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Field Margin Vegetation in Tropical African Bean Systems Harbours Diverse Natural Enemies for Biological Pest Control in Adjacent Crops

Prisila A. Mkenda [1,2,*], Patrick A. Ndakidemi [1], Philip C. Stevenson [3,4], Sarah E. J. Arnold [3], Steven R. Belmain [3], Maneno Chidege [5] and Geoff M. Gurr [6,7]

1 Department of Sustainable Agriculture, Biodiversity and Ecosystems Management, The Nelson Mandela African Institution of Science and Technology, P.O. Box 447, Arusha 23311, Tanzania; patrick.ndakidemi@nm-aist.ac.tz
2 Department of biosciences, Sokoine University of Agriculture, P.O. Box 3000, Morogoro 67129, Tanzania
3 Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent ME4 4TB, UK; P.C.Stevenson@greenwich.ac.uk (P.C.S.); S.E.J.Arnold@greenwich.ac.uk (S.E.J.A.); S.R.Belmain@greenwich.ac.uk (S.R.B.)
4 Royal Botanic Gardens, Kew, Richmond Surrey TW9 3AB, UK
5 Department of Research, Plant Protection Division, Tropical Pesticide Research Institute (TPRI), P.O. Box 3024, Arusha 23201, Tanzania; mchidege@yahoo.com
6 Graham Centre for Agricultural Innovation, Charles Sturt University, P.O. Box 883, Orange, NSW 2800, Australia; GGurr@csu.edu.au
7 Institute of Applied Ecology, Fujian Agriculture and Forestry University, Fuzhou 350002, China
* Correspondence: mayoprisca@gmail.com

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Abstract: Non-crop vegetation around farmland can be valuable habitats for enhancing ecosystem services but little is known of the importance of field margins in supporting natural enemies of insect pests in tropical agriculture. This study was conducted in smallholder bean fields in three elevation zones to assess the importance of field margin vegetation to natural enemy populations and movement to the bean crop for biological pest control. The pests and natural enemies were assessed using different coloured water pan traps (to ensure the capture of insects with different colour preferences) and the interactions of the two arthropod groups with the margin vegetation and their movement to the bean crop were monitored using fluorescent dye. Sentinel plants were used to assess predation and parasitism levels. A total of 5003 natural enemies were captured, more in the field margin than within the bean field for low and mid elevation zones, while in the high elevation zone, they were more abundant within the bean field. Pests were more abundant in the crop than margins for all the elevation zones. The use of a dye applied to margin vegetation demonstrated that common natural enemy taxa moved to the crop during the days after dye application. The proportion of dye-marked natural enemies (showing their origin to be margin vegetation) sampled from the crop suggest high levels of spatial flux in the arthropod assemblage. Aphid mortality rates (measured by prey removal and parasitism levels on sentinel plants) did not differ between the field edges and field centre in any of the three elevation zones, suggesting that for this pest taxon, the centre of the fields still receive comparable pest control service as in the field edges. This study found that field margins around smallholder bean fields are useful habitats to large numbers of natural enemy taxa that move to adjacent crops providing biological pest control service.

Keywords: ecosystem service; pest regulation; predators; parasitoids; non-crop vegetation

1. Introduction

Natural pest regulation by beneficial arthropods can be enhanced through habitat management close to and within the crop [1,2] or at the landscape level [3,4], an approach known as conservation biological control [5]. However, manipulation within the crop habitat such as planting flower strips or the use of cover crops pose challenges as these approaches compromise farming practices and may lead to competition with the crops and difficulties during harvest, especially where mechanized. Currently, much attention is given to the features that are already present around the crop lands which can be well managed or preserved for enhancing natural pest control [6,7]. Evidence of the importance of field margin plants and the effect of various management practices to different arthropod groups is described by Anderson et al. [6]. The influence of field margin plants on different natural enemy taxa, including syrphids and tachinids [8], carabids [9] and spiders [10], is also reported. Landscape structure and composition, together with non-crop vegetation along the field margin, contribute significantly to the abundance and diversity of the natural enemies within the crop land [11–13]. This is due to the fact that natural and semi-natural habitats are less disturbed in comparison to crop land. Thus, they can act as reservoirs of natural enemies to recolonize the crop area after disturbance. González et al. [14] found that natural enemies moved from native forest to the crop to a greater extent than did herbivores.

Most predators and parasitoids require a variety of resources beyond those the crop alone can provide. Thus, plant diversification close to agricultural land is important for their survival. Landscape simplification together with monoculture farming systems reduces farmland biodiversity, including biological control agents such as predators and parasitoids, while promoting insect pests [15,16]. The quality and complexity of the non-crop vegetation around crop fields, as well as its spatial arrangement, influence the movement of natural enemies into and between agricultural lands [17–19]. Boetzl et al. [20] found beneficial edge effects for predatory carabid beetles, irrespective of the margin type, signifying the importance of non-crop habitats around arable farms.

Synchronization between natural enemy and insect pest assemblages is important for effective biological control [21]. The timely arrival of natural enemies into crop fields, relative to the arrival of pests, is essential to maintain insect pest populations below economically damaging thresholds. For example, Brosius et al. [22] and Pfab et al. [23] found maximum efficiency of control when predator populations were established before aphid colonization. One of the key factors that determines the timely arrival of natural enemies to the crop is distance between the crop land and donor habitat [24]. Field margin vegetation may therefore act as an important feature on arable farms to provide shelter and food resources to natural enemies, thereby enhancing their local density and persistence, potentially leading to pest suppression provided that movement into crops from margins takes place.

Some field margins have, however, been reported to be a source of insect pests to the crop, especially where the field margins contain an alternative host plant for a particular insect pest. For example, the cassava pest, *Stictococcus vayssierei*, is reported to be enhanced in proximity to some field margin plants in the Congo basin [25]. Other insect pests reported to benefit from field margin plants include fruit flies, *Drosophila suzukii* [26,27] and spider mites [28]. Therefore, understanding the net effects of field margin vegetation on natural enemies and pests is key to effective habitat management for pest management. The reasons for semi-natural habitats failing to promote natural pest control include a lack of natural enemies in the area and low levels of dispersal into crops from the non-crop vegetation [29]. Accordingly, work in poorly studied regions, such as the smallholder bean system of the present study, must determine as early steps the extent to which field margin vegetation harbours natural enemies and whether these move into crops for biological pest control.

Much information on the importance of non-crop vegetation to natural enemy populations, biological control activity and other ecosystem services is reported in Europe, e.g., [30–32], but there is limited information from Africa and other tropical countries and this is an important information gap addressed in the present study. Related to this, while the manipulation of cropping systems by planting strips of flowering plants along the field margin or within the field crop [33,34] or by the use of cover crops [35] is well known to enhance beneficial insects in developed country cropping systems, there is limited information relevant to smallholder tropical farming systems. Accordingly, this study was carried out to characterise the pest and natural enemy assemblages of bean crops and field margin vegetation in Tanzanian smallholder farms. The first objective involved surveys of farms in three elevation zones in order to assess insect abundance and their distribution within the field margin and the bean fields over two years (2016 and 2017), each spanning the major (long rain) and minor (short rain) growing seasons. The second objective involved the application of fluorescent dye to the field margin followed by sampling insects within the bean crop to determine the extent of movement among taxa and sites. The third objective assessed the biological control activity of natural enemies by measuring predation and parasitism on sentinel plants, close to the field margin and within the bean field during bean flowering.

2. Materials and Methods

2.1. Study Sites

The field sites (Supplementary Materials Table S1, Figure S1) were located in three elevation zones of the Moshi rural district in Northern Tanzania. The low zone was below 1000 m asl, the mid zone was between 1000 and 1500 m asl and the high zone was between 1500 and 1800 m asl. In each zone, eight fields of common bean (*Phaseolus vulgaris*) were studied; one 'major' site (involved intensive data collection) and seven 'minor' sites (involved less intensive data collection) were surveyed to characterise the natural enemy and insect pest assemblages in the field margin and the crop. The spatial distribution of the natural enemies and insect pests at different distances from the margin into the bean fields was studied for major sites only. Field experiments on biological control involved five of the eight sites (one major and four minor sites) in each zone and a fluorescent dye experiment to monitor the movement of insects from margin to crop habitats was performed on three of the minor sites.

2.2. Insects Sampling in the Field Margin and at Different Distances into the Bean Field

This study was conducted during 2016 and 2017, each consisting of two bean seasons, the long rain season (March to June) and short rain season (July to October). The densities of natural enemies and insect pests in the field margin and within the bean field were determined using trios of coloured water pan traps (blue/white/yellow + UV paint), with a diameter of 18 cm and depth of 10 cm (henceforth "traps" used to refer to the trio of pans on the stand) in 8 sites within each zone, making a total of 24 sites in all zones—3 major sites and 21 minor sites. The choice of the three colours of the traps were based on the fact that they are the most preferred by different insect taxa as also reported in similar studies [36,37], and hence maximized the capture of insects with different colour preferences. The assessment of the spatial and temporal distribution of insects involved sampling at different distances within the bean field, and several crop growth stages, in the three major sites, one at each elevation. All sites were smallholder farmers' bean fields fully managed by them according to their normal farming practices for planting, weeding and other activities, with the single exception that they all agreed not to apply any kind of pesticide in the selected bean field during the experimental period.

At each major site, ten traps were deployed: five placed equidistantly along a 50 m transect at the border of the field margin and crop, along the longest edge of the field. A further five traps were arranged equidistant along a 50 m transect perpendicular to the field margin into the bean crop, starting at the centre of the first transect and running into the field. The aim of the field transect was to assess the spatial distribution of the natural enemies at different distances from the field margin. On minor sites, two traps were used: one at the border of the field margin and crop and another at the centre of the bean field. Each trap consisted of one pan of each colour containing 300 mL of water with a drop of detergent to reduce surface tension and prevent trapped insects from escaping.

Sampling at major sites was conducted at: seedling (when the bean plants had the first true leaves), flowering (when the crop has open flowers), podding (when pods are developing and flowering ceased) and post-harvest (after bean harvest with only weeds present in the fields) stages of each of the two seasons on the year, for two years. For minor sites, the seedling stage was not included. Traps were left in the field for 48 h on major sites and 24 h in minor sites for logistical reasons but comparability was achieved by converting catches to a daily basis. The collected insects were then removed from the traps using Pasteur pipettes and forceps and transferred to labelled vials containing 70% ethanol for later identification.

2.3. The Assessment of Predominant Field Margin Plants in Each Elevation Zone

The predominant plant species in each elevation zone were also identified by a transect approach surveying plant species in 1 m × 1 m quadrats along the field margin. The transects were 50 m in length for major sites and at each 10 m, two quadrats of 1 m × 1 m were measured, one before the 10 m mark, and one after, making a total of 10 quadrats per margin. In the minor sites, two quadrats at the centre of the field margin were measured. The plant species present in each quadrat were identified and the % coverage was recorded. The plant species which were difficult to identify in the field were collected for herbarium preparation and, thereafter, they were sent to Tropical Pesticide Research Institute (TPRI) and Royal Botanic Gardens, Kew, for identification.

2.4. Fluorescent Dye Experiment to Monitor the Movement of the Natural Enemies and Pests

Fluorescent dye spray was used to monitor the movement of insect pests and natural enemies from non-crop vegetation to the bean crop on three sites in each zone, making a total of nine sites. The sites contained native and non-native plants naturally growing along the field margins. The yellow fluorescent pigment was purchased from Spray Shop, Adelaide, Australia (www.sprayshop.com.au). It was prepared as per the manufacturer's instruction with 1 L of pigment diluted in 100 L of water. The dye was applied using a clean backpack sprayer of 12 L capacity (Taizhou Kaifeng Plastic and Steel Co. Ltd, Taizhou Zhejiang, China). Spraying was performed on the non-crop vegetation in a 3 m wide and 50 m long strip when beans were at 50% flowering. The spraying time was 10:00 ± 1 h. The sampling of the insects was performed after 24 h using sweep nets on three consecutive days along 2 m wide and 50 m long transect lines. The sampling time was 10 min per transect. Samples were collected at 0, 10, 20, and 40 m from the field margin after Perović et al. [38], except in the high zone, where it ended at 20 m only because of the smaller size of the bean fields. The samples from each distance were immediately placed in separate tubes containing cotton wool sprayed with ethanol to make the arthropods inactive and minimise contaminating and consuming each other. Samples were then kept in a fridge at 4 °C for later identification. Sampled insects were inspected for traces of fluorescent dye under UV light in a dark room. The insects were considered marked if a drop pattern of the dye was observed on any part of the body reflecting the original application but the insects with small scattered flecks were considered contaminated during sampling and disregarded.

2.5. Field Predation Experiment

Field predation was assessed using 45 potted bean plants, each infested with 60 ± 10 aphids (*Aphis fabae*), as sentinel prey. The potted bean plants were placed into 5 bean fields in each zone, one at the field centre, one near the field margin and one control (caged to prevent entry of natural enemies) placed randomly within the bean field, making a total of 3 potted plants per field and 15 potted plants per zone. This resulted in exposure of the sentinel plants to the natural enemies present in each part of the field. The sentinel aphids were exposed for two days during the bean flowering period. The assessment of the predation rate was performed by counting the number of aphids before and after 2 days of exposure. The removal of aphids in the caged sentinels was used to partition predation caused by factors other than predation.

2.6. Field Parasitism Experiment

Parasitism levels were assessed using a different set of potted bean plants to those in 2.4, again infested with 60 ± 10 aphids, which were reared in green house inside cages to prevent them from experiencing any parasitism before field exposure. This was followed by controlled field exposure under caged and open conditions in same fields used for the predation experiment. A total of 20 potted bean plants were exposed in each zone—in five bean fields, four potted bean plants per field, where two plants (caged and uncaged) were placed near the field margin and other two at the field centre for 2 days. The cages were made of a coarse mesh (1 mm) which allowed the entry of parasitoids while preventing the entry of predators. After field exposure, the potted bean plants were all placed in individual cages measuring 30 × 30 × 60 cm (L × W × H) in size with a fine mesh and were placed in the green house. After 7 days, the parasitized aphids (mummies) were counted and later, the emerging parasitoids were collected using an aspirator and preserved in 98% ethanol for later identification. Parasitoids were identified based on morphological features at TPRI, Arusha, Tanzania.

2.7. Data Analysis

All data were analysed in R program Version3.5.1 [39]. The overall field insect survey data set was grouped into different categories for the testing of different variables according to the sampling design involved. First, data from the minor sites were used to assess the effect of margin vs. field and field size on natural enemy and pest abundance. Second, data from the major sites were used to test the effect of distance from the margin (10 to 50 m) and sampling stages (seedling, flowering, podding and post-harvest) on arthropod abundances. Third, data collected in the second season of both years were used to test the effect of elevation on arthropod numbers. The dye experiment data was used to assess the movement of natural enemies and pests from the field margin plants to field crops at different distances. Biological control activity of the natural enemies was assessed from predation and parasitism data.

The insect survey data were analysed by a generalized linear model with a negative binomial for describing the interactions and associations of different categorical variables using the MASS package [40] and glm () function in R. The model selection was based on its application to count data that are not normally distributed. Different independent variables including the elevation zones, margin vs. field transect, field size, distance, growth stage and season were tested against the dependent variable, which was the abundance of different insect taxa. The non-significant terms and their interactions were dropped stepwise by comparing the models using the likelihood ratio test (LRT) until the final model was obtained. Analysis of variance (ANOVA) was used to test the significant difference of the different variables to the abundance and distribution of insects in the field.

Data from the dye experiment were analysed by a generalized linear regression model with Gaussian family. The model was used to test for the significance of distance from margin, elevation zone, farm size and time from dye application on the proportion of captured natural enemies and insect pests that were marked. The same model was also applied in predation and parasitism experiments. ANOVA was used to test the statistical significance of the different variables in the model. Tukey's honestly significant difference (HSD) post hoc test was used to check where the significant difference occurred in different parameters by pairwise comparison.

3. Results

3.1. Sampled Insects in the Three Coloured Pan Traps across the Three Zones

A total of 5003 natural enemies were collected, comprising predatory wasps (Hymenoptera: Vespidae) and parasitic wasps (Hymenoptera: Braconidae and Ichneumonidae), ants (Hymenoptera: Formicidae), robber flies (Diptera: Asilidae), long legged flies (Diptera: Dolichopodidae), tachinid flies (Diptera: Tachinidae), hoverflies (Diptera: Syrphidae), lacewings (Neuroptera: Chrysopidae), rove beetles (Coleoptera: Staphylinidae), carabid beetles (Coleoptera: Carabidae), lady beetles (Coleoptera: Coccinellidae), assassin bugs (Hemiptera: Reduviidae), and spiders (Araneae: Araneidae). Lady beetles were very abundant through field observation, but they were not easily trapped by the water pan traps. Parasitic wasps and ants were the most sampled in the low and mid zones, while long legged fly and rove beetle were more sampled in the high zone (Figure 1). Though hoverflies are predators only as larvae, adults were included in the analyses since these produce eggs that will hatch and provide pest regulation services and so are a proxy for the predatory larvae.

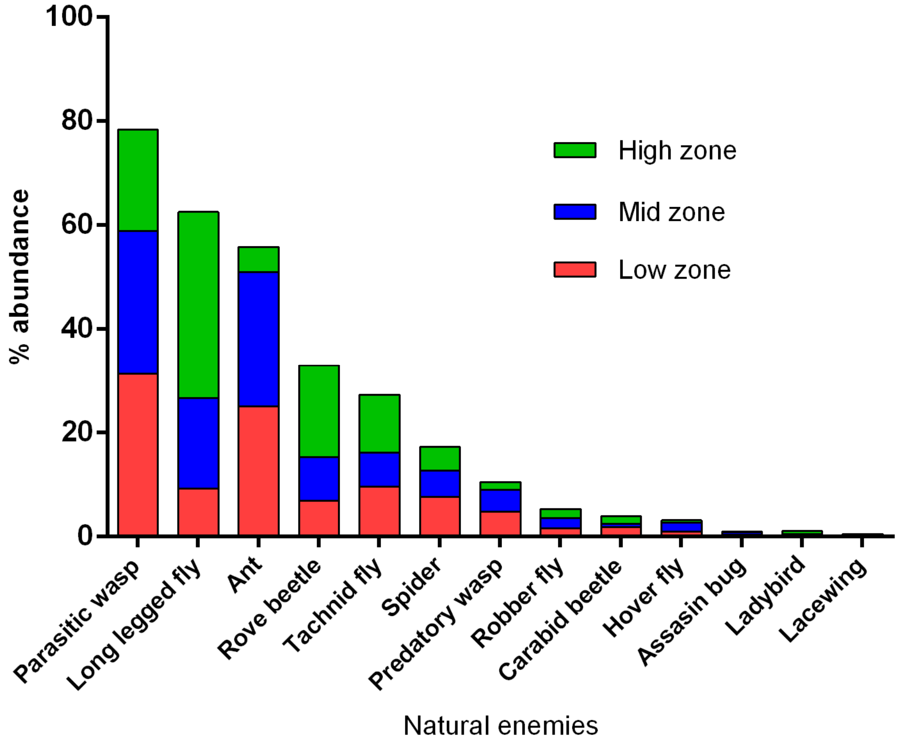

Figure 1. Percent abundance of natural enemies in smallholder bean fields across three elevation zones of the Moshi rural district, Tanzania.

There was a significant difference in the abundance of the natural enemies among the three elevation zones (F = 15.817, df = 2, $p < 0.001$); most numerous in the high zone (50.4% of catch) and declining with elevation, 31.7% and 18.0% in the mid and low zones, respectively (Figure 2). Tukey's post hoc test showed significant differences in the natural enemy abundance between low and mid elevation zones ($p = 0.047$), mid and high elevation zones ($p = 0.004$) and low and high elevation zones ($p < 0.001$).

A total of 2594 insect pests were captured in the pan traps. With the exception of aphids, the most sampled insect pests that were present in the field were: blister beetles (Coleoptera: Meloidae), bean leaf beetles, *Ootheca* sp. (Coleoptera: Chrysomelidae), bean weevil (Coleoptera: Chrysomelidae), caterpillars (Lepidoptera: Crambidae), thrips (Thysanoptera: Thripidae), and whiteflies (Hemiptera: Aleyrodidae). Aphids are considered the most damaging insect pests in the area, but they usually do not enter into the water pan traps. *Ootheca* were abundant during the seedling stage, observed in the field in the low and mid zones, but they were similarly less likely to enter into the pan traps. Thrips and blister beetles were the most trapped insect pests in the low and mid zones, while in the high zone, caterpillars were the pests most often trapped (Figure 3).

There was a significant difference in the abundance of the insect pests between zones (F =11.983, df = 2, $p < 0.001$); most numerous in the mid elevation zone (50.62% of catch) followed by the low elevation zone (30.44%) and the least in the high elevation zone (18.93%) (Figure 4). Tukey's post hoc test showed no significant difference in pest abundance between the low and mid elevation zones ($p = 0.191$), but with significant difference between the mid and high elevation zones ($p = 0.012$) and the low and high elevation zones ($p < 0.001$).

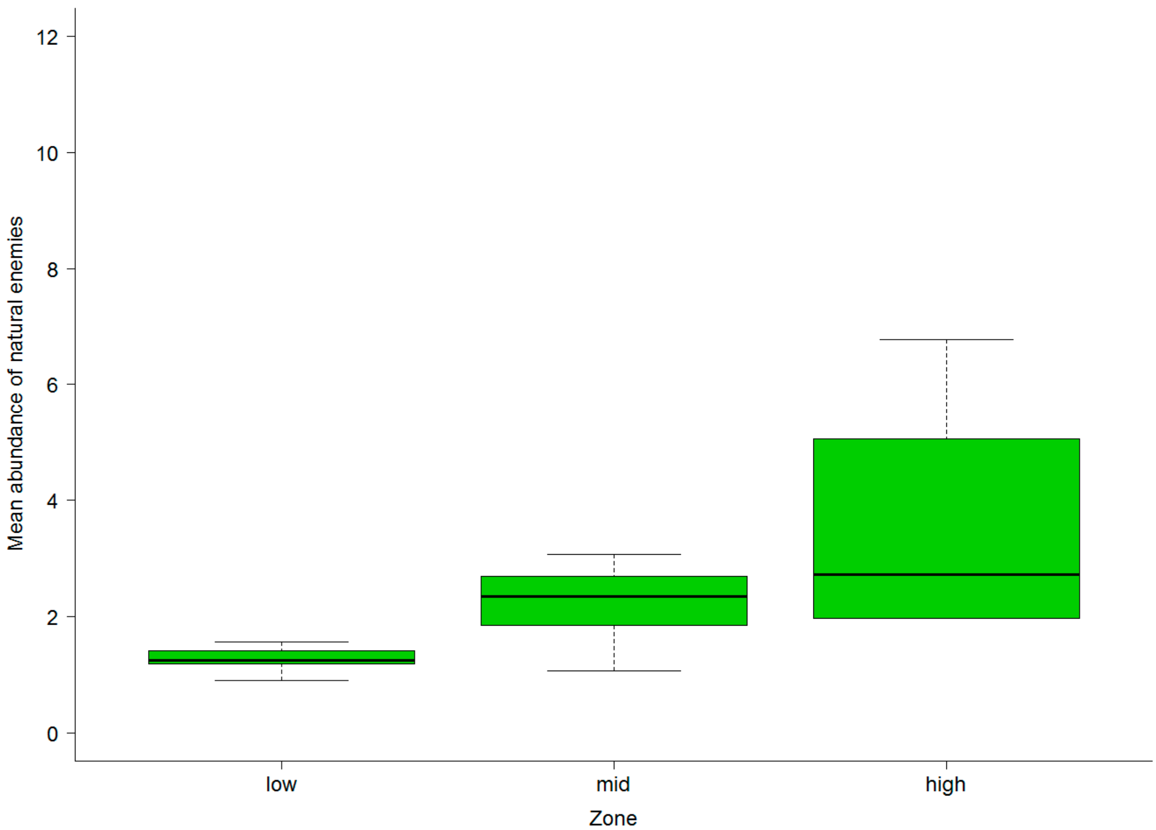

Figure 2. The effect of elevation on catches of natural enemies in smallholder bean fields in northern Tanzania.

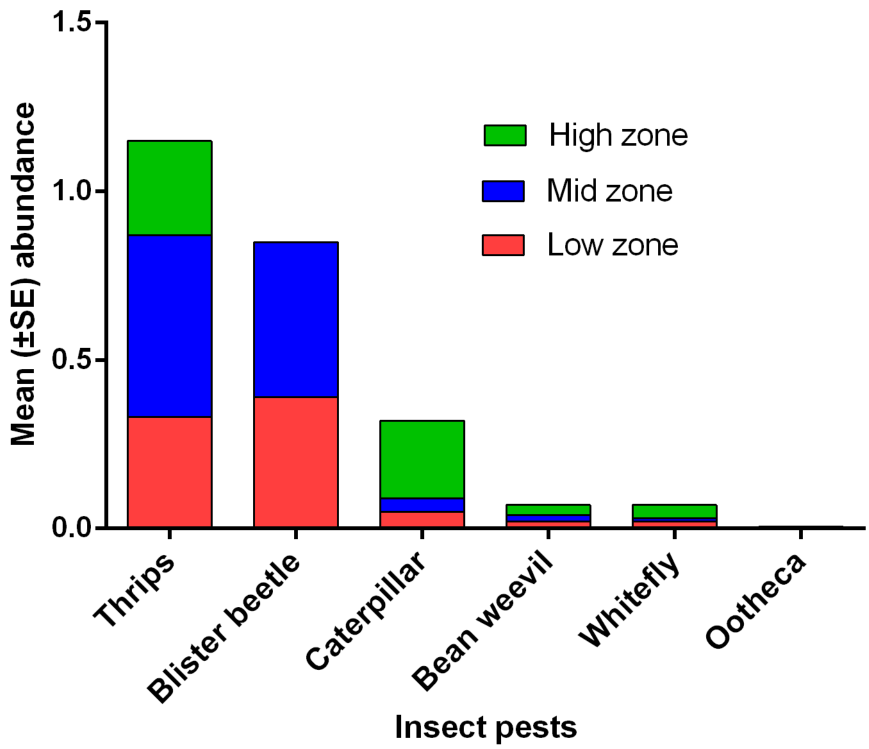

Figure 3. The effect of elevation on different insect pests in smallholder bean fields in northern Tanzania.

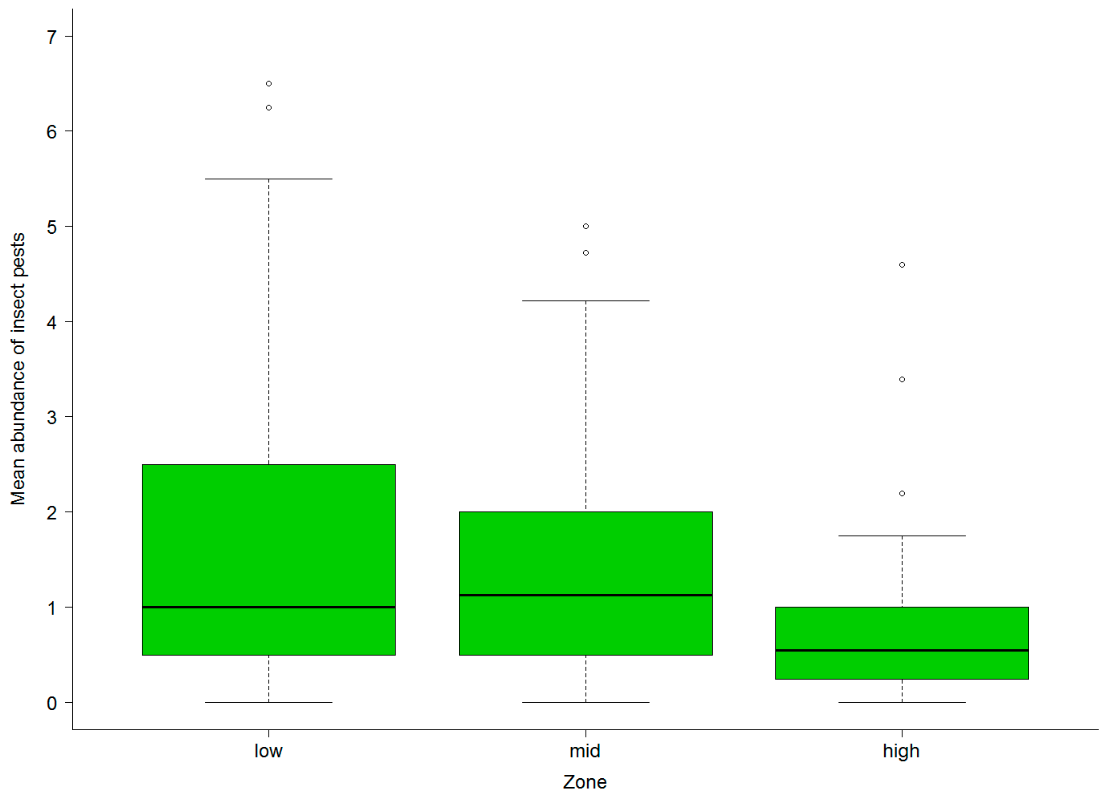

Figure 4. The effect of elevation on catches of insect pests in smallholder bean fields in northern Tanzania.

3.2. Abundance of Natural Enemies and Insect Pests in Field Margins and Crops

Catches of natural enemies differed significantly between the margins and fields across the elevation zones (F = 30.978, df = 1, $p < 0.001$) where the majority of the natural enemies were found along the field margin than within the bean field for the low and mid elevation zones, while in the high elevation zone, they were more abundant within the field (Figure 5). The majority of the natural enemy taxa were more abundant in margins than fields with significant difference only for parasitic wasps (F = 7.854, df = 1, p = 0.005) in all elevation zones. Though the size of the fields in the three elevation zones was significantly different (Table 1), there was no statistical significance (F = 0.590, df = 1, p = 0.443) of the influence of field size on the abundance of the natural enemies across the three zones.

Table 1. Mean size of smallholder bean fields in three elevation zones of the Moshi rural district in Tanzania.

Zone	Number of Farms (N)	Mean Farm Length (m)	Mean Farm Width (m)	Mean Farm Size (m^2)
Low	8	73.8 ± 2.79 a	55.1 ± 6.68 a	4167.5 ± 648.85 a
Mid	8	71.0 ± 3.69 a	56.6 ± 4.98 a	4116.4 ± 568.37 a
High	8	38.5 ± 2.78 b	29.5 ± 1.09 b	1132.9 ± 79.82 b
ANOVA, F value		39.534 ***	9.828 ***	12.068 ***

Each value is the mean ± standard error. *** is significant at $p < 0.001$. Means within the same column followed by the same letter are not significantly different at p = 0.05 from each other.

Catches of pests differed significantly between margins and fields (F = 9.478, df = 1, p = 0.002). Unlike natural enemies, insect pests were generally more abundant within the fields than margins in all the three zones (Figure 6). Similarly, the margin and field abundance was the same for the majority of individual pest species with significant difference (F = 8.221, df = 1, p = 0.004) in thrips, which were also significantly more abundant in the field than margin in all elevation zones.

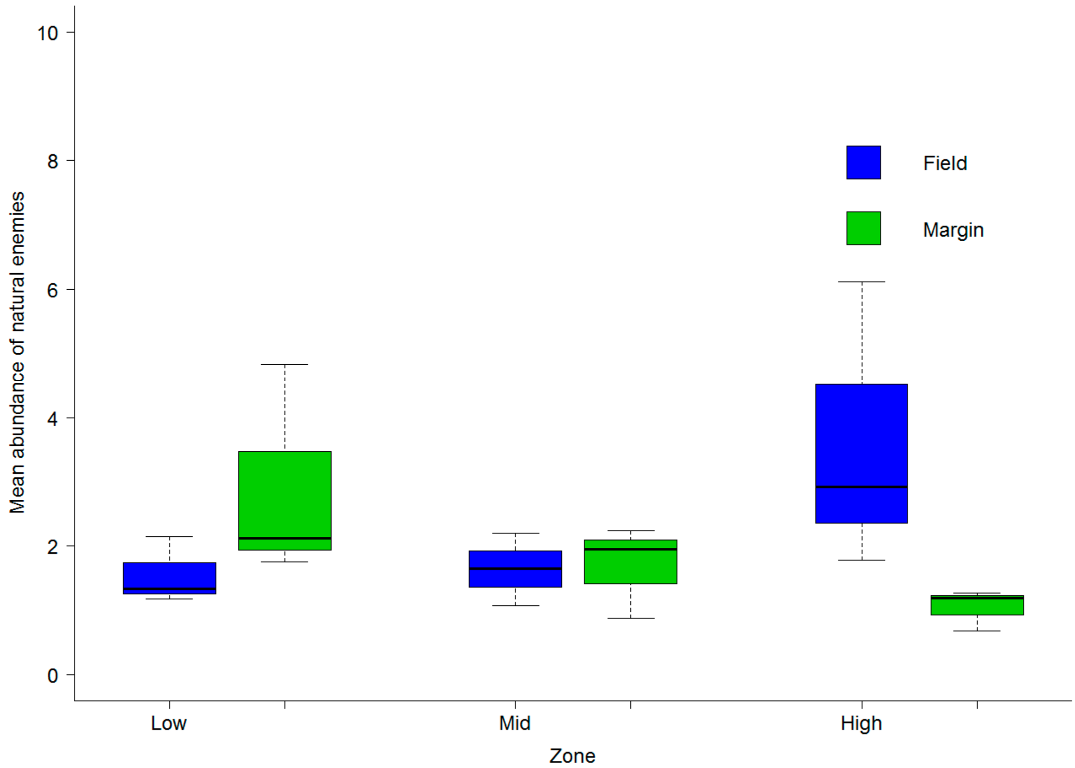

Figure 5. Margin and field abundance of natural enemies in smallholder bean fields across three elevation zones in northern Tanzania.

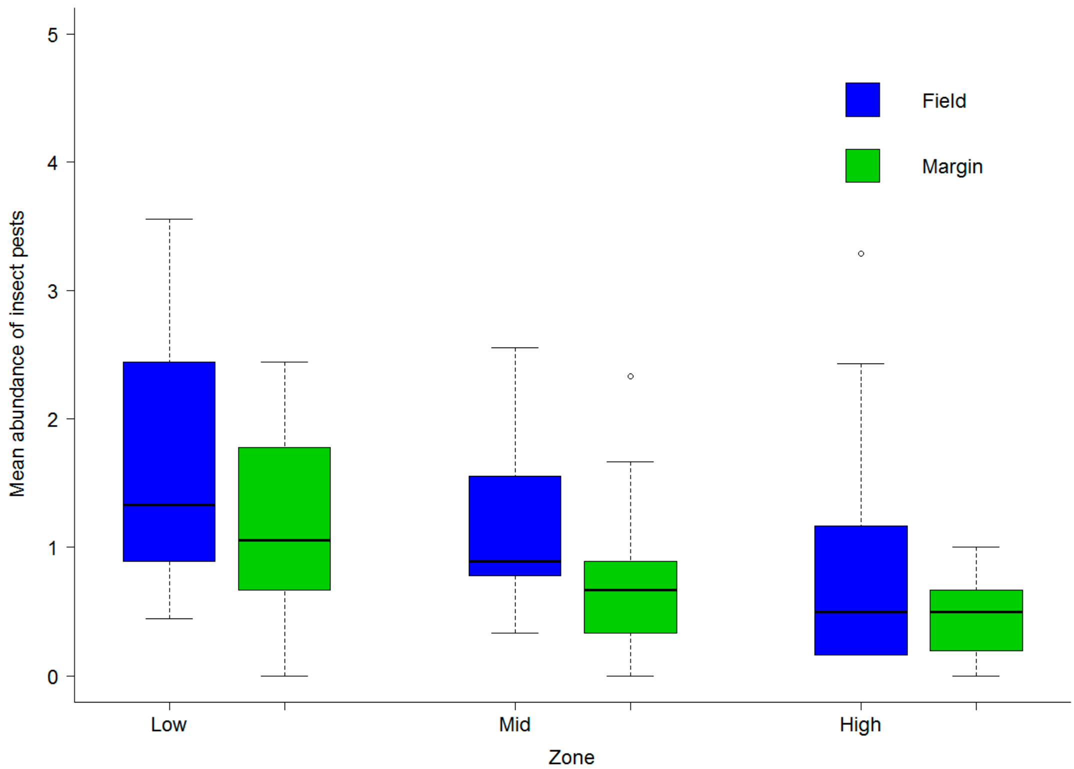

Figure 6. Margin and field abundance of insect pests in smallholder bean fields across three elevation zones in northern Tanzania.

3.3. Spatial Variation of the Sampled Insects in the Bean Field

Overall, there was no spatial signal in insect distribution at 10 to 50 m (F = 0.597, df = 4, p = 0.665) from the field margin into the bean field. Spatial trends in insects caught in traps in the three elevation zones at different stages in the cropping cycle were analysed to find out whether the time in the cropping cycle significantly affected the distribution of the natural enemies. The results show the insect migration patterns within the field did not change according to the point in the cropping cycle (F = 0.678, df = 3, p = 0.566), meaning that they were uniformly distributed within the bean field from 10 to 50 m distance throughout the season.

3.4. Fluorescent Dye Experiment

3.4.1. Movement of the Natural Enemies from the Field Margin to the Bean Crop

Generally, more of the natural enemies captured were marked (71%) compared with unmarked (29%), showing high interaction with the margin vegetation (Table 2). The most abundant natural enemies sampled were parasitoid wasps (Hymenoptera: Braconidae and Ichneumonidae), followed by predatory wasps (Hymenoptera: Ichneumonidae), assassin bugs (Hemiptera: Reduviidae), hoverflies (Diptera: Syrphidae) and spiders (Araneae: Araneidae).

Table 2. Total natural enemies and marked proportions, sampled in smallholder bean fields at three days after dye application in field margin plants.

Natural Enemies	Total Sampled	Marked Insects after Spray			Total Marked
		Day 1	Day 2	Day 3	
Parasitoid wasps	65 (27.5%)	19	17	13	49 (75.4%)
Predatory wasps	51 (21.6%)	10	19	11	40 (78.4%)
Assassin bug	28 (11.9%)	9	8	6	23 (82.1%)
Hover fly	19 (8.1%)	5	3	4	12 (63.2%)
Spider	19 (8.1%)	3	3	6	12 (63.2%)
Tachinid fly	12 (5.1%)	4	6	2	12 (100%)
Carabid beetle	10 (4.2%)	3	1	1	5 (50%)
Lady beetle	10 (4.2%)	3	0	2	5 (50%)
Long legged fly	7 (3.0%)	1	1	1	3 (42.3%)
Robber fly	5 (2.1%)	1	3	1	5 (100%)
Dragonfly	4 (1.7%)	0	0	0	0 (0%)
Rove beetle	3 (1.3%)	0	0	1	1 (33.3%)
Ants	3 (1.3%)	0	0	0	0 (0%)

There was a significant effect of elevation on the marked proportion of natural enemies (F = 8.398, df = 2, $p < 0.001$), with more marked in the high elevation than other zones (Figure 7). Overall, distance from the field margin significantly influenced the proportion of marked insects ($F_{3, 178} = 7.144$, $p < 0.001$), with more marked close to the field margin than towards the field centre. Within each zone, the effect of distance to proportion marked was significant in the low (F = 2.982, df = 3, p = 0.039) and mid (F = 3.598, df = 3, p = 0.018) elevation zones but not in the high elevation zone (F = 1.764, df = 2, p = 0.181) where the sampling distance ended at 20 m due to small field size (Figure 7). There was no significant effect of time from dye application to sampling of insects (F = 2.679, df = 2, p = 0.071) (Table 3). Post hoc testing showed that the low and mid elevation zones were not significantly different (p = 0.450) in terms of marked proportions of natural enemies but the two zones were significantly different from the high elevation zone.

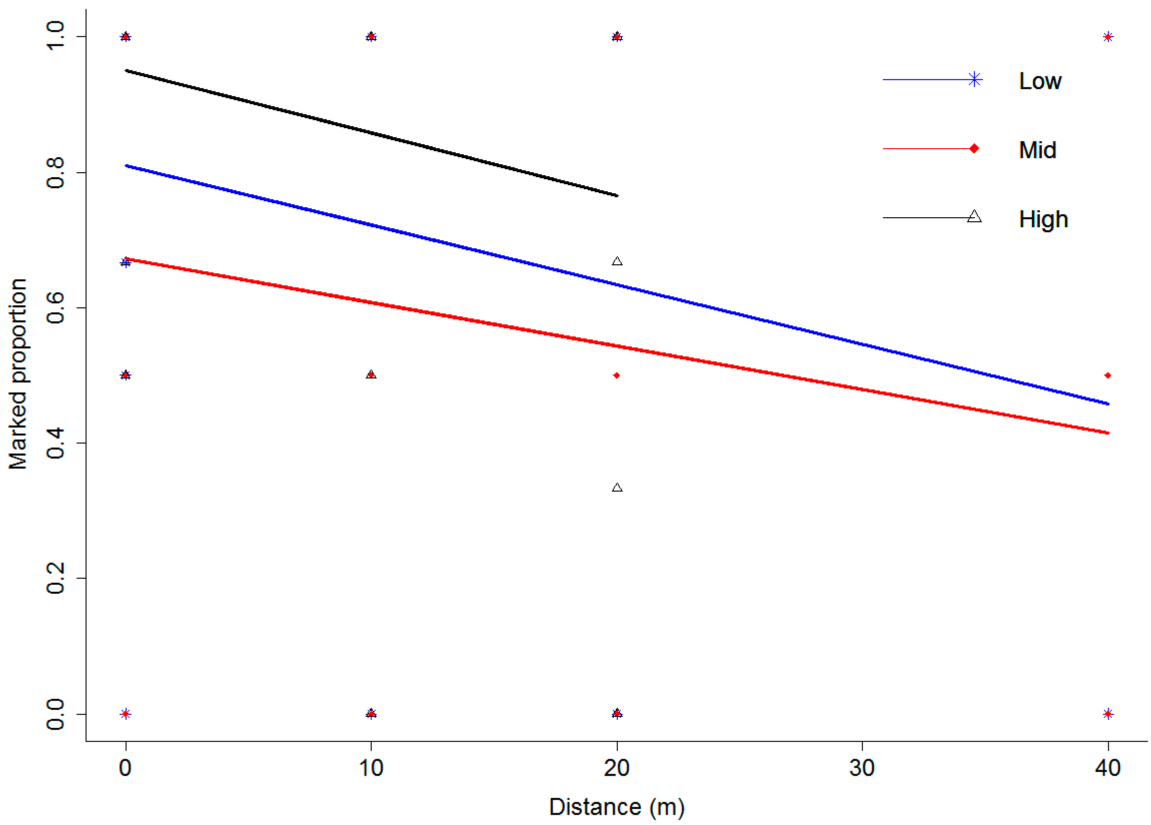

Figure 7. Effect of distance from the field margin to the marked proportion of natural enemies in smallholder bean fields across three elevation zones in northern Tanzania.

Table 3. Results obtained from the generalized linear regression model on the effect of elevation zone, distance from the margin to the field, field size and sampling day to the marked proportion of natural enemies sampled after dye application on field margin plants in smallholder bean fields in Tanzania.

Dependent Variable	Independent Variable	Sum of Squares	Mean Squares	Df	F Value	*p* Value
Marked proportions of natural enemies	Zone	2.594	1.297	2178	8.398	<0.001
	Distance	3.309	1.103	3178	7.144	<0.001
	Farm size	1.242	1.241	1178	8.040	0.005
	Day	0.870	0.435	2178	2.816	0.063

The degrees of freedom, F and p values were obtained from ANOVA at $p = 0.05$.

3.4.2. Movement of the Insect Pests from the Field Margin to the Bean Crop

The insect pests captured were blister beetles (Coleoptera: Meloidae), bean leaf beetles (Coleoptera: Chrysomelidae), leaf hoppers (Hemiptera: Cicadellidae), stink bugs (Hemiptera: Pentatomidae), bean brown bugs and leaf-footed bug (Hemiptera: Coreidae), fruit fly (Diptera: Drosophilidae and Tephritidae), locust (Orthoptera: Acrididae) and other plant bugs.

Fewer insect pests were marked (25.5%) compared with unmarked (74.5%), indicating that only a minority of them were in the margin during dye application (Figure 8). There was a significant effect of elevation on the marked proportion of insect pests (F = 4.125, df = 2, p = 0.020). Few insect pests were marked in the mid elevation zone (20%) compared with low and high elevation zones (37% and 50% respectively). Overall, distance from the field margin significantly influenced the proportion of marked insects (F = 12.506, df = 3, p < 0.001) but with significant effect only in the mid elevation zone (F = 8.410, df = 3, p < 0.001). There was no significant effect of time from dye application to sampling for low and high elevation zones, but only in the mid elevation zone (F = 4.430, df = 2, p = 0.018), where more marked insect pests were captured on the first day compared with the other days.

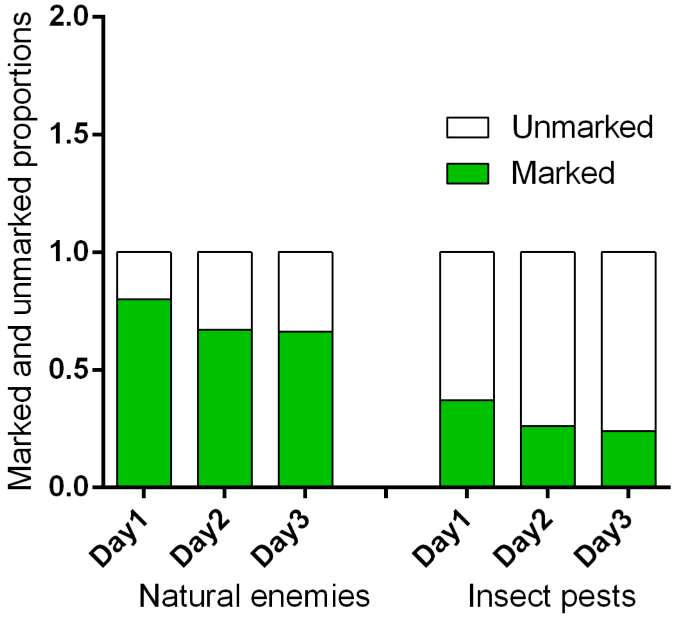

Figure 8. The difference between marked and unmarked proportions of natural enemies and insect pests sampled in smallholder bean fields after dye application to margin plants in northern Tanzania.

3.5. Field Predation and Parasitism

Aphid parasitism was higher on exposed sentinel plants placed in fields compared to caged plants (F = 8.456, df = 1, p = 0.005). Aphid mortality rates on the exposed plants, measured by parasitism levels on sentinel plants, did not differ between the three elevation zones (F = 2.704, df = 2, p = 0.076) and between field edges and field centres (F = 0.229, df = 1, p = 0.634). Mean parasitism rates varied between a maximum of 15%, which was observed on open sentinel plants in the low elevation zone, and a minimum of 0.5% observed on caged plants in the high elevation zone. The identification of parasitoids that emerged from the parasitized aphids showed that 90% were *Aphidius* species (Hymenoptera: Braconidae: Aphidiinae).

There was a significant difference between the aphid mortality recorded from control and exposed sentinel plants in the predation experiments (F = 28.973, df = 1, $p < 0.001$), whether at the field centre or near the field margin, indicating that there is a significant pest control service coming from the biodiversity on farm. In the control (caged) plants, aphid numbers increased over the course of the experiment, indicative of reproduction, whereas in the exposed plants, in all cases, the aphid numbers decreased—in some cases, by nearly half (Figure 9). The predation rate between the three elevation zones (F = 0.991, df = 2, p = 0.385) and between field edges and field centre (F = 0.914, df = 1, p = 0.348) was statistically not significant, indicating the centre of the fields in the three elevation zones still receive equivalent pest control service as the field edge.

3.6. Predominant Field Margin Plants in Each Elevation Zone

Considering the margin plants that were common in all zones, *Ageratum conyzoides* and *Cyperus rotundus* were more abundant in the high zone, while *Commelina benghalensis* was more abundant in the low zone. The three herbs (*A. conyzoides*, *C. rotundus* and *C. benghalensis*) which were abundant in the low and high elevation zones were also more abundant compared with the other plant species in the mid elevation zone (Figure 10). Some field margin plants occurred in only two of the three elevation zones while others occurred in either of the three zones. Considering each zone separately, the most abundant margin plant in the high zone was *Tripsacum laxum* followed by *A. conyzodes*; in the mid zone, the most abundant plant was *Asystasia mysorensis* followed by *Sida rhombifolia*; in the low zone, the most abundant plant was *S. rhombifolia*.

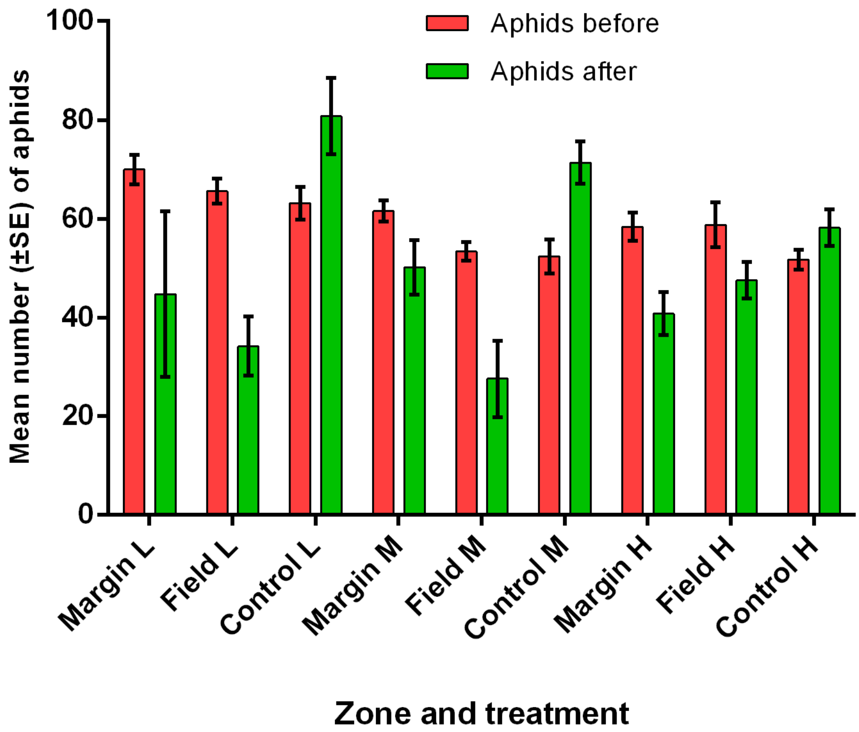

Figure 9. Margin and field predation and a control after 2 days exposure of sentinel aphids in smallholder bean fields in the low (L), mid (M) and high (H) elevation zones of the Moshi rural district in Tanzania.

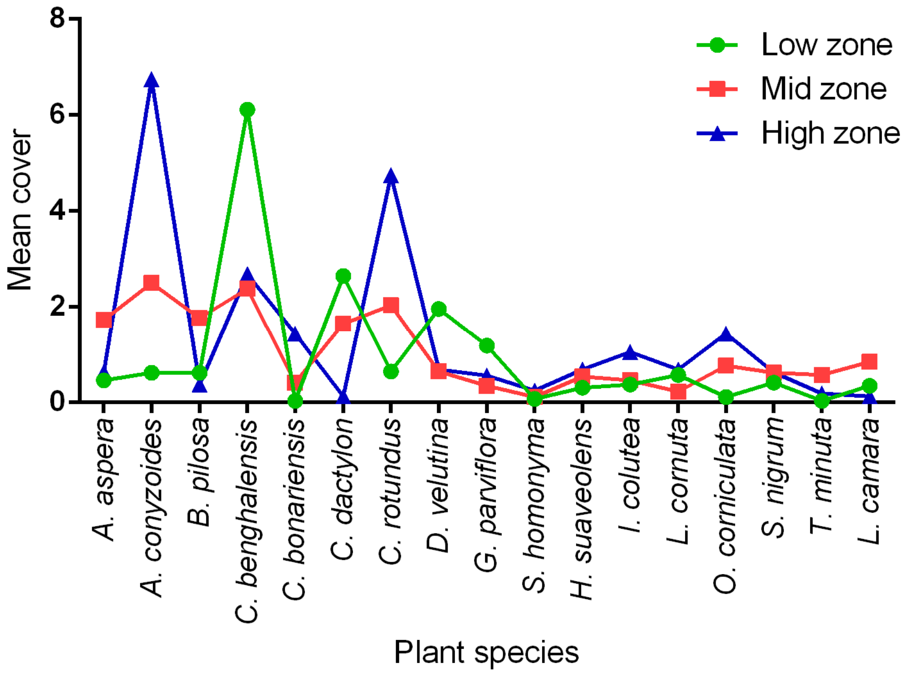

Figure 10. The common herbs and shrubs in the three elevation zones of the Moshi rural district in northern Tanzania.

4. Discussion

Margin vegetation around the smallholder bean fields in this tropical system plays a significant role of harbouring natural enemies that move into the field crop to exert biological pest control. This conclusion is in general agreement with other reported studies that non-crop vegetation areas around agricultural fields in other types of systems provide habitats for natural enemy populations [10,41,42]. Some of the factors reported to enhance the natural enemy community include the provision of alternative food resources like nectar and pollen from the flowering field margin plants and refuge sites during field disturbance like pesticide application or crop harvesting. In contrast to the present study, in which there were weak spatial differences in the strength of in-crop natural enemy activity, Bianchi and Wäckers [43] found biological control activity was enhanced adjacent to the field margin as a result of the attraction of parasitoids to flowers and nectar of the margin plants. In the present study, parasitoids were significantly more abundant along the field margin compared with the field centre. However, parasitism was not significantly different between the two locations. The habitat quality of field margin [44] as well as the farm size and distance from the field margin may play a significant role in enhancing the beneficial insects and their activity within agricultural systems.

Generally, low and mid elevation zones differed significantly from the high elevation zone in terms of the natural enemy and pest abundance and their distribution between margins and fields. Natural enemies were more abundant in the high elevation zone compared with mid and low elevation zones, whereas the insect pests were more abundant in the low and mid elevation zones compared with high elevation zone. In addition, the majority of the natural enemies were found along the field margin than within the bean field for low and mid elevation zones while in the high elevation zone, they were more abundant in the bean field. Several factors including differences in vegetation cover and farming practices in the three zones, climate factors and biological control implications may explain the observed variations. It is reported that non-crop vegetation around agricultural lands increases the natural enemy population while decreasing the insect pest population [45,46]. *A. conyzoides*, which was abundant in the high zone, is one of the known plant species with several floral visitors searching for pollen and nectar [47–49], signifying its importance as a food resource to beneficial insects when grown around agricultural land. *S. rhombifolia* which was abundant in the low and mid elevation zones is among the reported spontaneous plants that attract natural enemies for pest control in the field [50]. Other reported plants that support beneficial insects include species of *Commelina*, *Cyperus*, *Drymaria* and *Oxalis* [51]—all of which were abundant along the field margins of smallholder farmers. The differences in the quality of the pollen and nectar [52] as well as the overall vegetation cover [53] along the margin may significantly influence the natural enemy population in the three elevation zones. This requires further studies on the quality of the floral resources from the different field margin plants as well as the effect of farming practices, habitat management and other landscape and environmental factors to arthropod population in the three zones.

The difference among elevation zones in the spill over of the dye-marked natural enemies from the margin to field was associated with the marked difference in field sizes. The bean fields in the high zone are around one quarter the size of fields in other zones. This small size increases the possibility for any given insect from the field population being in the margin at the time of spray of dye with less distance to move between the two locations, hence higher proportions of marked insects were found in the high elevation zone than in the low and mid elevation zones. The fact that the dye-marked insect proportions decreased with distance from the margin to the field in the low and mid elevation zones with no significant difference in the high elevation zone can also be explained by the effect of field size. This is supported by Denisow and Wrzesien [54], where the effect of distance from the field margins to the crops was considered to influence the movement of beneficial insects and hence the ecosystem services provision in the crop.

The effect of small field size which is a characteristic of many smallholder farming systems [55] resulted in more uniform distribution of the insects sampled by the pan traps at different distances within the bean fields. This is contrary to other studies [9,20,56] that reported a significant edge effect in the distribution of ground-dwelling natural enemies that were sampled by pitfall traps within 60 m distance from the margin. The use of pan traps in this study biased the catch of more mobile natural enemies compared to less mobile, which may account for the relatively uniform distribution within the field. Previous studies have established that some beneficial insects are highly mobile and can move up to 100 m into a field away from the margin [7]. Therefore, the edge effect to the natural enemies mainly depends on the mobility of the natural enemies, the distance or farm size as well as the sampling technique involved as revealed in this study.

Margin and field predation and parasitism of the exposed sentinel aphids were not significantly different in the three zones, indicating equivalent pest control service between margin and field as a result of small field size of smallholder bean farming systems. Field size in relation to non-crop vegetation abundance explains the ecosystem services provided within the agricultural land. Other reported factors that may influence both predation and parasitism rates in field include weather conditions, plant composition, pest density, age structure of the pests, intraguild predation and poor dispersal of the biocontrol agents from the field margin vegetation [29,57,58]. Therefore, understanding the fundamentals of interactions between prey and predator and the influence of other environmental factors on biological activity is important for effective pest control. Exposed sentinel plants, whether at the field centre or near the field margin, showed a significant parasitism and predation levels unlike the controls, demonstrating the importance of farm biodiversity in pest control service.

The fact that the field margin plants harboured more natural enemies than insect pests indicates that the margin plants play an important role in enhancing biocontrol agents for conservation biological control. This is further supported by the fluorescent dye experiment, which implies that margin plants act as a habitat or resource for the natural enemy population and that they can move from the margin plants to the field crop to provide a pest control service. However, field margins may be the source of insect pests in the field, especially when they consist of alternative host or susceptible plants which lead to a build-up of insect pests and subsequent infestation in the field [26,27,59]. Therefore, understanding the field margin plants and their ecological contribution in agricultural systems is important for proper management.

The value of field margins and ecosystem service provision in the cropland may be affected by local, landscape or regional factors, leading to inconsistency in different settings, hence the need to test a specific agricultural system. Much information is available based on data collected in temperate (North America, Europe, and southern Australasia) environments, with limited information from most tropical countries. The findings of this study which was undertaken in tropical smallholder agricultural systems gives useful information on the field margin usage by the natural enemies and pest control service within the cropland. Understanding the field margin usage and movements of beneficial insects within small, low-input farms in tropical environments is necessary for the proper management of these features to enhance pest control services.

5. Conclusions

The findings of this study provide evidence of the existence of a rich community of natural enemies likely responsible for pest control services within the smallholder tropical farming systems. Natural enemies were found to move from the non-crop vegetation into the bean crops quickly (over 24 h periods) and there was strong evidence of penetration to the centre of fields. The high mobility of the natural enemies captured by the pan traps facilitated their interaction with the margin plants and their movement throughout fields. Predation and parasitism levels near the field margin compared with the field centre were similar, indicating high levels of spatial flux of the natural enemies between the margin plants and crop land within the smallholder bean farms. Further studies on the manipulation of specific vegetation types in comparison with fields in which where there is no margin vegetation over longer periods, together with assessment of the quality of floral resources to predators and parasitoids, are important to determine the contribution of margin vegetation to biological pest control within smallholder bean farming systems. Understanding the potential factors that affect the abundance and activity of the natural enemies and insect pests within the agricultural lands is key for effective pest control service.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/11/22/6399/s1: Figure S1: A map of the Moshi rural district showing the study sites located in three elevation zones in northern

Tanzania; Table S1: Location of the survey and experiments in the respective study sites; sites with a star are where predation and parasitism experiments were performed and sites with an additional # sign are where fluorescent dye experiments were performed. The overall insect survey was performed in all major and minor sites.

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