Biodiversity and Dynamics of Planthoppers and Their Natural Enemies in Rice Fields with Different Nitrogen Regimes

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Abstract: A field experiment was conducted to study the effect of different nitrogen fertilizer rates i.e. 200, 100 and 0 kg N/ha in paddy fields at International Rice Research Institute, Manila, Philippines. Biodiversity of arthropods sampled by Blower-Vac, and dynamics of planthoppers, egg parasitoids of Homoptera trapped by rice plants with eggs of brown planthoppers (BPH) *Nilaparvata lugens* (Stål), and web spiders on rice canopy collected by sweeping net, were analyzed at different rice growth stages. The most abundant arthropods were sampled at the milking stage of rice, totalling 116 species identified into 14 insect orders and 15 species of spider in all samples. Meanwhile the number of arthropod species significantly increased with rice growth and the diversity indices increased with the increase of nitrogen rate at the booting stage. On the other hand, in the dominant predators, *Pardosa pseudoannulata*, *Callitrichi formosana*, *Micraspis* sp., *Cyrtorhinus lividipennis*, *Veliidae* sp. and *Mesoveliidae* sp., only *C. lividipennis* and *Micraspis* sp. were increased significantly in abundance following the application of nitrogen at the milking stage of rice. The egg parasitoids of plant-hoppers were predominated by *Anagrus flaveolus* and *Oligosita* sp. and their densities in the field without nitrogen fertilizer were markedly higher than those in fields with 100 and 200 kg N/ha at both booting and milking stages of rice. The number and web area of dominant residential spiders *Tetragnatha* sp. and *Araneus* sp. in rice canopy significantly reduced with the increase of nitrogen fertilizer. The population density of planthoppers, included BPH and the white-backed planthoppers (WBPH) *Sogatella furcifera* Horváth, peaked during the booting stage, however, the number of BPH in rice field with 200 kg N/ha was considerably higher than those in other two rice fields with 100 kg N/ha and 0 kg N/ha at the booting as well as the milking stage. These results indicated that the rapid growth in populations of planthopper due to excessive nitrogen might be attributed to the combination of reduction in control capacity of natural enemies and strong simulation of nitrogen to planthoppers.

Key words: *Nilaparvata lugens*; nitrogen fertilizer; biodiversity; natural enemy; rice field; biological control

Biodiversity both theoretically and practically has relevance in addressing many problems of contemporary agriculture and allows the formation of functional groups that drive key ecosystem processes [1]. One of the most important processes in agroecosystems is pest regulation, because biodiversity is related closely to host-plant resistance, pest management attributes, natural biological control agents and their impacts, and stability as the ecological basis for pest management [2-3]. On one hand, intensive rice cultivation has led to a substantial increase in yield, but on the other hand, it caused a huge damage to biodiversity, resulting in the spread of insects, pests and other plant diseases. The solid evidences proved that biodiversity is closely related to the incidences of pests in rice ecosystems [4], and was strongly affected by the application of nitrogen fertilizer [5-6].

The rice brown planthoppers (BPH) *Nilaparvata lugens* Stål is an occasional insect pest in rice growing areas of Asian. In 1960s and 1970s, with the beginning of green revolution, the cropping systems and cultural practices were mostly focused to achieve higher yield using huge amount of chemical fertilizers in rice varieties [7], while the excessive use of nitrogen fertilizer was considered to be one of the key factors in shifting of BPH from minor to major insect pest [8]. Moreover, it also markedly increased the populations of other planthoppers, such as the white-backed planthoppers *Sogatella furcifera* Horváth [9-13] and small brown planthopper *Laodelphax striatellus* Fallén [10]. The application of nitrogen fertilizer not only improved the nutrients availability and

arthropods habitats for arthropods, but also modified the components of floodwater ecology in wetland rice fields, and altered the dynamics and structure of food chain through the change in aquatic invertebrate community [5, 14]. The roles of nitrogen addition may be paid directly and indirectly in herbivores and their natural enemy abundance [15], and may be complicated in insect diversity because the changes in the plant community may have opposing effects on insect species richness [16]. However, the exact role of nitrogen nutrients on the arthropod biodiversity, community structures, predators and parasitoids dynamics are not well described in rice fields.

The main objective of this study is to compare the changes of arthropod biodiversity and to qualify the dynamics of predators and parasitoids of planthoppers in temporal and spatial in paddy fields using different nitrogen regimes to evaluate the influences of excessive application of nitrogen fertilizer on the relationship between natural enemies and planthoppers.

MATERIALS AND METHODS

Preparation of experimental fields

During experiment three nitrogen rates 200, 100 and 0 kg/ha (abbreviated as 200N, 100N and 0N, respectively) with four replications were used in randomized complete block design at experimental farm of International Rice Research Institute (IRRI), Manila, Philippines (13°14′ N, 121°15′ E, 22 m above sea level) during dry season. Each treatment consisted of a plot divided into 12.5 m × 33.0 m. The fully separated sub-irrigation cannels were built up around each plot, which were connected with main irrigation cannels around the fields for irrigation and drainage. Seedlings of IR64 were transplanted at the rate of 2 or 3 seedlings per hill with 20 cm × 20 cm spacing. The nitrogen fertilizer was applied in three fractions i.e. 30% were used just one day before transplanting, another 30% were used 10 d after transplanting and the remaining 40% were used at the booting stage.

Arthropods sampled by Blower-Vac

In each plot, five sampling points were setup randomly using "Z" type model. Four hills at vegetative growth stage and one hill at reproductive growth stage in each sampling point were covered by using a plastic enclosure with nylon mesh on the top. All arthropods in the enclosure were completely sucked up by Blower-Vac suction machine and then kept in vials containing 75% alcohol. The plant samples were collected at the tillering, booting and milking stages. The arthropods were identified into species, genera and families based on their roles in rice ecosystem, and sorted into guilds as used by Moran et al [17].

Egg parasitoids of Homoptera trapped by rice plants with BPH eggs

Five sampling points from each plot were selected randomly in "Z" type pattern and marked by short bamboo sticks. Rice plants were uprooted with soils from each hill from all selected points and put into a clay pot (Dia. 14 cm) labeled with the point number. The rice plants growing in pots were washed with water and covered by mylar cages after removal of outer leaf sheaths (to remove all hopper eggs). Five, five and eight gravid BPH females were introduced into cages with rice plants from 200N, 100N and 0N rice field, respectively. After 24 hours of infestation, plants removed mylar cages and the gravid BPH females were replaced following the point numbers. After 3 d of exposure in fields, potted rice plants with parasitized and unparasitized BPH eggs were re-collected and covered immediately with mylar cages again, and kept in a greenhouse for development of parasitoids. When BPH nymphs were observed, the mylar cages were replaced by another mylar cages with tapered top attached an inverted screw glass vial, and then worn with black jackets to get complete darkness. The parasitoids attracted by light were captured from the glass vials and kept in the vials containing 75% alcohol.

Web spiders on rice canopy collected by sweeping net

All residential or temporal arthropods on rice canopy were collected by sweeping around 180° using a sweep net (Dia. 33 cm) with a 65 cm-length handle and kept in a vial containing 75% alcohol. Ten nets were swept in each plot at the tillering, booting and milking stage of rice, respectively. Number of spider

webs and their diameter in total of 80 m^2 rice canopy were determined in each plot during 6:00-8:00 am at the booting stage of rice.

Statistical analyses

Enumerative data were transformed by logarithm before analysis and the parametric statistics were generally used to compare means. Two-way analysis of variance (ANOVA) and Duncan's multiple range tests were performed with SAS package using PROC ANOVA or PROC GLM.

The diversity indices, Hill (1973) proposed:

$$N_0 = S \tag{1}$$

where S is the total number of species

$$N_1 = \exp(H') \tag{2}$$

where H' is Shannon's index, and

$$N_2 = 1/\lambda \tag{3}$$

where λ is Simpson's index.

N_1 measures the number of abundant species and N_2 represents the number of very abundant species.

For species evenness or equitability, Pielou's (1969) index of

$$J = \ln N_1/\ln N_0 \tag{4}$$

and Alatalo's (1981) index of E_5 were used,

$$E_5 = (N_2-1)/(N_1-1) \tag{5}$$

E_5 will approach zero as a single species becomes more dominant.

RESULTS

Community structures of arthropods

During the experiment the most abundant arthropods with maximum number of 116 species were found and identified into 14 insect orders and 15 species of spider in all samples collected by Blower-Vac at the milking stage of rice, which were further categorized into 4 guilds, 34 phytophages, 36 predators, 22 parasitoids and 24 scavengers of aquatic arthropods. Both species and individual number of natural enemies of insect pests were much higher than those of phytophages. More than two-thirds of species, and all economically important species including insect pests and natural enemies were recorded in all the three tested rice fields with different nitrogen regimes. The absent species were minor in economic importance or uncommon, and in 0N rice field the highest number of absent species was followed by in 100N rice field.

Arthropod diversity

Species richness

The number of arthropod species increased significantly with the rice growth, however, at the same rice growth stage, there was no obvious difference in species richness among three tested rice fields with different nitrogen regimes (Fig. 1). Two-way ANOVA results had shown a significant difference at rice growth stage ($P<0.0001$), but not in nitrogen regime and its interaction with rice growth stage.

Species diversity

Most of the diversity indices showed an increasing trend with rice growth in three tested samples with different nitrogen regimes, except special high values of N_1, N_2 and E_5 in 0N rice field at the tillering stage (Table 1). All indices increased significantly with the increase in nitrogen rate at the booting stage ($P<0.05$), while no differences were found among rice fields at the tillering and milking stages. The two-way ANOVA results showed no marked differences in nitrogen regimes, growth stages and their interactions in all indices.

Dynamics of arthropod populations

Predators of planthoppers sampled by Blower-Vac

Most of the natural enemies collected by Blower-Vac were predators, due to the limitation of the sampling method and instrument. Spiders,
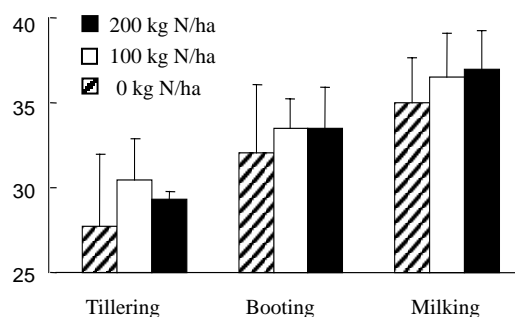

Fig. 1. Species richness in rice fields with different nitrogen regimes.

Table 1.   Arthropod diversity indices in rice fields with different nitrogen regimes.

Rice stage	N regime (kg N/ha)	H'	N_1	N_2	J	E_5
Tillering	0	2.2044	9.6393	7.4090	0.6456	0.8443
	100	2.4347	12.1067	7.0930	0.6936	0.5408
	200	2.3907	12.5019	8.6720	0.6384	0.5886
	P	0.5438	0.3715	0.6221	0.5661	0.3070
Booting	0	2.2411 b	9.4740 c	5.3486 b	0.6778 b	0.5116 b
	100	2.4344 a	11.4930 b	7.2259 ab	0.7128 ab	0.5901 ab
	200	2.4903 a	12.2060 a	8.5021 a	0.7379 a	0.6595 a
	P	0.0418	0.0405	0.0245	0.0411	0.0115
Milking	0	2.4916	12.1144	8.1489	0.7013	0.6437
	100	2.5587	13.0723	8.1231	0.7145	0.5772
	200	2.6722	14.4838	9.1854	0.7403	0.6064
	P	0.1629	0.1396	0.4806	0.1067	0.2022

Pardosa pseudoannulata and *Callitrichi formosana*, lady beetle, *Micraspis* sp., mirid bug, *Cyrtorhinus lividipennis*, predatory Hemiptera, *Veliidae* sp. and *Mesoveliidae* sp. were dominant species in rice fields. Amongst all the dominant species, only *C. lividipennis* and *Micraspis* sp. differed significantly in abundance at the milking stage of rice among three tested rice fields, while the others were not affected by the nitrogen regimes and kept the relative constant abundance, though their individual number increased greatly with the rice growth (Fig. 2).

On the other hand, the scavenger Collembola served as the alterative prey for generalist predators increased slowly at the early rice growth stage and elevated strongly at the milking stage of rice in all three tested rice fields, when 45.6, 74.0 and 77.9 Collembola per hill were collected in 0N, 100N and 200N rice fields, respectively. The results of two-way ANOVA had shown significant differences between N regimes (P=0.0226), rice growth stages (P<0.0001) and their interaction (P=0.0357).

Egg parasitoids trapped by rice plants with BPH eggs

During this experiment three egg parasitoids, *Anagrus flaveolus, Oligosita* sp. and *Gonatocerus* sp. were trapped in all tested rice fields, dominated by *A. flaveolus* and *Oligosita* sp. Abundance of the total egg parasitoids trapped in 0N was significantly higher than those trapped in 100N and 200N fields both at the booting and milking stages of rice. However, an

Table 2. Two-way ANOVA results for planthoppers and their natural enemies at different rice growth stages.

Arthropod[a]	N regime	Rice stage	Interaction
Predatory spiders	0.0635	<0.0001	0.0529
P. pseudoannulata	0.0709	<0.0001	0.1687
C. formosana	0.2519	<0.0001	0.1902
C. Lividipennis	**0.0108**	<0.0001	**0.0043**
Predatory Hemiptera	0.1247	0.0005	0.4927
Micraspis sp.	**0.0152**	<0.0001	**0.0021**
N. lugens	**0.0003**	<0.0001	**0.0207**
S. furcifera	**0.0311**	<0.0001	0.0609
N. virescens	0.6666	<0.0001	0.5429
Egg parasitoids	**0.0020**	0.001	**0.0474**
Anagrus sp.	**0.0143**	<0.0001	**0.0026**

[a]Predatory spiders include *P. pseudoannulata, C. formosana, Tetragnatha* sp.*, Dischiriognatha* sp.*, Araneus* sp. and *Salticidae*. Predatory Hemiptera include *Veliidae* and *Mesoveliidae*, and Egg parasitoids include *Anagrus* sp., *Gonatocerus* sp. and *Oligosita* sp.

obviously abundant population of *A. flaveolus* was only found at the booting stage (Fig. 2). The two-way ANOVA results showed that all factors of nitrogen regimes, rice growth stages and their interactions were significantly affected the abundance of egg parasitoids (P<0.05)(Table 2).

Plant- and leaf-hoppers collected by Blower-Vac

More than 99.9% individuals in total plant- and leaf-hopper captured were green leafhoppers (GLH),
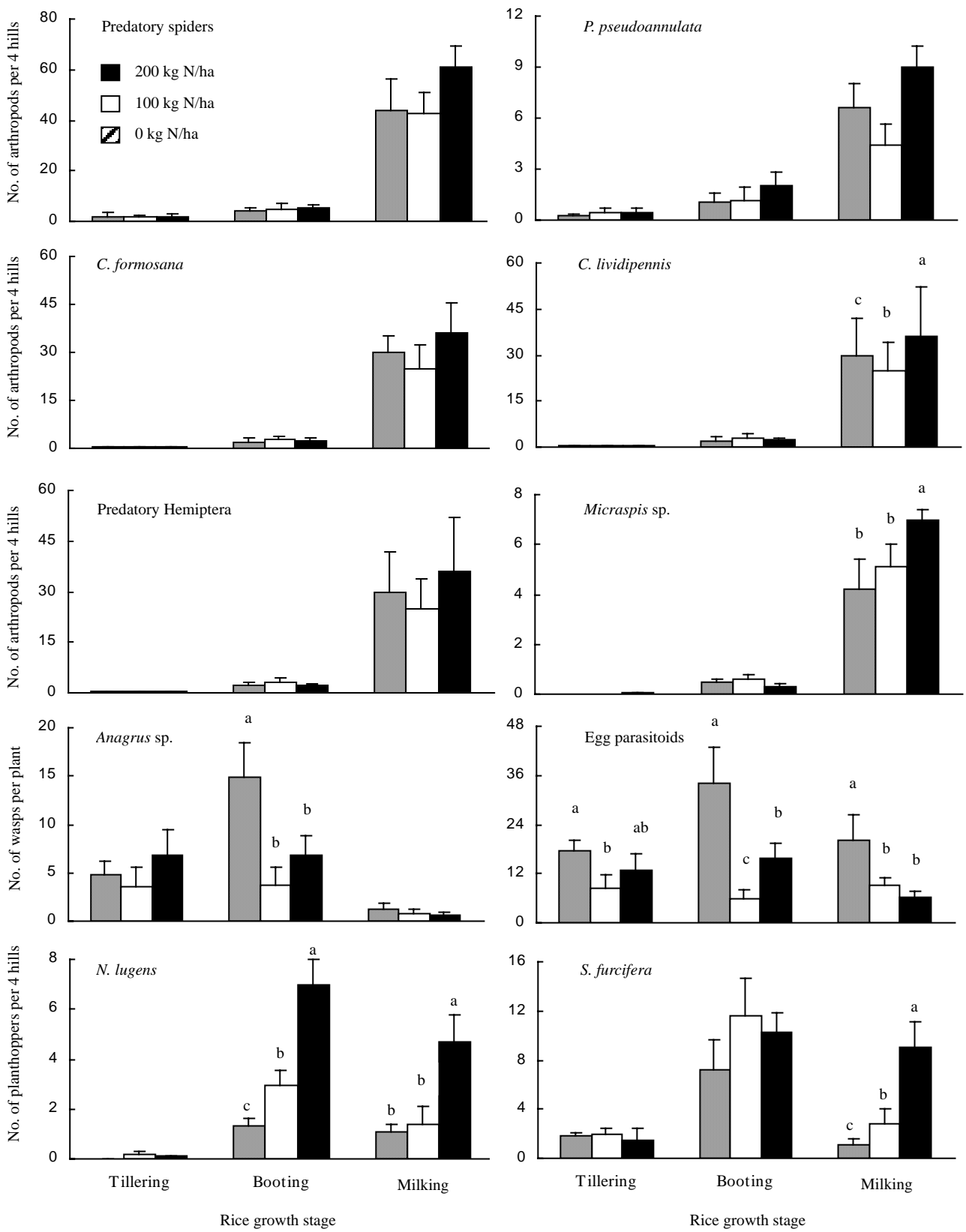
Fig. 2. Dynamics of planthoppers and their natural enemies in rice fields with different nitrogen regimes (sampled in 4 hill or trapped by a potted rice plant with BPH eggs).

Nephotettix virescens, and *N. nigropictus*, BPH and WBPH. The densities of GLH at all rice growth stages were not significantly different among three tested rice fields and different nitrogen regimes, although their abundance increased with rice growth. The two-way ANOVA results showed that GLH abundance was not affected by N regimes ($P=0.6666$) and its interaction with rice growth stage ($P=0.5429$) (Table 2).

The number of BPH and WBPH fluctuated greatly and humped typically ($P<0.0001$) with the change in rice growth, with the greatest increase at the booting stage and then start to decrease at the milking stage of rice. Moreover, significant differences have been noted among BPH and WBPH populations under different nitrogen regimes ($P=0.0003$ for BPH, $P=0.0311$ for WBPH). On the whole, the number of BPH in 200N rice field was strongly higher than those in 100N and 0N rice fields at the booting and milking stages, whereas no significant difference was found at the tillering stage, while the BPH densities were much lower than WBPH, especially at the booting stage in all the three tested fields (Fig. 2).

Web spiders on rice canopy

The main predators of planthoppers on rice canopy in all three tested fields were *Tetragnatha* sp., *Araneus* sp., *Cyrthorhinus lividipennis*, *Micraspis* sp. and *Agriocnemis* sp., while the web spiders, *Tetragnatha* sp. and *Araneus* sp. were the most dominant in rice canopy. The number of *Tetragnatha* sp. at the booting stage in 0N rice field was significantly higher than those in 100N and 200N rice

fields ($P=0.015$), however, no obvious difference in abundance of *Araneus* sp. was recorded among three tested rice fields ($P=0.2975$) (Fig. 3). There was significant reduction in number ($P=0.003$) and area ($P=0.0153$) of webs with the increase of nitrogen rate at the booting stage.

DISCUSSION

Intensive cultivation, predominated by pesticide spraying and severe use of fertilizer topdressing, has led to substantial increases in crop yield, but at the cost of losing much biodiversity and, consequently, vulnerability to outbreaks of pests. The application of nitrogen fertilizer not only improved the nutrients availability and habitats for arthropods, but also altered the dynamics and structure of food web by changing the invertebrate community [5,14], and increased the arthropod species and abundance. The differences in dynamics performances of various arthropod species through the influences of host plants, natural enemies and aquatic invertebrates determined the biodiversity indices in irrigated rice field, moreover, the use of additional nitrogen resulted in increase of most measured biodiversity indices, especially at the booting stage (Table 1). The Fig. 1 shows that the number of invertebrate species increased with the growth of rice plants. However, the species component of dominant arthropods, phytophage and natural enemies, did not differ during all rice growth stages, though their abundance fluctuated strongly and increased greatly with the rice growth (Fig. 2). Those results were not in agreement
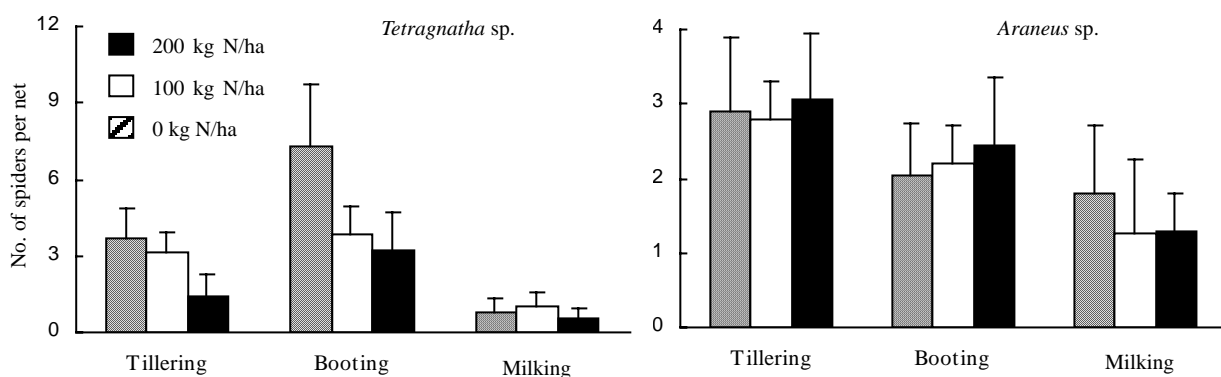

Fig. 3. Dynamics of web spiders on rice canopy in rice fields with different nitrogen regimes.

with the previous findings that insect species richness and diversity decreased, and insect abundance increased in long-term nitrogen loading grassland [18], since the duration of excessive nitrogen fertilizer usage in rice fields was much shorter in this experiment.

Mortality is one of the major biotic factors, which fluctuate the number of insect pests caused by invertebrate predators and parasitoids [19]. Natural enemies of leaf- and plant-hoppers were mostly predominated by common predators and specific parasitoids. In general, the common predators may play a more important role in suppression of BPH population growth than specific parasitoids in tropical rice growing areas of Asia with more complex food webs [19, 20-27]. The application of nitrogen fertilizer in paddy field can enrich the nitrogenous chemicals in flooded water and the availability of nutrients for aquatic invertebrate community, thus resulting in abundant number of aquatic invertebrates such as aquatic scavengers Collembola and chironomids. Collembola and chironomid midge larvae allow general predators to establish and multiply in unsprayed paddies before herbivores immigration [6, 22, 27-29]. The scavenger Collembola abundance increased with nitrogen fertilizer with significant difference only at the booting and milking stages. It implied that Collembola could not considerably enhance the predator populations at the early growth stage. On the contrary, aquatic scavengers trigger prey-switching by predators that otherwise control rice insect pests switch to aquatic scavengers because they are more numerous and easier to capture than pests [30]. Collembola may profoundly decrease the predatory efficiency of general predators by additional supply of preys and the interference with general predators' foraging for target preys [19]. However, the dynamics of predators in rice fields was affected both directly and indirectly by the application of nitrogen fertilizer, moreover it also characterized by their own biological properties, as the resultants, the abundant differences caused by nitrogen fertilizer differed greatly among all monitored predators.

Egg parasitoids were clearly demonstrated its role in overall BPH mortality. Their enhancement and manipulation in non-rice habitats was considered to be an effective tool in improvement of natural BPH control [20, 23, 33]. The importance in host finding and location of leaf reflectance properties or yellow leaf color was demonstrated in many insect species, Aleyrodidae, Aphididae, Thrpidae, dipterans and Coleopteran, and reviewed by Bernays & Chapman [32]. *Anagrus* sp. was also found to be attracted effectively by higher bright or yellow color traps, and by rice plants with low nitrogen fertilizer [33]. The application of nitrogen fertilizer changed the color of rice leaf, which strongly attracted the *Anagrus* sp. and resulted in wasps flied toward low nitrogen fields (unpublished data). This may be a partial reason that abundance of *Anagrus* trapped by rice plants with BPH eggs in 0N rice field was much higher than in others (Fig. 2). However, the low host egg density in 0N rice field might be the other reason since the higher egg densities on trap rice plants may increase the parasitic probability for egg parasitoids including *Oligosita* sp.

C. lividipennis is a plant sucker and insect predator [34]. The better nourishment and more succulent rice plants produced by nitrogen nutrient increases the rate of survival and reproduction of *C. lividipennis* due to availability of sufficient nutrient, which provides ideal conditions to maintain the greater population and bigger size in high nitrogen fields than those in other fields (Fig. 2). On the other hand, this biological property of *C. lividipennis*, may not be beneficial for *C. lividipennis* as a biological control agent due to decrease in their predatory efficiency by continuous feeding on rice plant [35]. The availability of higher succulent rice plants with softer leaf blades makes it difficult for web spiders to use the leaf blade as frames for webbing, moreover, the thicker canopy make them difficult to find enough space for webbing. As a result, more webs and bigger web size were found in fields with low nitrogen fertilizers (Fig. 4).

It has been noted that leaf- and plant-hoppers were highly affected by multiple environmental factors, and suppressed by many natural enemies in rice ecosystem. However, multiple step-wise regressions to describe the abundance of several pest species including BPH was successfully used to find the relationship between pests and natural enemies in unsprayed and sprayed fields [36]. Similar to this model,
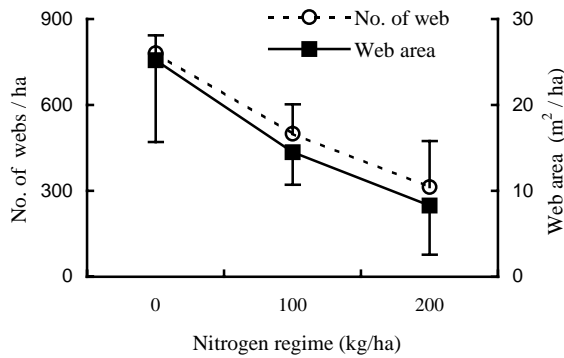
Fig. 4. Number of spider webs and their area in the fields with different nitrogen regimes at rice booting stage.
ANOVA for No. of spider webs, $P = 0.0030$; Web area, $P = 0.0153$.

during this experiment no significant relation was found between BPH population and single natural enemy in rice plants grown under different nitrogen regimes. In addition, the initial phytophage population was low at the tillering stage and build-up in time, which was closely followed by natural enemies. The ratio of spider to BPH was used popularly to evaluate the natural biological control capacity in several rice growing Asian countries [37-38]. Though the densities of planthoppers increased drastically at the booting stage of rice, but the ratio of natural enemies against BPH decreased significantly with the increase of nitrogen fertilizer (unpublished data), as a consequence, the differences in planthopper populations enlarged among fields. The rapid growth in planthopper populations due to excessive application of nitrogen fertilizer may be attributed to the combined reduction in capacity of natural enemies to control pests and strong simulation of nitrogen to planthoppers.

ACKNOWLEDGEMENTS

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