T. emma*, *T. japonica* and *B. ferruginea* showed the similar

$\delta^{13}$C value, they were aggregated into a signal trophic group called ‘herbivores’, *Proisotoma* sp. and *Hypogastrura* sp. showed the similar $\delta^{13}$C value, they were aggregated into a signal trophic group called ‘collembola’, and as the $\delta^{13}$C range of four species (*P. rapae*, *P. xylostella*, *A. agnate* and *S. litura*) of pests was from -28.0% to 30.0%, they were also aggregated into a signal trophic group called ‘pests’. Relative contributions of the potential food sources are reported as ranges and means of food source proportions in the food menu.

According to calculation, histogram of Figure 5.5 shows all the possible food source proportions (X-axis) and their frequency percentage (Y-axis). Table 5.2 shows the 50% and 70% of total frequency centered on average value. During the occurrence period of pests, when the 50% of total frequency centered on average value was considered, in the introduction plot, the relative contribution of potential preys in the menu of wolf spider was: 12-31% (mean=22.6%) of collembolan animal, 11-36% (mean=22.3%) of dipteran larvae, 12-32% (mean=21.0%) of herbivores, 11-22% (mean=17.0%) of *Harpalus* larvae and 9-26% (mean=17.0%) of lepidopteran pests, and in no-introduction plots, the proportion was: 13-32% (mean=22.1%) of collembolan animal, 12-35% (mean=21.9%) of dipteran larvae, 10-25% (mean=17.6%) of herbivores, 17-23% (mean=20.5%) of *Harpalus* larvae and 9-28% (mean=18.0%) of lepidopteran pests. When 70% of total frequency centered on average value was considered, the range of food source became larger (Table 5.2).

Combine the above result with the relative abundance of alternative preys in two plots, some valuable indications was obtained. As shown in Table 5.3 and Figure 5.6, when 50% of total frequency centered on average value was considered, from the no-introduction to introduction plot, the ratio of ‘pest ： (pest＋alternative preys)’ decreased and the decrement was expressed as:

$$R_1 = \frac{\text{Pest proportion}_{\text{No-intro.}} - \text{Pest proportion}_{\text{Intro.}}}{\text{Pest proportion}_{\text{No-intro.}}}$$

(from 52% to 10%, decreased by 77%);

On the other hand, the largest decrement of pest proportion in wolf spider’s food menu was expressed as:

$$R_2 = \frac{\text{Max}_{\text{pest proportion}}(\text{No-intro.}) - \min_{\text{pest proportion}}(\text{Intro.})}{\text{Max}_{\text{pest proportion}}(\text{No-intro.})}$$

(from 28% to 9%, decreased by 68% for pest proportion in wolf spider's food menu).

It was expected that, when the ratio of $R_1/R_2$,

>1, predators specialize on pests, and the predator was available for pest control;

≒1, predators randomly feeding, and the predator was at least available for pest control;

<1, predators more specialize on alternative preys, and the predator was not available for pest control.

In the present analysis, $R_1/R_2$=77%/68%=1.13, and $R_1/R_2$=0.95 when when 50% of total frequency centered on average value was considered. Therefore, in this case, the decrement of the ratio of 'pest：(pest＋alternative preys)' was almost consistent with the decrement of pest proportion in spider's food menu from the no-introduction to introduction plot. It reflected that, at least, with the existence of low density of pest, the wolf spider could still prey on target pest randomly in the introduction plot.

However, this was only an extreme case. In most cases, in no-introduction plot, the pest proportion in spider's menu was less than 28%, and in the introduction plot, the pest proportion in spiders' menu was more than 9% when 50% of total frequency centered on average value was considered. From the no-introduction to introduction plot, the preference of wolf spiders to pest was kept at some extent.

From this point, at least, we can deny that the wolf spiders prefer to prey on alternative preys when pest frequency is low in the introduction plot. Even if the wolf spiders do not prefer target pest and only prey on pest and alternative preys randomly, the enriched wolf spider population by introduced plant residue can still ensure the efficacy of pest suppression at some extent.

## Table 5.2 Estimated proportion of preys in wolf spider's food menu by using software Isosource version 1.3

### 50% of total frequency centered on average value

**Introduction (Spider, $\delta^{13}C$=-23.33±0.73‰, $\delta^{15}N$=10.37±0.28‰, $\Delta\delta^{15}N$=3.4, increment=1%)**

| Potential diets | Collembola (*Hypogastrura* sp.) | Dipteran larvae (*Chironomus* sp.) | Herbivores (*T. emma*, *T. japonica*, *B. ferruginea*) | *Harpalus* larvae | Pest (*P. rapae*, *P. xylostella*, *A. agnate*, *S. litura*) |
|---|---|---|---|---|---|
| Average $\delta^{13}C$ (‰) | -21.86 ± 0.67 | -27.62 ± 0.10 | -23.97 ± 0.41 | -13.20 ± 0.15 | -28.98 ± 0.64 |
| Average $\delta^{15}N$ (‰) | 8.88±0.31 | 8.32±0.1 | 5.35±0.40 | 5.36±0.03 | 6.27±0.20 |
| Range (%)* | 12～31 | 11～36 | 12～32 | 11～22 | 9～26 |
| Mean (%)* | 22.6±0.7 | 22.3±0.9 | 21.0±0.8 | 17.0±0.4 | 17.0±0.7 |

**No-introduction (Spider, $\delta^{13}C$=-24.34±0.32‰, $\delta^{15}N$=10.50±0.33‰, $\Delta\delta^{15}N$=3.4, increment=1%)**

| Potential diets | Collembola (*Hypogastrura* sp.) | Dipteran larvae (*Chironomus* sp.) | Herbivores (*T. emma*, *T. japonica*, *B. ferruginea*) | *Harpalus* larvae | Pest (*P. rapae*, *P. xylostella*, *A. agnate*, *S. litura*) |
|---|---|---|---|---|---|
| Average $\delta^{13}C$ (‰) | -25.27 ± 0.10 | -28.52±0.3 | -25.24±1.03 | -13.7±0.10 | -29.37±0.15 |
| Average $\delta^{15}N$ (‰) | 8.91±0.02 | 8.50±0.30 | 5.21±0.62 | 5.69±0.07 | 6.63±0.20 |
| Range (%) | 13～32 | 12～35 | 10～25 | 17～23 | 9～28 |
| Mean (%) | 22.1±0.8 | 21.9±0.9 | 17.6±0.6 | 20.5±0.3 | 18.0±0.7 |

### 70% of total frequency centered on average value

**Introduction (Spider, $\delta^{13}C$=-23.33±0.73‰, $\delta^{15}N$=10.37±0.28‰, $\Delta\delta^{15}N$=3.4, increment=1%)**

| Potential diets | Collembola (*Hypogastrura* sp.) | Dipteran larvae (*Chironomus* sp.) | Herbivores (*T. emma*, *T. japonica*, *B. ferruginea*) | *Harpalus* larvae | Pest (*P. rapae*, *P. xylostella*, *A. agnate*, *S. litura*) |
|---|---|---|---|---|---|
| Average $\delta^{13}C$ (‰) | -21.86 ± 0.67 | -27.62 ± 0.10 | -23.97 ± 0.41 | -13.20 ± 0.15 | -28.98 ± 0.64 |
| Average $\delta^{15}N$ (‰) | 8.88±0.31 | 8.32±0.1 | 5.35±0.40 | 5.36±0.03 | 6.27±0.20 |
| Range (%) | 8～34 | 8～42 | 8～39 | 8～24 | 6～31 |
| Mean (%) | 22.6±0.7 | 22.3±0.9 | 21.0±0.8 | 17.0±0.4 | 17.0±0.7 |

**No-introduction (Spider, $\delta^{13}C$=-24.34±0.32‰, $\delta^{15}N$=10.50±0.33‰, $\Delta\delta^{15}N$=3.4, increment=1%)**

| Potential diets | Collembola (*Hypogastrura* sp.) | Dipteran larvae (*Chironomus* sp.) | Herbivores (*T. emma*, *T. japonica*, *B. ferruginea*) | *Harpalus* larvae | Pest (*P. rapae*, *P. xylostella*, *A. agnate*, *S. litura*) |
|---|---|---|---|---|---|
| Average $\delta^{13}C$ (‰) | -25.27 ± 0.10 | -28.52±0.3 | -25.24±1.03 | -13.7±0.10 | -29.37±0.15 |
| Average $\delta^{15}N$ (‰) | 8.91±0.02 | 8.50±0.30 | 5.21±0.62 | 5.69±0.07 | 6.63±0.20 |
| Range (%) | 8～36 | 7～39 | 6～29 | 15～25 | 6～32 |
| Mean (%) | 22.1±0.8 | 21.9±0.9 | 17.6±0.6 | 20.5±0.3 | 18.0±0.7 |

*, the proportion of preys was shown as a range and its mean value (mean ± SE).

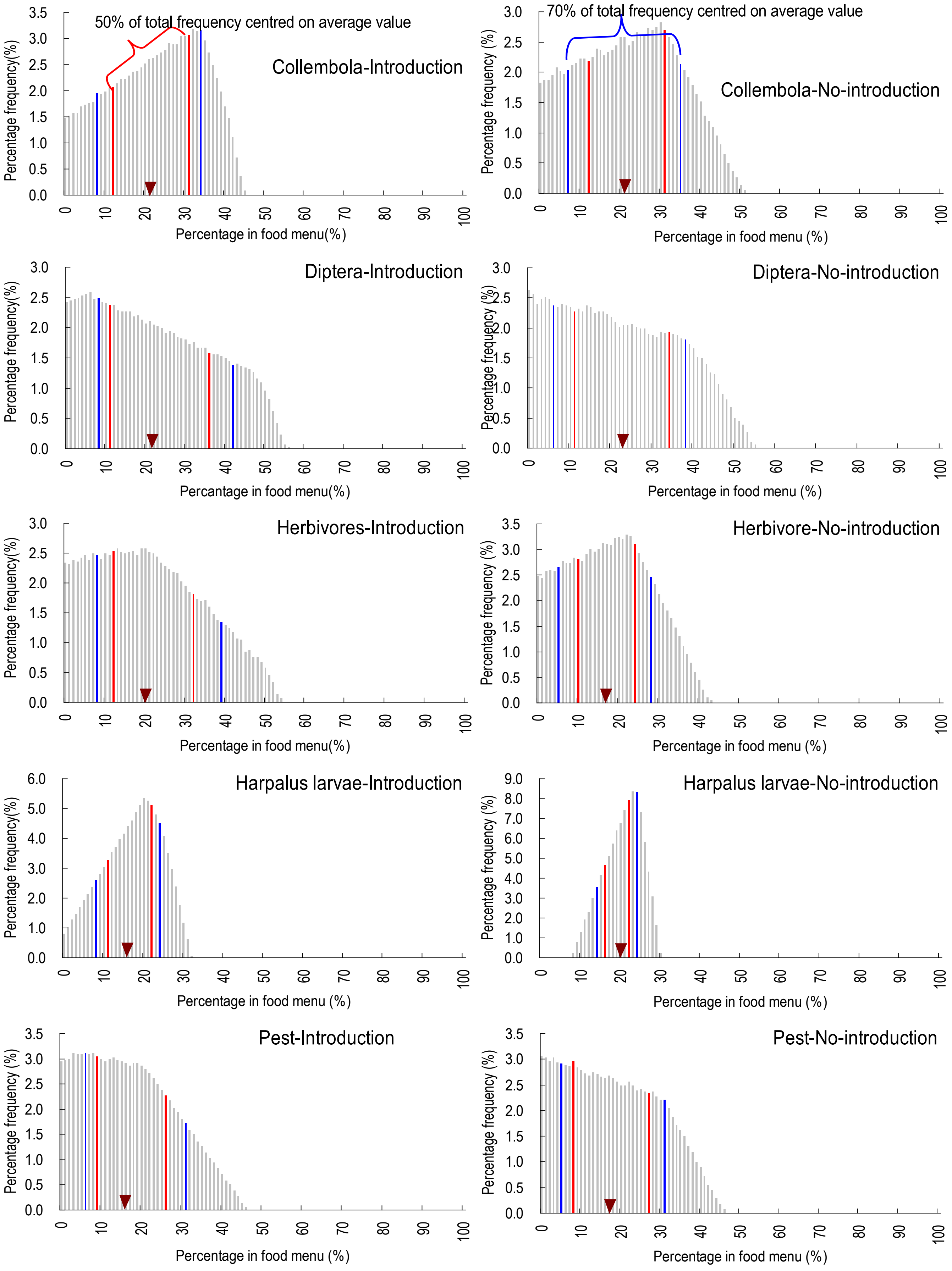


**Figure 5.5 Distribution of feasible contribution of five primary producers to wolf spider in two plots based on δ$^{13}$C signature.** The point with ▼ is average value, the area between two red bars is 50% of total frequency centered on average value, the area between two blue bars is 70% of total frequency centered on average value.

Table 5.3 **Biomass of organism in introduction and no-introduction plot during the period of pest occurrence**

| Taxa | Biomass of alternative preys (mg/50×50×10cm) and pest (mg/50×50cm) | | | |
|---|---|---|---|---|
| | Introduction | | No-introduction | |
| | 2010 | 2011 | 2010 | 2011 |
| Wolf spider | 48 | 56 | 12 | 16 |
| Alternative preys | 826 | 1067 | 201 | 234 |
| Pests | 110 | 106 | 230 | 240 |
| Pest:(pest + alternative preys) | 0.12 | 0.09 | 0.53 | 0.51 |

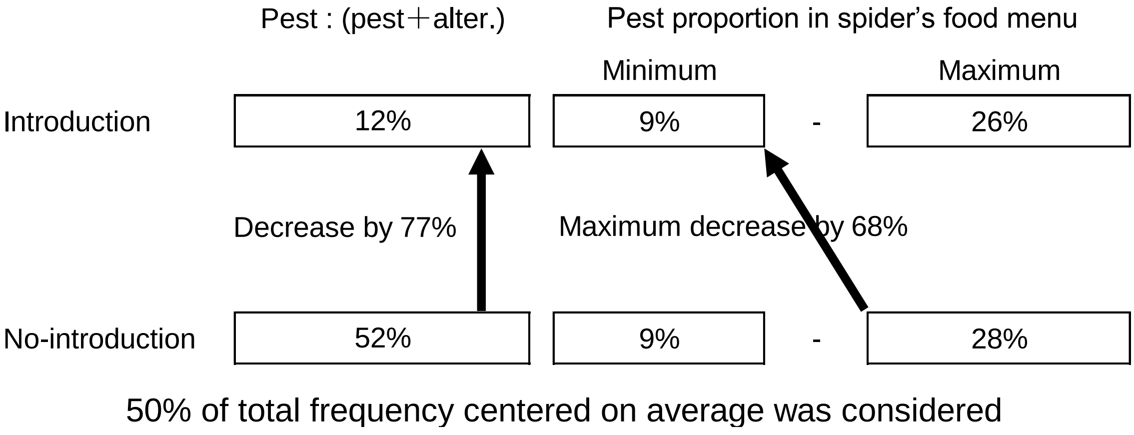


**Figure 5.6 Proportion of consumed target pest in wolf spider's food menu and the existing pest proportion (frequency) in two plots**

## 5.4 Discussion

### 5.4.1 Detritivores incorporated the carbon from plant residue

The $\delta^{13}$C values of consumers reflect $\delta^{13}$C signatures of their diet resource (Webb et al. 1998). In the present experiment, the C4 weeds held around one third amount of introduced plant residue, and the alternative preys including dipteran larvae (*Chironomus* sp.), *T. japonica*, *B. ferruginea*, collembolan animal (*Hypogastrura* sp.) and *Harpalus* larvae and predator of wolf spiders showed higher $\delta^{13}$C value in introduction than in no-introduction plot, with the difference as 0.90‰, 2.26‰, 2.05‰, 3.42‰, 0.5‰, 1.01‰ respectively, which indicated that the these animals incorporated the carbon from C4 plant residue that has a higher $\delta^{13}$C value (-12～-14‰). And in turn, the density of them in introduction plot significantly increased.

Therefore, it was concluded that the plant residue introduced into the field indeed provided strong energy flow of the food web from ‘organic material’, through alternative preys, to predators.

### 5.4.2 Stable trophic level of predators between two plots

The $\delta^{15}$N signatures of wolf spiders, predacious carabids and frogs were obviously distinct and divided into three trophic guilds, with the value range as 9‰ - 11‰, 7‰ - 9‰ and 4‰ - 5‰ respectively. Such differentiation indicated different use of diet resource among guild members. It was worth noting that frogs have relatively low $\delta^{15}$N signatures (from 4‰ to 5‰), such low value conflicted the gut analysis result of frogs in the previous literatures. The reason of such result was not clear and is being explored now.

There was no significant difference of $\delta^{15}$N signatures of most measured organisms between introduction and no-introduction plot. This might because introduction and no-introduction plot have been subjected to the same organic management for 20 years before our experiment. Both the introduction and no-introduction plot have been subjected to biofertilizer successively for many years, and the vegetation composition of two plots was similar. The $\delta^{15}$N value revealed the stable trophic level of predators in two plots, indicating that the feeding ways of predators was similar in the introduction and no-introduction plots.

Therefore, it was suggested that the low density of predators in the no-introduction plot and high density in the introduction plot was mainly due to the abundance of alternative preys which could be increased by introduced plant residue. In the no-introduction plot, the amount of alternative preys was the major limitation of the increase of predator populations. So, it was concluded that the introduced plant residue well functioned in increasing the abundance of indigenous generalist predators.

### 5.4.3 Stable isotope analysis is an effective method

Preference of generalist predators have been studied in many areas such as snail (*Thais emarginata* and *Acanthina spirata*) (Murdoch, 1969), coccinellid beetles (Murdoch and Marks, 1972) and some birds (Manly et al., 1972). Murdoch (1972) assumed two possible cases: 1) a predator showed the same degree (same rate) of 'preference' to one of the species regardless of the relative abundance; 2) a predator may specialize on the most abundant prey type available (Cornell, 1976; Hughes et al., 1993).

The above results were obtained in laboratory condition. However, under field condition, the complexity may affect the feeding 'preference' of natural enemies (Kareiva, 1990). Murdoch (1972) also stressed that the effect of a predator on prey depends on environment factors in addition to the basic form of the response itself. Therefore, the true situation of feeding 'preference' is needed to be cleared by new approach. The method of stable isotope analysis was a step forward compared to the traditional feeding experiment. By the stable isotope analysis, it was reflected that, even with low frequency of pest, the wolf spider could prey on pest and did not only concentrate to alternative preys in the introduction plot. It was confirmed that wolf spiders can at least randomly prey on target pest and can prefer target pest to certain extent. This result showed some consistence with a feeding experiment performed by Heong (1991), which found that the proportion of rice brown plant hopper attacked by wolf spider (*Pardosa pseudoannulata*) was greater at a lower prey proportion.

Therefore, stable isotope analysis can directly determine the trophic characteristic of predators in the actual farmland and may become a more effective and easy-to-do way to check the generalist predators as pest control agent. For the checking, it is better to combine the field investigation and stable isotope analysis, and further works for explanation of stable isotope analysis is needed.

# Chapter 6 General discussion

## A new trial of conservation biological control

As one of strategies of integrated pest management in the organic field, conservation biological control was considered. Indigenous generalist predators were employed as pest control agent. For the best efficacy, conservation practice for natural enemies with high density is required. For this purpose, a new trial by introducing plant residue into field of which the main objective is not to provide crop fertilizer but to enrich the alternative preys for indigenous generalist predators.

To perform a conservation biological control, at least three items are needed to be considered: 1) what types of predators well adapt to the local area. Populations of natural enemy species in existence should be spatially and temporally available (Landis et al., 2000); 2) feeding response of predators to target pests; 3) whether predator populations can be enriched by artificial conservation practices especially in period without pest occurrence.

As the first step, it is needed to check the local environmental characteristics and observe the main species of native predators. In the present experiment plot, the activities of generalist predators were observed for many years before the start of the present study. These predators included lacewings, rove beetles, assassin bugs, wolf spiders, carabids and frogs. Many characteristics of these organisms have been described by entomologist or ecologist, which provide necessary basic information for the present study.

Among these predator species, the response of them to target pest species can be tested at least tested in laboratory (Murdoch, 1972). If a predator well adapt in the local environment and showed response to the target pest, it is promising to be employed as a pest control agent (O'Neil, 1990). First we can get the implications of generalist predators’ diet spectrum from previous literatures and observation in the local area. And on this basis, it is possible to increase the abundance of predators by enriching abundance of alternative preys of them. Previously, practices of living mulch and sown weed strips were adopted to increase the abundance of herbivores because they can serve as alternative prey for predator. In the present study, for predator such as wolf spiders, not only herbivore but also detritivores can be as alternative prey. Therefore, a new trial of plant residue introduction into field was explored. The feasibility of such method was based on a premise that, some generalist predators in the farmland belong to either grazing food web (crop-pests-predator) or detrital food web (plant residue-detritivores/herbivores-predator). When pests disappear, the persistence of generalist can be maintained by enhancing the energy source of detritus food web, and the enhanced predators are expected to suppress pests (Chen and Wise, 1999; Halaj and Wise, 2002).

By performance of plant residue introduction, it was found that the plant residue can enrich the abundance of alternative preys and predators, and the low pest density was observed. Therefore, it initially proved that the selected predators have the potential to be pest control agent in the conservation biological control.

In many cases, it might be a challenge to build a rather high biodiversity in farmland and the high level of biodiversity does not necessarily benefit the development of natural enemies (Rosenheim, 1993; Baggen, 1998). This study rethinks the effects of plant residue mulching and specially enriched the abundance of several species of natural enemies (Emden and Williams, 1974; Andow and Risch, 1985; Wratten et al., 1998). Because the main aim of such practice is to suppress the crop pest, the most important point is that whether the predator densities can be regulated before the pest appearance of target pests. Although we have proved that using plant residue for pest management was effective in organic field, the details of experiment design were not sufficient. Therefore, this needs more repeats and trials in future. According to my observation, such practice also benefited the pest suppression of some other vegetable such as komatsuna (*Brassica rapa* var. perviridis) and the possibility needs more studies in future.

## Utilization of stable isotope analysis

The heterogeneity of actual farmland sometimes invalidates or conflicts with results obtained in laboratory (Lucas et al., 1997). Therefore, stable isotope analysis, previously widespread used in aquatic system and proved to be a good indicator of trophic characteristic (Hobson, 1994, 1999; Inger et al., 2006), was adopted to check the trophic characteristics of predators in the present study.

By stable isotope analysis, in many cases, it has been found that, the proportion of preys in predator’s food menu or the feeding preference of predators changed with the varying of predators’ habitat environment (Inger et al., 2006), because of the temporal or spatial variation of preys (Dalerum and Angerbjörn, 2005). In the present study, it was found that with the low frequency of pest, the wolf spiders could prey on pest at least randomly in the introduction plot. From this point, at least, we can deny that the wolf spiders only concentrated on more alternative preys when pest density is low in the introduction plot, conversely, wolf spiders may show certain preference to target pest. This conclusion was in agreement with previous studies performed by feeding experiment at some extent (Heong, 1990). However, stable isotope analysis is more easy-to-do and can directly reveal the actual trophic characteristics of communities in farmland as shown in the present study. Therefore, through this study, it was strongly suggested that the stable isotope analysis is useful to determine the feeding characteristics of the potential pest control agent. Furthermore, it is best to combine the result of gut analysis, laboratory test or field observation with the stable isotope analysis to determine the efficacy of predators (Mantel, 2004).

For a potential pest control agent, the feeding response is important because it determines that whether a predator can control pest into low density or not. If a predator has only two food sources, the contribution of each resource can be calculated accurately. However, if there are more than three diet sources, the accuracy of estimation would reduce. The proportion of food sources is not a certain value but a range of solutions. Therefore, it is better to combine the feeding experiment in the laboratory and field to get more confidence.

## Ecological considerations for plant residue introduction

Surrounding environment affects the effectiveness of conservation biological control (Bianchi et al., 2006). A noticeable point that, the present experiment was carried out in the context of organic farmland environment. The experiment plot was surrounded by other organic farmland that also harbors some number of predators, and predators in surrounding area may colonize or visit to the target experiment plot (Letourneau, 1998). It is unclear that whether our method can be successful in the conventional farmland where pesticide is usually applied. According to our preliminary observation, the effectiveness of pest control by plant residue introduction in the conventional farmland was worse than that in organic farmland. Reducing the use of toxic pesticides can minimize their negative impacts on natural enemies (Pimentel, 1991; Landis, 2000).

As the conservation biological control is one component of integrated pest management, it is considered that introduction of plant residue can be used combining with other practices such as living mulch and bank plants (Wiedenmann and Smith, 1997), and such integration needed to be explored in detail in future. Furthermore, it was preliminarily observed that, the generalist also showed predacious ability to some other pest such as aphids and this need more study in detail.

Certainly there is some limitation of plant residue introduction enhancing parasitical natural enemies because their alternative diet is mainly from the nectar of some flowering plants (Lill et al., 2002). Plant residue mulching can not only protect natural enemies, but also serve as refuge or food resource for some pest insects. In the present plot, some pest insect such as Scarabaeidae larvae and Bibionidae larvae increased in introduction plot, but they had not become a threat pest. Further observation and improvements are needed in the future work.

### Points needing attention of the plant residue introduction

Different manners of plant residue application would lead to different effects to soil and environment. For the effect of enriching natural enemies, several suggestions of plant residue introduction into organic farmland should be noticed.

1) Layout. It is best to place the plant residue on to the surface of field ground in the form of strips with certain intervals, which not only provide food source for decomposers but also serve as shelter place or overwintering site for predators. Further, this can increase the farmland heterogeneity and enhance the activities of fauna. Predators can move between the habitat sites and cropping area (Hausammann et al., 1996). In the present study, the layout was designed as width of 50 – 100 cm and interval of 1 - 3 m.

2) Amount, time and types of plant residue. It is needed to ensure micro environment of the junction part between plant residue strip and the field ground, because this area contains most of soil fauna species and abundance. For this, plant residue strip with the width of 50 - 100 cm, thickness of 5 - 10 cm, and the eventual amount of 1.5 – 3.0

kg/m$^2$ was adopted based on the experience. In the present study, plant residue mulching was kept through the year which can keep the persistence of alternative preys and enable the colonization of predator. Straw of wheat, rice, soybean, corn and some weeds were confirmed effective for enriching soil fauna including earthworm, mite, millipedes, collembolan animals, etc (Tian, 1992; Afun et al., 1999; van Gestel et al., 2003). In the present study, it was confirmed that wheat straw and cereal weeds straw could enhance at least 15 groups of soil fauna/herbivores. However, it was observed that lavender of Lamiaceae and some tree leaves are not suitable for introduction into fields because such materials have special smell and some predators showed an aversion to them. The present design of plant residue introduction was conducted based on experience and previous literatures. Further studies on it are needed to find the optimum strategy of enhancing the indigenous natural enemies for pest control.

# Chapter 7 Conclusion

In the present study, as a new trial of conservation biological control in organic farmland, a series of observational studies and experiments was explored.

From observational data and laboratory test, three groups of predators (wolf spiders, predacious carabids and Japanese tree frogs) showed strong predatory capacity on the Lepidoptera larvae. The introduction of plant residue into organic farmland could enrich populations of alternative preys, subsequently enrich the abundance of generalist predators, and consequently the pest density was suppressed obviously. Before the rapid increase of pest, the enriched alternative preys caused the predator population a ‘head start’ , which contributed to the pest suppression.

By stable isotope analysis, it was found that the wolf spiders could prey on pest even when the ratio of pest : (pest + alternative prey) become low in the introduction plot. It was suggested that the wolf spiders did not only concentrate to alternative preys when pest density is low in the introduction plot. Even if the wolf spiders only prey on pest randomly, the enriched wolf spider population can still ensure the efficacy of pest suppression at some extent. It was suggested that the stable isotope analysis could be utilized as a useful tool to evaluate the natural enemies in conservation biological control.

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# Abstract

One way of conservation biological control aims to suppress pest by employing indigenous natural enemies in organic farmland under local environment characteristics. For this, as a new approach, the plant residue was introduced to increase the alternative preys for indigenous predators in organic field and the efficacy of pest suppression by the predators was examined from various viewpoints.

In farmland, many generalist predators have a potential to be biological control agents such as wolf spiders, predacious carabids and frogs. However, sometimes because of insufficient diets, their densities do not well increase to control pest to a satisfactory level. During the growing period of crops, predators can prey on pests in crops, while during the period without pest, they had to search other alternative diets for their survival. Thus, it is worthy considering how to establish the alternative diets for these predators before and after pest occurrence to enhance their persistence. To do so, although there are several tactics, in the present study, a more economic and easy-to-do method — enriching alternative preys of indigenous predators by introducing plant residue into organic farmland was adapted. When plant residue is mulched in field, it is expected that it could provide food source and refuge place for some decomposers and detritivores, and enrich their density. Many species of them could be as alternative prey for indigenous predators, which may support their survival before and after pest appearance.

By the observation in present organic field, indigenous predators including wolf spiders (*Pardosa* spp.), predacious carabids (*Dolichus halensis*, *Chlaenius pallipes* and *Harpalus* spp.) and Janpaese tree frog (*Hyla japonica*) were selected as the potential control agent of lepidopteran pest in cabbage cultivation (Chapter 2). By laboratory testing, the three predators showed strong predatory capacity on the Lepidoptera larvae. The functional response of these predators to pest fitted into the Holling type II (Chapter 4).

The field experiment was designed in both open field and greenhouse where cabbage was planted from 2009 to 2011. In the open field, through the year, plant residue including wheat straw and cereal weeds straw were mulched in the form of strips along

the direction of cabbage rows at a rate of 2500g $m^{-2}$ with the width of 50 -100 cm and with the interval between strips as 3-5 m. In the greenhouse, the same amount of plant residue was mulched along the two sides of borders (Chapter 2).

From observation, both density and biomass of alternative preys were significantly higher in introduction plot than that in no-introduction plot (Chapter 2). Population density of the predators in the introduction plot was relatively high and that of pests was low in the same plot (Chapter 3).

By the stable isotope analysis ($\delta^{13}C$), the contribution of potential preys to wolf spider's food menu was estimated. It was reflected that, even with low frequency of pest, the wolf spiders could prey on pest in the introduction plot. It was believed that the wolf spiders did not only concentrate to alternative preys when pest density/frequency was low in the introduction plot. Even if the wolf spiders only prey on pest randomly, the enriched wolf spider population can still ensure the efficacy of pest suppression at some extent (Chapter 5).

It was concluded that the strategy of introduction of plant residue into organic field could be one of effective ways to enrich alternative preys for indigenous predators, which result in the suppression of pests. It was demonstrated that the stable isotope analysis could be utilized as a useful tool for the evaluation of response of generalist predators to pest suppression.

**Key words** conservation biological control, alternative prey, indigenous predators, plant residue introduction, stable isotope analysis

# References

Afun, J. V. K., Johnson, D. E. and Russell-Smith, A. 1999. The effects of weed residue management on pests, pest damage, predators and crop yield in upland rice in Cote d'Ivoire. *Biological agriculture & horticulture*, 17(1): 47-58.

Agustí, N., Shayler, S. P., Harwood, J. D., Vaughan, I. P., Sunderland, K. D. and Symondson, W. O. C. 2003. Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. *Molecular Ecology*, 12(12): 3467-3475.

Aitchison, C. W. (1984). Low temperature feeding by winter-active spiders. *Journal of Arachnology*, 12: 297-305.

Amir, J. and Sinclair, T. R. 1996. A straw mulch system to allow continuous wheat production in an arid climate. *Field crops research*, 47(1): 21-31.

Andow, D. A. and Risch, S. J. 1985. Predation in diversified agroecosystems: relations between a coccinellid predator Coleomegilla maculata and its food. *Journal of Applied Ecology*, 22: 357-372.

Baggen, L. R. and Gurr, G. M. 1998. The Influence of Food on *Copidosoma koehleri* (Hymenoptera: Encyrtidae), and the Use of Flowering Plants as a Habitat Management Tool to Enhance Biological Control of Potato Moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Biological Control*, 11(1): 9-17.

Barbosa, P. A. 1998. *Conservation biological control*. Academic Press. San Diego, California, USA.

Bianchi, F. J. J. A., Booij, C. J. H. and Tscharntke, T. 2006. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society B: Biological Sciences*, 273(1595): 1715-1727.

Birkhofer, K., Wise, D. H. and Scheu, S. 2008. Subsidy from the detrital food web, but not microhabitat complexity, affects the role of generalist predators in an aboveground herbivore food web. *Oikos*, 117(4): 494-500.

Blackman, R. L. 1967. The effects of different aphid foods on *Adalia bipunctata* L. and Coccinella 7 ‐punctata L. *Annals of applied Biology*, 59(2), 207-219.

Blanchart, E., Villenave, C., Viallatoux, A., Barthès, B., Girardin, C., Azontonde, A. and Feller, C. 2006. Long-term effect of a legume cover crop (*Mucuna pruriens* var.*utilis*) on the communities of soil macrofauna and nematofauna, under maize cultivation, in southern Benin. *European Journal of Soil Biology*, *42*: S136-S144.

Brévault, T., Bikay, S., Maldes, J. M. and Naudin, K. 2007. Impact of a no-till with mulch soil management strategy on soil macrofauna communities in a cotton cropping system. *Soil and Tillage Research*, 97(2): 140-149.

Browne, S., Vickery, J. and Chamberlain, D. 2000. Densities and population estimates of breeding Skylarks Alauda arvensis in Britain in 1997. *Bird Study*, 47(1): 52-65.

Cabras, P. and Angioni, A. 2000. Pesticide residues in grapes, wine, and their processing products. *Journal of Agricultural and Food Chemistry*, 48(4): 967-973.

Campbell, C. A., Lafond, G. P., Zentner, R. P. and Biederbeck, V. O. 1991. Influence of fertilizer and straw baling on soil organic matter in a thin Black Chernozem in western Canada. *Soil Biology and Biochemistry*, 23(5): 443-446.

Cao, T. W., Wang, R. and Li, C. A. 2007. Hemiptera, Insects of Natural Enemies in Shanxi Province. *Journal of Shanxi Agricultural Sciences*, 8: 22-35.

Chamberlain, D. E., Wilson, A. M., Browne, S. J. and Vickery, J. A. 1999. Effects of habitat type and management on the abundance of skylarks in the breeding season. *Journal of Applied Ecology*, 36(6): 856-870.

Chen, B. and Wise, D. H. 1999. Bottom-up limitation of predaceous arthropods in a detritus-based terrestrial food web. *Ecology,* 80(3): 761-772.

Chen,J. X., Ma, Z. C., Yan, H. J. and Zhang, F. 2007. Roles of springtails in soil ecosystem. *Biodiversity Science,* 15 (2):154–161.

Cheng, B. and Wise, D. H. 1997. Responses of forest-floor fungivores to experimental food enhancement. *Pedobiologia*, 41(4): 316-326.

Chung, S. W., and Chen, B. L. 2011. Determination of organochlorine pesticide residues in fatty foods: a critical review on the analytical methods and their testing capabilities. *Journal of Chromatography A*, 1218(33), 5555-5567.

Coat, S., Monti, D., Bouchon, C. and Lepoint, G. 2009. Trophic relationships in a tropical stream food web assessed by stable isotope analysis. *Freshwater Biology,* 54(5): 1028-1041.

Collier, T., and Van Steenwyk, R. 2004. A critical evaluation of augmentative biological control. *Biological Control*, 31(2): 245-256.

Cornell, H. 1976. Search strategies and the adaptive significance of switching in some general predators. *The American Naturalist*, 110(972): 317-320.

Crinnion, W. J. 2009. Chlorinated pesticides: threats to health and importance of detection. *Alternative medicine review*, 14(4): 347-359.

Crossley Jr, D. A., Coleman, D. C. and Hendrix, P. F. 1989. The importance of the fauna in agricultural soils: research approaches and perspectives. *Agriculture, ecosystems and environment,* 27(1): 47-55.

Dalerum, F. and Angerbjörn, A. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia*, 144(4): 647-658.

Dennis, P., Wratten, S. D. and Sotherton, N. W. 1991. Mycophagy as a factor limiting predation of aphids (Hemiptera: Aphididae) by staphylinid beetles (Coleoptera: Staphylinidae) in cereals. *Bulletin of entomological Research*, 81(1): 25-31.

Donald, P. F., Green, R. E. and Heath, M. F. 2001. Agricultural intensification and the collapse of Europe's farmland bird populations.*Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268(1462): 25-29.

Edgar, W. D. 1969. Prey and feeding behaviour of adult females of the wolf spider *Pardosa amentata* (Clerck). *Netherlands Journal of Zoology*, 20(4): 487-491.

Egert, M., Stingl, U., Bruun, L. D., Pommerenke, B., Brune, A. and Friedrich, M. W. 2005. Structure and topology of microbial communities in the major gut compartments of Melolontha melolontha larvae (Coleoptera: Scarabaeidae). *Applied and environmental microbiology*, 71(8): 4556-4566.

Ehler, L. E. 1998. Conservation biological control: past, present, and future. In: Conservation biological control, pp.1-8.

Emden, H. V. and Williams, G. F. 1974. Insect stability and diversity in agro-ecosystems. *Annual Review of Entomology*, 19(1): 455-475.

Frank, S. D. and Shrewsbury, P. M. 2004. Effect of conservation strips on the abundance and distribution of natural enemies and predation of Agrotis ipsilon (Lepidoptera: Noctuidae) on golf course fairways. *Environmental entomology*, 33(6), 1662-1672.

Fujita, M. 1990. Population dynamics of Oribatida sakamorii AOKI (Acari: Oribatei) in a field of natural farming. *Edaphologia*, 42: 31-35.

Fuller, R. J., Gregory, R. D., Gibbons, D. W., Marchant, J. H., Wilson, J. D., Baillie, S. R. and Carter, N. 1995. Population declines and range contractions among lowland farmland birds in Britain. *Conservation Biology*, 9(6): 1425-1441.

Gardner, B. 1996. European agriculture: policies, production, and trade. Psychology Press.

Ge, Y. M. and Zhao, S. X. 2009. Predation of Predominant Natural Enemies on the Larvae of Prodenia litura Fabricius in the Field for Alisma orientalis (Sam.) Juzep. *Journal of Zhangzhou Normal University (Natural Science)*, 4: 19.

Gethi, M., Omolo, E. O. and Mueke, J. M. 1993. The effect of intercropping on relative resistance and susceptibility of cowpea cultivars to Maruca testulalis Geyer when in mono and when intercropped with maize. *Insect Science and Its Application*, 14(3): 305-313.

Gitonga, L. M., Overholt,W. A., Lohr, B., Magambo ,J. K. and Mueke, J. M. 2002. Functional response of Orius albidipennis (Hemiptera: Anthocoridae) to Megalurothrips sjostedti (Thysanoptera: Thripidae). *Biological Control* 24:1–6

Grundy, P. and Maelzer, D. 2000. Predation by the assassin bug Pristhesancus plagipennis (Walker) (Hemiptera: Reduviidae) of Helicoverpa armigera (Hübner)(Lepidoptera: Noctuidae) and Nezara viridula (L.) (Hemiptera: Pentatomidae) in the laboratory. *Australian Journal of Entomology*, 39(4): 280-282.

Gupta, M., and Pawar, A. D. 1989. Biological control of rice leafhoppers and planthoppers in Andhra Pradesh. *Plant Protection Bulletin (Faridabad)*, 41(1-2): 6-11.

Halaj, J. and Wise, D. H. 2002. Impact of a detrital subsidy on trophic cascades in a terrestrial grazing food web. *Ecology*, 83(11): 3141-3151.

Halaj, J., Cady, A. B. and Uetz, G. W. 2000. Modular habitat refugia enhance generalist predators and lower plant damage in soybeans.*Environmental Entomology*, 29(2): 383-393.

Hättenschwiler, S., Tiunov, A. V. and Scheu, S. 2005. Biodiversity and litter decomposition in terrestrial ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, 36: 191-218.

Haughton, A. J., Bell, J. R., Boatman, N. D. and Wilcox, A. 1999. The effects of different rates of the herbicide glyphosate on spiders in arable field margins. *Journal of Arachnology*, 27: 249-254.

Hausammann, A. 1996. The effects of weed strip-management on pests and beneficial arthropods in winter wheat fields. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, 103(1): 70-81.

Heong, K. L., Bleih, S. and Rubia, E. G. 1990. Prey preference of the wolf spider, *Pardosa pseudoannulata* (Boesenberg et Strand). *Researches on population ecology*, 33(2): 179-186.

Hill, R. H., Head, S. L., Baker, S., Gregg, M., Shealy, D. B., Bailey, S. L. and Needham, L. L. 1995. Pesticide residues in urine of adults living in the United States: reference range concentrations. *Environmental Research*, 71(2): 99-108.

Hirai, T. 2007. Diet composition of *Hyla japonica* in soybean [Glycine max] fields: Is a euryphagous predator useful for pest management? *Japanese Journal of Applied Entomology and Zoology*, 51(2): 103-106.

Hobson, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, 120(3): 314-326.

Hobson, K. A., Piatt, J. F. and Pitocchelli, J. (1994). Using stable isotopes to determine seabird trophic relationships. *Journal of animal ecology*, 786-798.

Holland, J. and Fahrig, L. 2000. Effect of woody borders on insect density and diversity in crop fields: a landscape-scale analysis. *Agriculture, ecosystems and environment*, 78(2): 115-122.

Holling, C. S. 1959. Some characteristics of simple types of predation and parasitism. *The Canadian Entomologist*, 91(7): 385-398.

Holling, C. S. 1959. The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. *The Canadian Entomologist*, 91(05): 293-320.

Http://www.insects.jp/konbunhankame.htm

Hughes, R. N. and Croy, M. I. 1993. An experimental analysis of frequency-dependent predation (switching) in the 15-spined stickleback, Spinachia spinachia. *Journal of Animal Ecology*, 62: 341-352.

Inger, R., Ruxton, G. D., Newton, J., Colhoun, K., Robinson, J. A., Jackson, A. L. and Bearhop, S. 2006. Temporal and intrapopulation variation in prey choice of wintering geese determined by stable isotope analysis. *Journal of Animal Ecology*, 75(5): 1190-1200.

Janssen, A. 1999. Plants with spider‐mite prey attract more predatory mites than clean plants under greenhouse conditions. *Entomologia Experimentalis et Applicata*, 90(2): 191-198.

Jiang, J.X., Liang, G.W. and Wang K.W. 2001. Predation of several predators to common cutworm, Spodoptera litura. *Shanghai Nongye Xuebao,*17(4): 78-81.

Kareiva, P. 1990. The spatial dimension in pest-enemy interaction. In: Mackauer, M., Ehler, L. E., Roland, J. (eds) Critical issues in biological control. Intercept, Anover, Hants, pp. 213–227.

Koch, R. L. 2003. The multicolored Asian lady beetle, Harmonia axyridis: a review of its biology, uses in biological control, and non-target impacts. *Journal of Insect Science*, 3: 32.

Koppenhöfer, A. M. 1994. Observations on the bionomics of *Thyreocephalus interocularis* (Eppelsheim) (Col., Staphylinidae), a predator of the banana weevil. *Journal of Applied Entomology*, 117(1‐5): 388-392.

Kossou, D. K., Gbèhounou, G., Ahanchédé, A., Ahohuendo, B., Bouraïma, Y. and van Huis, A. 2001. Indigenous cowpea production and protection practices in Benin. *International Journal of Tropical Insect Science*, 21(02): 123-132.

Lal, R. 1988. Effects of macrofauna on soil properties in tropical ecosystems. *Agriculture, Ecosystems & Environment*, 24(1-3): 101–116.

Landis, D. A., Wratten, S. D. and Gurr, G. M. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology,* 45(1): 175-201.

Lang, A. 2003. Intraguild interference and biocontrol effects of generalist predators in a winter wheat field. *Oecologia*, 134(1): 144-153.

Lang, A., Filser, J. and Henschel, J. R. 1999. Predation by ground beetles and wolf spiders on herbivorous insects in a maize crop. *Agriculture, ecosystems and environment*, 72(2): 189-199.

Lao, X. R., Wu, Z. Y. and Gao, Y. C. 2002. Effect of Long-Term Returning Straw to

Soil on Soil Fertility. *Transactions of The Chinese Society of Agricultural Engineering*, 2: 49-52.

Lavelle, P., Bignell, D., Lepage, M., Wolters, W., Roger, P., Ineson, P., Heal, O. W. and Dhillion, S. 1997. Soil function in a changing world: the role of invertebrate ecosystem engineers. *European Journal of Soil Biology*, 33(4): 159-193.

Lawrence, K. L. and Wise, D. H. 2004. Unexpected indirect effect of spiders on the rate of litter disappearance in a deciduous forest. *Pedobiologia*, 48(2): 149-157.

Letourneau, D. K. 1998. Conservation biology: lessons for conserving natural enemies. In: *Conservation Biological Control. Academic Press, San Diego*, pp. 9-38.

Li, L.S. and Li ,Y. D. 1988. Acarology. *Chongqing University Press*, pp. 1222 - 228.

Lill, J. T., Marquis, R. J. and Ricklefs, R. E. 2002. Host plants influence parasitism of forest caterpillars. *Nature*, 417(6885): 170-173.

Liu, F., Zhang, C. S., Chen, A. U., Zhou, G. S. and Wu, J. S. 2012. Technology research and application prospect of straw returning. *Crops*, 2: 18-23.

Lucas, E., Coderre, D. and Vincent, C. 1997. Voracity and feeding preferences of two aphidophagous coccinellids on Aphis citricola and Tetranychus urticae. *Entomologia Experimentalis et Applicata*, 85(2): 151-159.

Maloney, D., Drummond, F. A. and Alford, R. 2003. Spider predation in agroecosystems: Can spiders effectively control pest populations. Maine Agricultural and Forest Experiment Station Technical Bulletin 190.

Manley, G. V. 1977. *Paederus fuscipes* [Col.: Staphylinidae]: A predator of rice fields in west Malaysia. *Entomophaga*, 22(1): 47-59.

Manly, B. F. J., Miller, P. and Cook, L. M. 1972. Analysis of a selective predation experiment. *American Naturalist*, 106(952): 719-736.

Mantel, S. K., Salas, M. and Dudgeon, D. 2004. Foodweb structure in a tropical Asian forest stream. *Journal of the North American Benthological Society*, 23(4): 728-755.

Martin, J. 2000. The development of modern agriculture: British farming since 1931. Macmillan Press Ltd.

Mellbrand, K. 2009. The spider and the sea: effects of marine subsidies on the role of spiders in terrestrial food webs (Doctoral dissertation, Linköping).

Møller Marcussen, B., Axelsen, J. A. and Toft, S. 1999. The value of two Collembola species as food for a linyphiid spider. *Entomologia experimentalis et applicata*, 92(1): 29-36.

Moore, J. C., Walter, D. E. and Hunt, H. W. 1988. Arthropod regulation of micro-and mesobiota in below-ground detrital food webs. *Annual Review of Entomology*, 33(1): 419-435.

Moreno, C. R. 2006. The role of relative abundance and identity in the effectiveness of generalist predators as biocontrol agents of Pieris rapae L.(Lepidoptera: Pieridae) (Doctoral dissertation).

Mtiller, H., Nuessly, G. S. and Goeden, R. D. 1990. Natural enemies and host-plant asynchrony contributing to the failure of the introduced moth. *Agriculture, ecosystems and environment*, 32: 133-142.

Murdoch, W. W. 1969. Switching in general predators: experiments on predator specificity and stability of prey populations. *Ecological Monographs*, 39(4): 335-354.

Murdoch, W. W. 1972. The functional response of predators. *Biological Control*, 15: 237-40.

Murdoch, W. W. and Marks, J. R. 1973. Predation by coccinellid beetles: experiments on switching. *Ecology*, 54(1): 160-167.

Neher, D. A. 1999. Soil community composition and ecosystem processes: comparing agricultural ecosystems with natural ecosystems. *Agroforestry Systems, 45*(1-3): 159-185.

Nentwig, W. 1983. The prey of web-building spiders compared with feeding experiments (Araneae: Araneidae, Linyphiidae, Pholcidae, Agelenidae). Oecologia, 56(1): 132-139.

New, T. R. (1975). The biology of Chrysopidae and Hemerobiidae (Neuroptera), with reference to their usage as biocontrol agents: a review.Transactions of the Royal Entomological Society of London, 127(2), 115-140.

Nyffeler, M. 1999. Prey selection of spiders in the field. *Journal of Arachnology,* 27: 317-324.

Nyffeler, M. and Benz, G. 1988. Feeding ecology and predatory importance of wolf spiders (*Pardosa* spp.) (Araneae, Lycosidae) in winter wheat fields1.*Journal of Applied Entomology*, 106(1 ‐5): 123-134.

Oatman, E. R., Badgley, M. E., and Platner, G. R. 1985. Predators of the two-sDotted sDider mite on strawberry. *California Agriculture*, January-February, 9-12.

O'Neil, R. J. 1990. Functional response of arthropod predators and its role in the biological control of insect pests in agricultural systems. In *UCLA symposia on molecular and cellular biology* (Vol. 112).

Palokangas, P. and Neuvonen, S. 1992. Differences between species and instars of Phratora leaf beetles (Coleoptera, Chrysomelidae) in the probability of being preyed on. *Annales Zoologici Fennici*, 29: 273-278.

Perumalsamy, K., Selvasundaram, R., Roobakkumar, A., Rahman, V. J., Babu, A. and Muraleedharan, N. N. 2009. Life table and predation of *Oligota pygmaea* (Coleoptera: Staphylinidae) a major predator of the red spider mite,*Oligonychus coffeae* (Acarina: Tetranychidae) infesting tea. *Biological Control*, 51(1): 96-101.

Phillips, D. L. and Gregg, J. W. 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia*, 136(2): 261-269.

Powell, W. 1986. Enhancing parasite activity in crops. In: J. Waage and D. Greathead (eds), Insect Parasitoids. Academic Press, London, pp. 319–340.

Raikow, D. F., Walters, D. M., Fritz, K. M. and Mills, M. A. 2011. The distance that contaminated aquatic subsidies extend into lake riparian zones. *Ecological Applications*, 21(3): 983-990.

Rainio, J. and Niemelä, J. 2003. Ground beetles (Coleoptera: Carabidae) as bioindicators. *Biodiversity and Conservation*, 12(3): 487-506.

Ratnadass, A., Michellon, R., Randriamanantsoa, R. and Se´guy, L. 2006. Effects of soil and plant management on crop pests and diseases. In: *Biological Approaches to Sustainable Soil Systems*, CRC Press, pp. 589–602.

Riechert, S. E. 1999. The hows and whys of successful pest suppression by spiders: insights from case studies. *Journal of Arachnology*, 27: 387-396.

Riechert, S. E. and Lockley, T. 1984. Spiders as biological control agents. *Annual Review of Entomology*, 29(1): 299-320.

Rioja, T. C., Vargas, H. E. and Bobadilla, D. E. 2006. Biology and natural enemies of Peregrinus maidis (Ashmead)(Hemiptera: Delphacidae) in the Azapa

Valley. *IDESIA*, 24(1): 41-48.

Rosenheim, J. A., Wilhoit, L. R., and Armer, C. A. 1993. Influence of intraguild predation among generalist insect predators on the suppression of an herbivore population. *Oecologia*, 96(3): 439-449.

Ruess, L., Zapata, E. J. G. and Dighton, J. 2000. Food preferences of a fungal-feeding Aphelenchoides species. *Nematology*, 2(2): 223-230.

Salim, M. and Heinrichs, E. A. 1986. Impact of varietal resistance in rice and predation on the mortality of *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae). *Crop Protection*, 5(6): 395-399.

Schmaedick, M. A. and Shelton, A. M. 2000. Arthropod predators in cabbage (Cruciferae) and their potential as naturally occurring biological control agents for Pieris rapae (Lepidoptera: Pieridae). *The Canadian Entomologist*, 132(5): 655-675.

Senior, L. J. and McEwen, P. K. 2001. The use of lacewings in biological control. In: *Lacewings in the Crop Environment*, Combridge University Press, Cambridge, Newyork, pp. 296-302.

Settle, W. H., Ariawan, H., Astuti, E. T., Cahyana, W., Hakim, A. L., Hindayana, D. and Lestari, A. S. 1996. Managing tropical rice pests through conservation of generalist natural enemies and alternative prey. *Ecology*, 77(7): 1975-1988.

Shultz, B. J., Lensing, J. R. and Wise, D. H. 2006. Effects of altered precipitation and wolf spiders on the density and activity of forest-floor Collembola. *Pedobiologia*, 50(1): 43-50.

Stephens, D. W. and Krebs, J. R. 1986. Foraging Theory. Princeton University Press.

Stern, V., Bosch, R. and Leigh, T. 1964. Strip cutting alfalfa for lygus bug control. *California Agriculture*, 18(4): 4-6.

Stigter, C. J. 1984. Mulching as a traditional method of microclimate management. *Archives for meteorology, geophysics, and bioclimatology, Series B*, 35(1-2): 147-154.

Stinner, B. R. and House, G. J. 1990. Arthropods and other invertebrates in conservation-tillage agriculture. *Annual Review of Entomology*, 35: 299–318.

Suenaga, H. and Hamamura, T. 1998. Laboratory evaluation of carabid beetles (Coleoptera: Carabidae) as predators of diamondback moth (Lepidoptera: Plutellidae) larvae. *Environmental entomology*, 27(3): 767-772.

Symondson, W. O. C., Sunderland, K. D. and Greenstone, M. H. 2002. Can generalist predators be effective biocontrol agents? *Annual review of entomology*, 47(1): 561-594.

Tangyuan, N., Bin, H., Nianyuan, J., Shenzhong, T. and Zengjia, L. 2009. Effects of conservation tillage on soil porosity in maize-wheat cropping system. *Plant Soil Environ*, 55(8): 327-333.

Thomson, L. J. and Hoffmann, A. A. 2007. Effects of ground cover (straw and compost) on the abundance of natural enemies and soil macro invertebrates in vineyards. *Agricultural and Forest Entomology*, 9(3): 173-179.

Thomson, L. J. and Hoffmann, A. A. 2010. Natural enemy responses and pest control: importance of local vegetation. *Biological Control*, 52(2): 160-166.

Tian YJ, liang GW, Zeng L, Lu YY. 2011. Predatory efficiencies of Pardosa Pseudoannulata on eggs and larvae of *Spodoptera litura*. *Journal of environmental entomology*, 33(4): 502-506.

Tian, G., Kang, B. T. and Brussaard, L. 1992. Biological effects of plant residues with contrasting chemical compositions under humid tropical conditions—decomposition and nutrient release. *Soil Biology and Biochemistry*, 24(10): 1051-1060.

Tiunov, A. V. 2007. Stable isotopes of carbon and nitrogen in soil ecological studies. *Biology Bulletin*, 34(4): 395-407.

Unger, P. W. 1978. Straw-mulch rate effect on soil water storage and sorghum yield. *Soil Science Society of America Journal*, *42*(3): 486-491.

van Gestel, C. A., Kruidenier, M. and Berg, M. P. 2003. Suitability of wheat straw decomposition, cotton strip degradation and bait-lamina feeding tests to determine soil invertebrate activity. *Biology and fertility of soils*, 37(2): 115-123.

Van Lenteren, J. C., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H. M. T., Kuske, S. and Tommasini, M. G. 2003. Environmental risk assessment of exotic natural enemies used in inundative biological control. *Biological Control*, 48(1): 3-38.

Van Lenteren, J. C., Bale, J., Bigler, F., Hokkanen, H. M. T. and Loomans, A. J. M. 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. *Annual Review of Entomology*, 51: 609-634.

Wang, Z. Y., Shi, Y. and Li, H. L. 2011. Effects of maize residue return in combination with S024 on soil microbial population and wheat sharp eyespot. *Chinese Journal of Ecology*, 2: 18.

Waring, R. H. 1980. Forests, fresh perspectives from ecosystem analysis: proceedings of the 40th annual Biology Colloquium. Oregon State University, Press, corvallis Oregon.

Webb, S. C., Simpson, S. J. and Hedges, R. E. 1998. The change in $\delta^{13}C$ and $\delta^{15}N$ in locust tissues with variations in the levels of dietary protein and carbohydrate. *Chinese Science Bulletin*, 43: 142-142.

Weibull, A. C., Bengtsson, J. and Nohlgren, E. 2000. Diversity of butterflies in the agricultural landscape: the role of farming system and landscape heterogeneity. *Ecography*, 23(6): 743-750.

Wiedenmann, R. N. and Smith, J. W. 1997. Attributes of natural enemies in ephemeral crop habitats. *Biological Control*, 10(1): 16-22.

Wilson-Rummenie, A. C., Radford, B. J., Robertson, L. N., Simpson, G. B. and Bell, K. L. 1999. Reduced tillage increases population density of soil macrofauna in a semiarid environment in central Queensland. *Environmental entomology*, 28(2): 163-172.

Wise, D. H., Snyder, W. E., Tuntibunpakul, P. and Halaj, J. 1999. Spiders in decomposition food webs of agroecosystems: theory and evidence. *Journal of Arachnology*, 27: 363-370.

Wratten,S. D., van Emden, H. F. and Thomas, M. B. 1998. Within field and border refugia for the enhancement of natural enemies. In: Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests. Berkeley, University California Press. pp. 422.

Wu, Z., Zhang, H., Xu, G., Zhang, Y. and Liu, C. 2002. Effect of returning corn straw into soil on soil fertility. *The journal of applied ecology*, 13(5): 539.

Yano, E. 1999. Risk assessment of introduced natural enemies for insect pest control. *Agriculture and Horticulture*, 74(4): 435-436.

Zamani, A., Talebi, A., Fathipour, Y. and Baniameri, V. 2006. Temperature-dependent functional response of two aphid parasitoids, Aphidius colemani and Aphidius matricariae (Hymenoptera: Aphidiidae), on the cotton aphid. *Journal of Pest*

*Science*, 79(4): 183-188.

Zhu, J., Obrycki, J. J., Ochieng, S. A., Baker, T. C., Pickett, J. A. and Smiley, D. 2005. Attraction of two lacewing species to volatiles produced by host plants and aphid prey. *Naturwissenschaften*, 92(6): 277-281.