Flowering margins support natural enemies between cropping seasons

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Scope Statement

Here we investigated how agricultural landscapes support natural pest regulating insects between cropping seasons; an important challenge in Pest Management by Small-Holder Farmers so aligns with this special issue. Smallholders in East Africa clear margins to provide fodder for cattle or livestock or simply to reduce the ingression of weeds into crop fields. We show that there was a significant seasonal variation in plant species richness and diversity around crops and the abundance of margin plants was strongly linked to abundance of natural enemies in the off season. The time since harvesting was also a significant factor influencing the overall abundance of natural enemies. Our paper reinforces our understanding of the importance flowering plants in agricultural systems as a refuge for natural enemies and other beneficial insects but uniquely emphasising the time between cropping seasons. Improving agricultural landscapes between crops to better support invertebrates will lead to more effective natural pest regulation early in the following crop with positive outcomes for the farmers and their families.

Conflict of interest statement

The authors declare a potential conflict of interest and state it below

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision

CRediT Author Statement

Erick Cheruiyot: Methodology, Project administration, Resources, Supervision, Writing – review & editing. Jane Nyaanga: Investigation, Methodology, Resources, Supervision, Writing – review & editing. Joshua Ogendo: Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing. Janet Obanyi: Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. Philip Charles Stevenson: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. Philip Bett: Formal Analysis, Investigation, Methodology, Writing – review & editing. Richard Mulwa: Data curation, Methodology, Supervision, Writing – review & editing. Steven Richard Belmain: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Supervision, Writing – review & editing. Sarah Arnold: Conceptualization, Formal Analysis, Methodology, Writing – review & editing. Victoria C Nash-Wooley: Conceptualization, Formal Analysis, Methodology, Writing – review & editing.

Keywords

natural enemies, field margins, Off-season, Smallholder farming systems, Sustainable pest management

Abstract

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Populations of natural enemies of insect pests are declining owing to agricultural intensification and indiscriminate use of pesticides and this may be exacerbated in agricultural systems that clear all margin plants after cropping season for other uses such as fodder. Retaining a diversity of non-crop flowering vegetation outside the cropping season may support more resilient and effective natural pest regulation. We tested the potential for non-crop vegetation to support natural enemies in fields across two locations after harvesting the primary crops of lablab and maize. A total of 54 plant species were recorded across the sites in Kenya with 59% of them being annuals and 41% perennials. There was a significant seasonal variation in plant species richness (ANOVA: $F_{1,16}$ = 33. 45; P<0.0001) and diversity (ANOVA: $F_{1,16}$ = 7.20; P=0.0511).While time since harvesting was a significant factor influencing the overall abundance of natural enemies (ANOVA: $F_{2,1133}$ = 8.11; P<0.0001) they were generally higher in abundance in locations with margin plants or where a diversity of margin plants was observed. These findings demonstrate that flowering plants in agricultural systems offer refuge and alternative food for natural enemies and potentially other beneficial insects between cropping seasons. The conservation of natural enemies between crops may lead to more effective natural pest regulation early in the following crop hence reducing reliance on insecticide application.

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Running title: Natural enemies between cropping seasons

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Abstract

Populations of natural enemies of insect pests are declining owing to agricultural intensification and indiscriminate use of pesticides and this may be exacerbated in agricultural systems that clear all margin plants after cropping season for other uses such as fodder. Retaining a diversity of non-crop flowering vegetation outside the cropping season may support more resilient and effective natural pest regulation. We tested the potential for non-crop vegetation to support natural enemies in fields across two locations after harvesting the primary crops of lablab and maize. A total of 54 plant species were recorded across the sites in Kenya with 59% of them being annuals and 41% perennials. There was a significant seasonal variation in plant species richness (ANOVA: $F_{1,16} = 33.45$; *P<0.0001*) and diversity (ANOVA: $F_{1,16} = 7.20$; *P=0.0511*). While time since harvesting was a significant factor influencing the overall abundance of natural enemies (ANOVA: $F_{2,1133} = 8.11$; *P<0.0001*) they were generally higher in abundance in locations with margin plants or where a diversity of margin plants was observed. These findings demonstrate that flowering plants in agricultural systems offer refuge and alternative food for natural enemies and potentially other beneficial insects between cropping seasons. The

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Kew words: Natural enemies, field margins, Off-season, smallholder farming systems, sustainable pest management.

1. Introduction

The effective use of natural pest regulation as an alternative to conventional pesticides requires conservation and effective management of landscapes to support natural enemies that move into crop fields targeting pest insects (Hatt et al. 2017; Sorribas et al. 2016). Natural or semi-natural flowering plants adjacent to croplands can be preserved or planted to enhance crop land, but the outcomes are often crop and system dependent (Landis, Wratten, and Gurr 2000; Perović et al. 2010; Ochieng et al. 2022; Obanyi et al. 2023). Natural enemies may obtain nectar, refuge, and other prey from non-crop vegetation, enhancing their abundance and effectiveness in pest management (Bianchi and Wäckers 2008). Beneficial arthropod populations, such as natural enemies and pollinators, have suffered significant reduction as a result of agricultural intensification characterized by indiscriminate pesticide use and reduction in suitable foraging and nesting habitats (Zhao et al. 2021; Tscharntke et al. 2005). In a study by Lundgren (2009), fecundity of female predatory and parasitic arthropods was attributed to the amount of food available at the adult stage. In the absence of flowering plants, the female parasitic wasps may reabsorb eggs and devote more energy to host seeking and survival decreasing their ability to reproduce (Kishinevsky et al., 2017). Therefore, cropping systems that include complete removal of biomass at harvest create conditions that hinder resilient and effective natural enemy populations thus reducing their potential to control pests (Nilsson et al. 2016). Crop harvesting cause rapid changes in the structure of habitats, making agroecosystems unstable for natural enemies (Obanyi et al.2023).

There are several groups of natural enemies known to provide control of aphid insect pests (Schmidt *et al*., 2003). These include parasitic wasps and larvae of syrphid flies, which feed on the aphids exerting natural control of aphids in the fields. The parasitic wasps are highly specific because they are able to locate aphid colonies from a greater distance via "alarm signals" emitted by an infested plant. After locating the aphid, the wasps use their ovipositor to lay eggs (oviposit) into the aphid abdomen where they grow inside and kill the aphid. More generalist natural enemies that also prey on aphids include carabid and staphylinid beetles and spiders, which mainly colonize plants from the ground (Schmidt *et al*., 2003). The other important natural enemies for aphids include lady beetles, lacewings; big eyed damsel and minute pirate bugs. These are predators and directly consume or feed on one or more aphid species (Desneux & Ramirez-Romero, 2009; Dixon, 2000). In addition, birds represent top predators for insects in many agricultural systems (Milligan et al. 2016). Adult hoverflies and some lacewings feed on nectar and pollen so floral resources are important although some lacewings also feed on soft bodied insects such as aphids (Samaranayake and Costamagna 2019). The ladybird beetles are common biological control agents of aphids in natural field settings. They delay and prevent aphid outbreaks and densities (Heimpel & Asplen, 2011). They regulate aphid populations as they are voracious with good searching ability, high predation capacity of both adults and larvae stages and high reproductive rates (Amorós-Jiménez *et al*., 2012). The carabids are typically polyphagous; however, they are also voracious feeders, consuming close to their own body mass in food daily. They have specialized feeding habits and feed mostly on bean aphids that fall to the ground.

Although certain invertebrate species have evolved a wide range of adaptive mechanisms in response to changes in habitat structure (Langellotto and Denno 2004; Gavish-Regev, Lubin, and Coll 2008), studies conducted by Opatovsky & Lubin (2012) showed that small crop-inhabiting arthropods such as spiders may be less able to adjust to unexpected changes in habitat quality as a result of harvest, while migratory vertebrates may seek shelter in nearby non-crop habitats. Furthermore, the study indicated that most insect pests adapt to these changes in habitat structure by having short life cycles that are synchronized with the cropping season. However, natural enemies such as predators and parasitoids tend to have longer life cycles than seasonal crop growth cycles, or may need to complete some generations outside the cropping season, prompting the need to assess the availability of non-crop habitats that can support these arthropod groups (Menalled et al. 2003). For example, Gurr et al., (2016) reported that ecologically engineered field margins in rice cropping systems using sesame plantings on rice bunds provided alternative prey and nectar for a parasitoid of the key rice pest the brown planthopper (*Nilaparvata lugens*) prior to the main rice crop that boosted populations of the natural enemy.

The distribution of natural enemies within fields changes throughout the crop's growth cycles. Kishinevsky et al. (2017) reported higher parasitoid abundance at the beginning of the season within natural habitats compared to later in the growing season where more parasitoids were abundant within the crops. Similar seasonal patterns were demonstrated for spiders in desert wheat fields and were explained by the migration of spiders into the crop field throughout the season, combined with the high reproductive rates within the crop fields

(Gavish-Regev, Lubin, and Coll 2008). Conversely, at the end of the season when the crop is harvested and the field is left largely bare, there is less cover and fewer resources for natural enemies (Gavish-Regev et al., 2008; Opatovsky & Lubin, 2012). Changes within crop habitats may make them less suitable for arthropods and lead to either high mortality or to the dispersal of crop dwelling arthropods into neighboring habitats.

Many natural enemies multiply in response to the availability of the food hence there is a time lag between pests and natural enemy fluctuations. Natural enemies are highly mobile their movement in and out of the crop will depend on how far their refuges are and the availability of prey and food. Therefore, throughout the season there will be fluctuation in population hence the need to assess their dynamics at several crop stages (Zhao *et al*., 2013). It is worth remembering that as plant communities change the associated organism species and population also changes, in this case as the crop stages and margin species change so do the natural enemies and pest and this influences their succession and colonization (Chaplin-Kramer *et al*., 2011). The crop vegetation is short-lived and must be recolonized by natural enemies at the beginning of each crop season, meaning that maintaining viable natural enemy populations in non-crop habitat outside the growing system is important to achieving reliable biocontrol without resorting to synthetic pesticides. To successfully build suitable non-crop habitat, a detailed understanding of the distribution, diversity and abundance of the plant species and how this links to plant–arthropod diversity after crop harvesting is vital. It is important that the non-crop habitat supports natural enemies preferentially over crop pests, or the benefits for natural pest regulation may be limited. This study aimed to measure how the abundance and diversity of field margin plants supported the abundance of natural enemies of bean aphid (*Aphis fabae*) between cropping season in a legume crop, lablab (*Lablab purpureus*) under different agroecological conditions.

2 Materials and methods

2.1 Study sites

This study was conducted during February-April 2020 and January-March 2021 succeeding the main cropping seasons of May-December 2019 and March -November 2020, respectively in sixteen farmers' fields in Kenya: eight in Njoro and eight in Rongai, sub-counties, Nakuru County. Njoro sub-county is located at 0° 10′ - 0° 29′ S and 34° 7′ - 34° 20′ E with an altitude range of 2000 to 2500 metres above sea level (m.a.s.l). The annual rainfall range is 1000 to 1250 mm and temperature ranges from 17° C to 30° C. Rongai is located at 0° 10′ - 0° 29′ S and 34° 7′ - 34° 20′ E with an altitude range of 1480 to 1550 m.a.s.l. The annual rainfall ranges of between 750 to 1000 mm and temperature range of 19° C to 32° C. The Njoro soils are well drained dark reddish clays, classified as Mollic Andosols whereas Rongai soils are well drained sandy clay loams, classified as Vitric Andosols (Jaetzold *et al*., 2012).

2.2 Experimental design and treatment application

The study used experimental plots previously planted with a lablab monocrop or a maize-lablab intercrop on plots measuring 10 m x 10 m with natural field margin vegetation along at least two sides of the plot (Supplementary resource 1). There were total of 16 farms, eight farms in each two of the location and each farm represented a replicate. The field margin vegetation along the harvested crop was at least 5 m in width and 2m away from the from the harvested experimental plots. Each of the harvested plots for the two cropping methods were evaluated as a treatment.

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Sampling of natural enemies of bean aphids (*A. fabae*)

The sampling of the natural enemies was done in field margins alongside the harvested experimental plots that were either planted as lablab monocrop or lablab-maize intercrop, both common cropping approaches in the region. Sampling was done for three months after harvesting the field crops. Trapping of natural enemies was conducted using yellow pan traps for ground dwelling insects, and yellow sticky cards and sweep nets for flying insects. Sticky and pan traps were set up at the field margins of each plot. We used pan and sticky traps to sample natural enemies as they have been widely adopted as efficient and effective approaches to field sampling (Shweta and Rajmohana 2018; Thant et al. 2016). In addition, pan and sticky traps can be placed and collected easily and can trap a wide range of insect taxa throughout the placement period.

A total four (2 sticky and 2 pan) traps were placed at the margin vegetation for each treatment spaced at 5m from one trap to another and the width of field margin was not exceeding 5m. The landscape of the study area was composed mostly of grasses, broad leaved annual weeds and woody vegetation. The woody vegetation was mainly preserved as either natural fence or windbreaks by the farmers. Woody vegetation consisted of trees and tall shrubs which were either remnants of existing vegetation, natural plant dispersal, or established through direct plantings by farmers. In collecting natural bean aphid natural enemies using pan trapping method, two yellow plastic pans measuring 20 cm in diameter and 5 cm high (Manfacturer: Kenpoly Manufacturers Limited) were placed at the ground level. They were filled with a premixed liquid solution containing 250 ml of water, 5 g of salt to preserve the natural enemies and 5 ml of odorless liquid detergent to break the surface tension of the water. The traps were left in the field for 48 hours. Thereafter, the trapped insects were retrieved by sieving and washing with clean water. The insects were picked from the sieve using a camel hair brush and placed in 50 ml plastic falcon tubes filled with 25 ml of 75% ethanol for preservation before being taken to the laboratory. The insects were placed under a dissecting microscope (Leica ZOOM 2000 Inc. Buffalo, NY U.S.A 14240-0123) at 200X magnification for counting and identification up to the family level using Simon and Schuster's identification key (Arnett and Jacques 1981).

Sticky trap sampling was performed by hanging two yellow sticky cards (8 cm width x 24 cm length) (Manufacturer: Real IPM, Kenya) 1 m above the ground level next to the pan traps in the field margin vegetation adjuscent to the harvested plots of the two croppping systems. The sticky traps were later collected from the field after 48 hours, placed in non-sticky lamination pouches of 25 cm x 10 cm and taken to the laboratory for identification to family level. Sweep net sampling was used to capture the natural enemies in the field margins according to Spafford and Lortie (2013). Sampling involved moving forward along the field margin vegetation and making 10 sweeps parallel within the margin. The sweep net bag was closed immediately and the insects caught were carefully transferred to a jar containing cotton wool soaked with formalin where they were left for 2 hours. The preparation and identification of the insects were the same as for pan and sticky trapping. The sampling of the natural enemies was done four times during the off-season period.

2.3 Sampling of the plant species abundance and richness

The experimental farm fields were left bare after harvesting lablab and lablab-maize intercrop and were not considered during sampling. However, this may not be the case since most of the smallholder farmers plant cover crops for soil conversation and fertility improvement purposes after harvesting. The composition of individual plant species was determined from 3 x 1 m^2 quadrat along the field margin. Plant species abundance and richness were determined by counting the number of individual plant species within the quadrat which were identified to species level using pictorial aids (e-library) and authentic identification was done by a plant taxonomist at the Department of Biological Science, Egerton University.

2.4 Bean aphids during the cropping season

During the cropping season data on aphid abundance, damage severity and percent incidence were collected. Across the 16 farms, each farm was considered a replicate that had lablab monocrop and maize-lablab intercrop with a natural field margin along at least two sides of the plot. Bean aphid infestation levels were only scored on lablab as the main crop for this study. Aphid abundance and damage severity were collected from ten randomly selected lablab plants from the inner five rows in each replicate. Aphid abundance were scored using a standard and widely adopted categorical scale where, 1= no aphids; 2 = 1-100; 3 = 101-300; 4 = 301-600; 5 = 601-1000; and 6 = >1000 (Aken et al., 2013; Mkenda et al., 2015). The severity of damage was determined by visually observing and scoring the level of damage on the selected plants. Again this is a widely adopted and used approach using a scoring scale of 1 to 5 was adopted, where; 1 = no infestation or damage; 2 = light damage and infestation, < 25 % plant parts damaged or infested; 3 = average damage and infestation, 26 - 50 % plant parts damaged; 4 = high infestation and damage, 51 - 75 % plants parts damaged showing yellowing of lower leaves; and 5 = severe infestation, > 75 % damage resulting to plants, with high infestation levels with yellow and severely curled leaves or dead plant (Mkenda et al., 2015). The incidence of aphids was determined by visually examining and counting the number of aphid damaged/infested plants by randomly sampling 30 plants from the inner five rows.

2.5 Lablab grain yield

Lablab grain yield was recorded at physiological maturity when pods turned brown. The pods were harvested separately from the middle rows falling within a sampling area of 36 m^2 for each treatment. Harvested lablab pods were sun-dried and threshed with the moisture content recorded using a digital moisture meter (Manufacturer: Dramiński S.A., Poland). At 13 % moisture content, grains from each treatment were weighed separately using a portable digital scale (Manufacturer: Comglobal Solutions, India) and converted to kg ha^{-1} using the following formula:

$$\text{Grain yield (kg ha}^{-1}) = \frac{\text{Grain weight per plot (kg)x 10,000m}^2\text{ha}^{-1}}{\text{Harvest area (m}^2)}$$

2.6 Data analysis

Data on plant species and natural enemies counts were subjected to arcsine and square root ($\sqrt{x+1}$) transformation, respectively to correct for heterogeneity of treatment variances. For plants species the unit for calculations was the number of plants counted one month after harvesting lablab crop. For natural enemies the unit for calculations were the number of natural enemy groups caught using sticky traps, pan traps and sweep net at different months post-harvesting lablab crop. To determine extent and how field margin vegetation conserve bean aphid natural enemies outside lablab main growing season, a species diversity index was calculated. To establish the diversity of either natural enemies or margin plants two of the most used indices that is, Shannon-Weiner index and Simpson's species diversity, respective, were adopted to quantify the diversity indices for each category. The diversity of the natural enemies was determined using Shannon-Weiner index of diversity (H) calculated at family-level for assessing landscape influence on the abundance of insect predators. While we acknowledge that calculating diversity indices based on family level determinations introduces a degree of uncertainty in our measures of natural enemy diversity, it was not possible to identify all insects to species level owing to the geographic region not being comprehensively researched. Previous studies have, however, demonstrated that the calculation of H, at family-level, is an appropriate proxy for species-level H (Osborne, Davies, and Linton 1980; Zou et al. 2020) and is more practical when data sets are large or the ecosystem is understudied. Field margin plant species diversity was determined using the Simpson's species diversity index.

The effects of cropping season, location, month after harvesting and their interactions for natural enemy population, species richness and diversity were subjected to analysis of variance (ANOVA) using the procedure for general linear model in SAS Institute version 9.4 (SAS Institute, 2011). Similarly, effects season, location and their interaction for margin plants populations, species richness and diversity, aphid infestation and grain were subjected to ANOVA. The treatments means were separated using Tukey's Honestly Significant Difference (HSD) test at $P < 0.05$. Simple linear correlation was carried out to determine relationships between the diversity of margin plants and populations of natural enemy months after crop harvest.

3. Results

3.1 Abundance and diversity of plant species at the field margins

A total 54 plant species were recorded from the field survey across Njoro and Rongai during the study periods. There was a significant seasonal variation for plant species richness (ANOVA: $F_{1,16}$ = 33. 45; *P<0.0001*) and species diversity (ANOVA: $F_{1,16}$ = 7.20; *P=0.0511*) (Table 1). A higher species richness was observed during 2021 across the farms in Njoro (12.3) and Rongai (13.9) as compared to 2020 (Njoro: 5.8; Rongai: 6.9). Although there was a significant difference for species richness and diversity due to location, on average farms in Rongai had higher species richness and diversity compared to Njoro during both 2020 and 2021 off-cropping seasons (Table 1).

Across all farms, 59% of the margin species were annuals and 41% perennials. In Njoro annual species were the most abundant in 2020 off-cropping season compared to perennials during the same period (Figure 3). In Rongai, perennial plant species were more abundant than annuals during the 2020 off-cropping season. During the 2021 season, low abundance of the plant species was observed across the two locations. Rongai had a lower abundance of perennial plant species (5%) compared to Njoro (6%). Annual plants across the two locations were at 8% during the 2021 off-season (Figure 1). In Njoro and Rongai during the 2020 and 2021 off seasons, the plant species abundance is shown in Table 2 and Table 3, respectively.

3.2 Distribution, abundance and richness of natural enemies during off and on cropping seasons

The abundance of natural enemies changed over time after harvest (ANOVA: $F_{2,1133}$ = 8.11; *P<0.0001*). Natural enemy abundance did not differ significantly between the two locations, or between 2020 and 2021(ANOVA: $F_{1,1133}$ = 1.26; *P=0.262*). The interaction between Season × Location × Months after harvest was not significant for overall abundance of NEs in the field margin (ANOVA: $F_{1,1133}$ = 0.10; *P=0.905*) (Table 4).

A total of 9,355 potential natural enemies of bean aphids belonging to nine families were collected across the two locations in 2020 and 2021 off seasons. During the 2020 off-cropping season, 4,859 individuals were collected, with Njoro having 2,511 and Rongai 2,348. In relation to individual count of trapped insects, Njoro over the three months after harvesting, the highest natural enemy numbers were collected two months after harvesting (999) and lowest at the third month (673). In Rongai, a similar trend was observed where the highest NEs were collected at the second month (900) and lowest at the month (562). During the 2021 off-season, a total of 4,496 individuals were collected. Njoro had a total of 2,393 with the highest

NEs collected at the second month (957) and lowest at the third month (621). In Rongai, a total 2,103 individuals were collected with the highest NEs at the second month (822) and lowest at third month (546) (Table 5).

In the two locations, species that were most abundance changed over the cropping seasons (Figure 2). The most common species were from families that are known to bean aphid specialists such as, Syriphidae, Nabidae and Tachinidae. During on-season natural enemies were most abundant at lablab flowering stage. Lablab monocrop had the highest abundance across all growth stages (Table 6).

3.3 Pearson correlation of field margin plants and natural enemy during off and on cropping seasons, aphid abundance and lablab grain yield

Pearson corelation analysis showed a nonsignificant positive association for margin richness, margin abundance on natural enemy abundance off season (*P>0.05*) (Table 7). However, for plant diversity there was a positive significant correlation for natural enemy abundance during the off season (*P=0.002*). Field margin richness had positive significant association with natural enemy abundance during on season. In addition, field margin richness had a negative signification association with aphid abundance (*P=0.04*) and lablab grain yield (*P=0.01*) (Table 7) (Supplementary resource 2 & 3, respectively).

3.4 Bean aphids and grain yield

Results showed that cropping season was significant for aphid abundance (ANOVA: $F_{1,892}$ = 131.50; $P<0.0001$), damage severity (ANOVA: $F_{1,892}$ = 18.08; $P<0.0001$) and percent incidence (ANOVA: $F_{1,892}$ = 227.23; $P<0.0001$). In 2019 cropping season, Njoro had the highest aphid abundance (245.4), damage severity (43.9) and percent incidence (9.1) compared to Rongai. In 2020 cropping season, Rongai had a higher aphid abundance (45.5) compared to Njoro (34.8). For damage severity and percent incidence Njoro was the highest 24.4 and 8.8, respectively compared to Rongai (Figure 3). There was no significant interaction effect between location and cropping seasons (ANOVA: $F_{1,16}$ = 0.64; $P=0.43$), however, Njoro had a higher grain yield of 1751.3kg/ha and 1725.6kg/ha for 2019 and 2020, respectively (Figure 4).

4. Discussion

The results from this study show that field margin habitats offer refuge for natural enemies and other beneficial insects outside the main cropping season. The data further demonstrate that plant species at crop borders may play a key role in supporting natural enemy biodiversity at the beginning of each growing season as the number of natural enemy groups increased in months after harvesting. Field margins plants and semi-natural habitats can serve as biodiversity reservoirs, providing complementary resources and refuges for many natural enemies of pests (Scott and Harmon-Threatt 2021; Fusser et al. 2017). These non-crop habitats are more often undisturbed, semi-permanent, permanent and or/ regenerative in case the plants are multipurpose compared to crop fields which are frequently manipulated and disturbed (Amoabeng et al., 2020; Holland et al., 2016). Beneficial organisms which includes natural enemies (predators and parasitoids) and pollinators require stable habitat with resources (prey, pollen and nectar) and shelter to sustainably deliver on natural pest regulation and pollination (Jado et al. 2019). Other studies from agricultural systems have highlighted the importance of continuity of resources to support beneficial insects, i.e. specifically ensuring that resources such as nectar, prey and shelter are available year-round, not just when the crop is growing (Schellhorn, Gagic, and Bommarco 2015). Many natural enemies multiply in response to the availability of the food hence there is a "time lag" between pests and natural enemy fluctuations. Natural enemies are highly mobile their movement in and out of the crop will depend on how far their refuge places are and availability of prey and food. Therefore, throughout the season there will be fluctuation in population hence the need to assess their dynamics at several crop stages (Zhao *et al*., 2013). It is worth remembering that as plant communities change the associated organism species and population also changes, in this case as the crop stages and margin species change so do the natural enemies and pest and this influences their succession and colonization (Chaplin-Kramer *et al*., 2011).

A majority of work on biocontrol and agroecology has historically focused on temperate regions (Steward et al. 2014). As these regions usually experience a cool or cold winter, this tends to relieve pest pressure, as well as potentially reducing natural enemy populations, for several months of the year. Conversely, in tropical systems such as where lablab is grown in sub-Saharan Africa, even outside the cropping season there is no comparable winter, and the ecology is typically influenced more by rainfall than temperature patterns. As a result, the role of off-season vegetation and refugia may be even more critical than in temperate systems, and yet there is very little research exploring this aspect of the agroecosystem.

A higher number of natural enemies were observed in the second month after crop removal, suggesting the contribution of stable habitat in population build up. This could be an indication that if the habitats were to remain undisturbed to the next crop cycle, relatively high numbers of natural enemies could be observed at the beginning of the crop season as described by (Opatovsky and Lubin 2012; Fountain 2022). Conservation biological control is a key strategy towards sustainable pest management with the main focus on enhancing diversity and populations of naturally occurring predatory and parasitic invertebrate taxa (Balzan, Bocci, and Moonen 2016). The presence of low numbers, species richness and diversity natural enemy groups at the onset of cropping seasons are less likely to regulate pest population (Rusch et al. 2010).

Obanyi et al. (2023) used fluorescent dye applied to margin flowers to demonstrate that natural enemies moved from margins into the lablab crop, indicating that margin plants can provide resources or refuge for natural enemies. In the same study colonization and population build up was higher at the beginning of the season (vegetation stage) as the natural enemies came into the ecosystem. The natural enemy populations reduced as resources started declining at flowering and were very high at podding stage as resources increased. Here we observed higher natural enemy numbers in the field margins that led to more natural enemy in the crop field which is supported by other studies in crop-non-crop habitat interactions (Bertrand, Baudry, and Burel 2016), and higher natural enemy numbers in margin plants was corelated with increased pest management (Mkenda et al. 2019).

In this cropping system it is likely that enhancing field margin vegetation will in turn support natural enemies populations at higher levels year-round and avoid bottlenecks in their population dynamics, which in turn can contribute to pest management (Obanyi et al. 2023). While lablab, during its flowering season, may provide nectar and pollen for beneficial insects in its own right (in common with other mass-flowering crops) (Holzschuh et al. 2013), outside the cropping season these invertebrate species continue to need food sources and alterative prey and so are reliant on non-crop plants for prey, pollen and nectar, such as from field margins. In a tropical system in particular, plant diversity will support a wider range of flowering phenology leading to continuity in resources where more species are present. Native and perennial plants may offer particular benefits as part of the assemblage (Pfiffner et al. 2019; Cahenzli et al. 2019). This could be the reason why the natural enemy populations were relatively uniform in abundance even though diverse in composition across the locations.

The composition of the natural enemy communities differed in Njoro and Rongai over the two off-seasons. Generally, flying insects were the most abundant compared to ground dwelling, most likely as a result of the sampling methods used or retained availability and population increase and survival in the habitat. During crop harvesting the habitats are highly disturbed and insect families which detect the changes are bound to rapid changes and dispersal (Skirvin et al. 2011; Opatovsky and Lubin 2012). Field margin plants and semi-natural habitat are the immediate dwelling options for these arthropods (Arnold et al., 2021; Mkenda et al., 2019). Availability of these non-crop habitats ensures a rapid shift promoting arthropod dispersal from the disturbed crop fields. In farmers' fields that were cleared immediately after harvesting, these clearance activities resulted in a simple habitat which supported only low numbers of natural enemies. This observation is supported by Cloyd (2020) who reported that availability of diverse plant species at the field margins was a clear indicator of higher diversity and abundance of natural enemy at the onset of cropping season.

There was a seasonal variation in the abundance and species richness of plants across the study areas. This difference to a greater extent can be related to agricultural practices carried out in these regions. For instance, Njoro region is classified as high agricultural zone with intense agricultural activities compared to Rongai which had reduced agricultural activities due to prevailing climatic conditions. However, recommending particular plants for field margins can be difficult, as plants vary in their nutritional requirements and seasonality (Lahiru & Costamagna, 2019) and some can be secondary hosts to pests or crop diseases (Buck et al. 2023). Furthermore, many plant herbivores are controlled by natural enemies that are habitat generalists, which makes it difficult to identify their requirements (Sorribas et al. 2016). The inconsistent results perhaps could be attributed to either failure of field margin plants to provide needed resources for the natural enemy community or competitive interactions among generalist predators (Fiedler and Landis 2007; Ramsden et al. 2014; Karp et al. 2018).

Conclusion:

Our study demonstrates that populations of agriculturally-relevant natural enemies are boosted by plant rich field margins outside the cropping period, and therefore that this habitat requires special consideration. In particular, *banking* natural enemies – particularly in warm climates where insect reproduction can take place year-round and beneficial populations can keep building – before a cropping season may protect the crop more effectively. Similarly, in temperate regions the presence of natural enemies early in the season in organic arable farms reduces pressure from major insect pests of corn, soybean and wheat (Costamagna et al., 2015;

Yang et al., 2017; Gontijo, 2019). Therefore, measures to sustain natural enemies outside the cropping season could allow farms to enter the main cropping season with better baseline populations, meaning crop pests are controlled more rapidly and may take longer to build to an economically damaging threshold (Stoddard et al. 2010).

This study has demonstrated that farms with higher abundance of non-crop vegetation in the off-season also have higher abundances of natural enemies, which previous work in this region has demonstrated can support pest management and improved yield (Obanyi et al. 2023). The composition of the natural enemy communities shifts across the season, and dissecting the reasons for this and the practical consequences for agriculture will be important in tailoring management for sustainable lablab production in future, especially in the face of changing climate and land-use in these regions.

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions: PCS, SRB, JOO and SEJA conceived the study. JOO, PKB, JGN, EKC, RMSM, SEJA, SRB, VCW and PCS were involved in the study design. JNO, VCW and SRB carried out the statistical analysis, JNO wrote the first draft of the manuscript. JNO carried out field and cage trials and data collection. All authors were involved in writing the manuscript and gave final approval for publication.

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Figure Legends

Figure 1: Mean abundance (counts per quadrat) of plant species in Njoro and Rongai during 2020 and 2021 off- cropping seasons. Vegetation type here means life cycle of plant species within the margins. Abundance= number of individual plant species within the quadrat.

Figure 2: Species group rank abundance curve for A) Rongai during 2020, B) Rongai during 2021, C) Njoro during 2020, D) Njoro during 2021 off seasons.
Rongai 2020 off season species rank: Tachinidae =1, Carabidae =2, Ichneumonidae =3, Geocoridae=4, Syriphidae =5, Nabidae=6, Coccinellidae=7, Braconidae=8; **Rongai 2021 off season species rank:** Nabidae=1, Syriphidae=2, Ichneumonidae=3, Geocoridae=4 Tachinidae=5, Braconidae=6, Coccinellidae=7, Carabidae=8; **Njoro 2020 off season species rank:** Geocoridae=1, Carabidae=2, Syriphidae=3, Tachinidae=4, Ichneumonidae=5, Nabidae=6, Coccinellidae=7, Braconidae=8; **Njoro 2021 off season species rank:** Syriphidae=1, Nabidae=2, Tachinidae=3, Ichneumonidae=4, Coccinellidae=5, Braconidae=6, Geocoridae=7, Carabidae=8

Figure 3: Bean aphid abundance, damage severity and percent incidence (Mean ±SE) in Njoro and Rongai location during 2019 and 2020 cropping seasons

Figure 4: Lablab grain yield kg/ha (Mean ±SE) in Njoro and Rongai location during 2019 and 2020 cropping seasons

Figure 1.JPEG

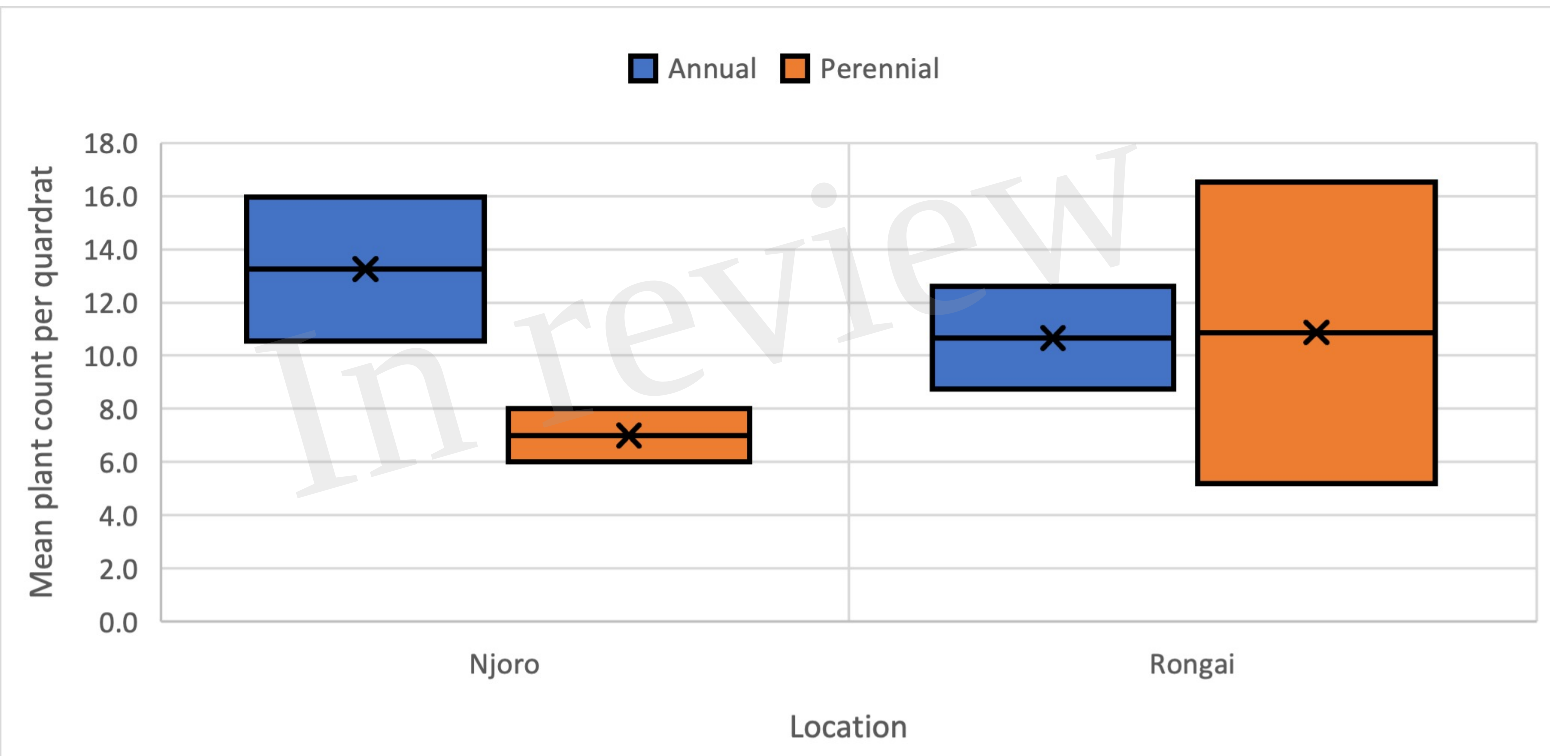

Figure 2.JPEG

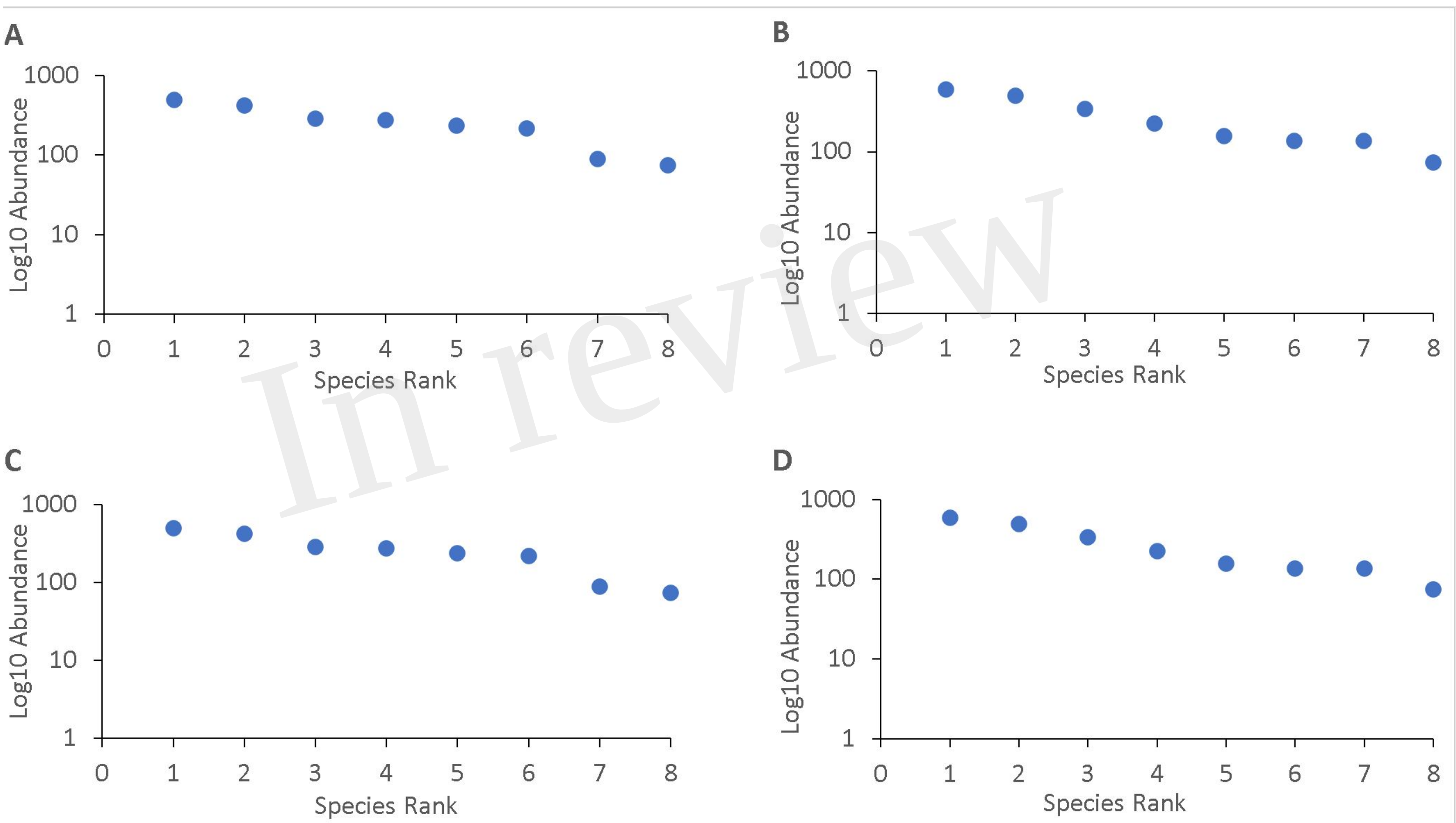

Figure 3.JPEG

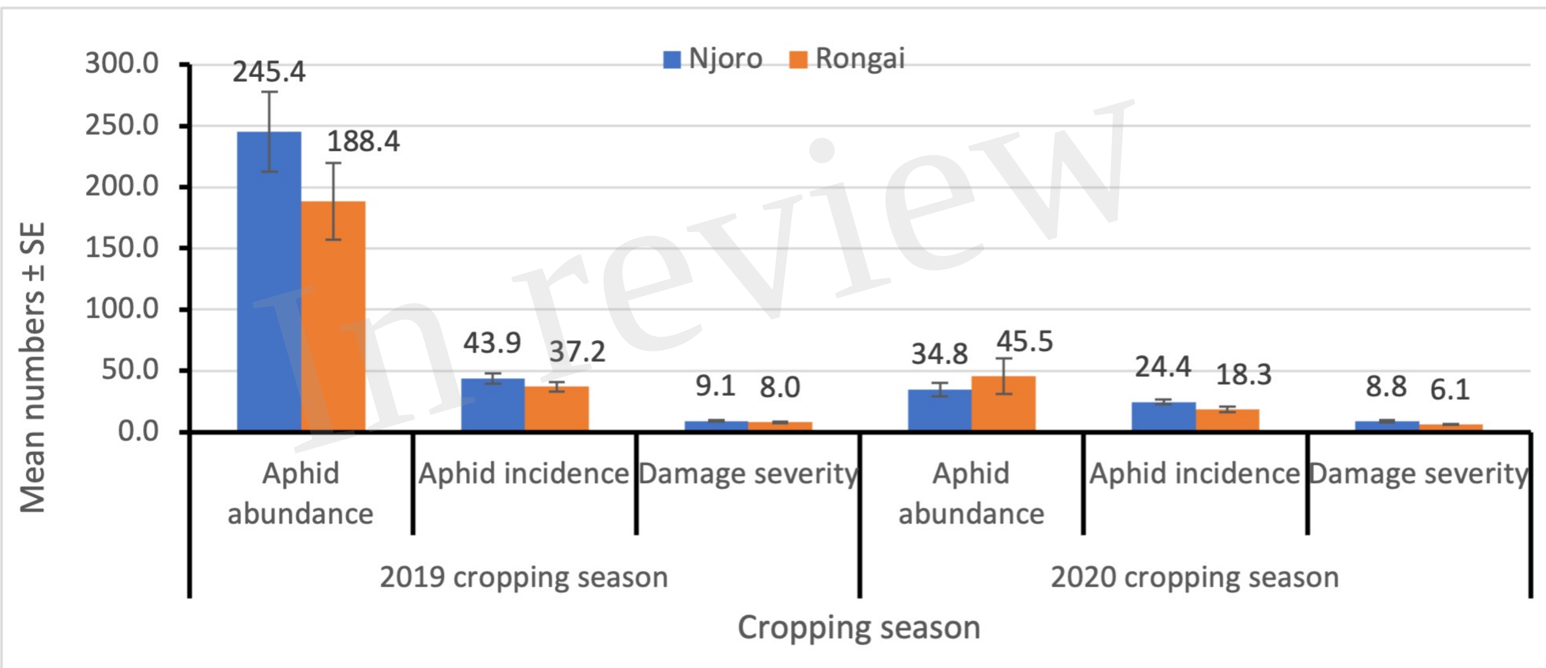

Figure 4.JPEG