**ORIGINAL ARTICLE**

# Field margins and cropping system influence diversity and abundance of aphid natural enemies in *Lablab purpureus*

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## Abstract

Field margin plants around crops are postulated to increase natural enemy abundance to enhance pest management. A trial was conducted to determine the contribution of field margin vegetation and cropping systems to natural enemies of bean aphids (*Aphis fabae*) on *Lablab purpureus*, an orphan crop legume. Natural enemy populations were surveyed in plots with a combination of lablab monocrop or maize-lablab intercrop and with or without planted field margins comprising four plant species (*Chenopodium album*, *Bidens pilosa*, *Galinsoga parviflora* and *Tagetes minuta*), arranged in a randomized complete block design with four replicates. A cage experiment was also conducted to understand the extent to which these field margin plants supported the parasitoid wasp, *Aphidius colemani*, to parasitize bean aphids. A total of 2029 insects from 10 families were collected using sticky cards and pan traps. In comparison to plots with no field margin plants, the presence of plant-rich field margins increased abundance of natural enemies by 9.5% and supported higher populations of Braconidae (parasitoids) and damsel bugs (predators). The maize-lablab intercrop had 15.5% lower abundance of natural enemies than the lablab monocrop. Higher grain yield was recorded in plots with a field margin vegetation (300 kg ha$^{-1}$) compared to plots without field margin vegetation (210 kg ha$^{-1}$). The presence of a companion plant did not significantly affect the performance of parasitoids, in the cage experiment, indicating that parasitoids were not limited by access to food. In supporting aphid parasitization, *B. pilosa* was associated with the highest number of mummies (8.28). The lowest number of mummies were observed in cages with *T. minuta* (3.44). These results demonstrate the potential of plant-rich field margins to augment natural enemy populations and enhance conservation biocontrol of aphids.

**KEYWORDS**

biological control, intercrop, margin vegetation, monocrop, natural enemies, sustainable pest management

# 1 | INTRODUCTION

Increased agricultural production is key to expanding global food and nutrition security (Ann & Tom, 2019). Agricultural expansion and intensification to increase food production has, however, led to natural habitat and biodiversity loss. Climate change on the other hand has exacerbated the impact of crop losses due to insect pests with far reaching consequences on both crop production and food security (Foley et al., 2005). Agricultural systems that rely heavily on mechanization and synthetic inputs such as pesticides disrupt non-crop habitats where such natural enemies of pests seek refuge (Balzan et al., 2016; Bommarco et al., 2013) This calls for approaches that will balance the benefit of agricultural intensification and restore biocontrol service through enhancing the role of natural enemies in pest management. Conservation of important biological control agents provide a sustainable pest management option that can reduce dependency on the application of synthetic pesticides (Ochieng et al., 2022).

Non-crop habitats provide refuge for invertebrate taxa and opportunities for the exchange of natural enemies between crop and non-crop areas (Balmer et al., 2013; Morandin et al., 2014; Tscharntke et al., 2005). Destruction of these habitats results in simplified landscapes with low plant diversity that directly or indirectly alters the invertebrate community in ecosystems, thus affecting the functioning of natural pest control (Bianchi et al., 2006). There is an urgent need for efficient interventions to increase food production sustainably and conserve diversity and increase the abundance of natural enemies in agricultural ecosystems (Amoabeng et al., 2020; Ratnadass et al., 2012).

Ecological intensification approaches include planting of flowering rich field margins and intercropping provides resources such as pollen and nectar and so can be associated with increased fecundity and longevity of natural enemies (Jacob & Evans, 2000). In particular, parasitoid wasps (e.g. *Aphidius colemani*, a key natural enemy in East Africa; Woolley et al., 2022) are important in the control of many insect pests. These parasitoids have been shown to benefit from access to nectar resources and alternative hosts (Gurr et al., 2017; Kishinevsky et al., 2017; Souza et al., 2019). Natural enemy diversity and abundance are dependent on non-crop vegetation providing prey, food, refuge and habitats to diapause (Alignier et al., 2014; Langellotto & Denno, 2004; Zhao et al., 2021).

Lablab (*Lablab purpureus* (L.) Sweet) is a drought resilient underutilized orphan crop legume with great potential to mitigate climate change-induced threats to global food security (Minde et al., 2021). Lablab production is, however, constrained by insect pests, specifically bean aphid (*Aphis fabae* Scopoli), which can result in 40%–90% yield losses (Abate & Ampofo, 2003; Nahashon et al., 2016). Integrated pest management, including the role of margin plants in supporting natural pest regulation in orphan legume crops such as lablab, has received little research attention. The main objective of the current study was to determine the pest management potential of natural enemies through ecological intensification of field margins. Here we investigated the different field margin plant species and cropping systems for their capacity to support natural pest regulation and improve yields. We hypothesized that by acting as alternative host for natural enemies, field margin vegetation and intercropping could reduce pest incidence in crop fields. We specifically addressed three research questions: (i) which plant species are important for natural pest regulation in orphan crop legumes (ii) which plant species provide effective field margin support for bean aphid natural enemies (iii) whether field margin vegetation and intercropping reduce pest incidence and increase crop yield. To test this hypothesis, we investigated how major weeds commonly found on farmlands support the diversity and abundance of a natural enemy of bean aphids in lablab. The study also focused on approaches to enhance natural enemy population using manipulated field margin vegetation in different cropping systems such as intercropping to manage bean aphids in lablab. The results from this study are aimed at providing information to farmers on how to implement a sustainable integrated pest management option for the control of bean aphids on lablab that can be extended to other arable crops.

# 2 | MATERIALS AND METHODS

## 2.1 | Study site

The study was conducted at the Agronomy Field Station, Egerton University, Nakuru County, Kenya. The site lies at 0°19′0″S and 36°06′0″E and an altitude of 2200m above sea level. The annual rainfall range is 1000–1250mm with a temperature of 17–22°C. The soils are classified as vintric mollic andosols (Jaetzold et al., 2012).

## 2.2 | Field experiment

In order to determine the effect of planted margin species and cropping systems on the abundance and diversity of bean aphid natural enemies, a field experiment was conducted during August–December 2020 cropping season. The first treatment factor was for experimental plots with field margin vegetation planted with four plant species (Appendix S1). The field margin plant species were selected based on their relative abundance in the ecosystem and previous studies, which indicated their role in supporting beneficial insects (Ditner et al., 2013; Ndakidemi et al., 2021). The plant species were *Chenopodium album* (Amaranthaceae), *Bidens pilosa* (Asteraceae), *Galinsoga parviflora* (Asteraceae) and *Tagetes minuta* (Asteraceae). The seeds of each species were mixed in equal proportions (by weight) and sown around each plot, which had plant margin treatments, two weeks before planting lablab crop. The margin species were planted 0.5m from the outer row of the lablab and the margin width was 2m. The plots which had no field margin

vegetation were left bare throughout the lablab growth cycle. The second treatment factor was cropping system where lablab was either planted as a monocrop or intercrop with maize. Lablab monocrops and a lablab-maize intercrop were, therefore, planted either in the presence or absence of field margin vegetation. The treatments were laid out using a $2 \times 2$ factorial arrangement in a randomized complete block design (RCBD) with four replications, giving a total of 16 plots. The experimental plots measuring $10 \times 10$ m were isolated from each other by a distance of 20 m. Lablab is a short crop; the 20 m separation was adopted as the compromise distance in conformity with typical smallholder land holdings (field sizes) as reported by Ochieng et al. (2022) that also minimizes insect movement between any two experimental plots. Lablab monocrop was planted at a spacing of $0.5 \times 0.3$ m. In the maize-lablab intercrop, maize was planted at a spacing of $0.75 \times 0.3$ m, 1 seed per hill and lablab was planted between the maize rows at an intra-row spacing of 0.3 m, 2 seeds per hill. No pesticides were applied on the crops during the season to allow for pest infestation and natural enemy population build-up. The planted field margin vegetation was left to grow up to flowering stage and coincide with the vegetative stage of the lablab crop.

## 2.3 | Cage experiment

To verify results from the field experiment on the potential of individual margin species *Chenopodium album* (Amaranthaceae), *Bidens pilosa* (Asteraceae), *Galinsoga parviflora*: (Asteraceae) and *Tagetes minuta* (Asteraceae) performance to support enhanced aphid natural enemy (parasitoids) abundance and bean aphid suppression, a cage experiment was also conducted in September–November, 2020. The cage experiment was laid out in an open field 4 km away from the crop fields, arranged in RCBD design with four replicates. Each of the four replicates had six metallic frame cages measuring $1 \times 1 \times 2$ m (length x width x height) set 2 m apart giving a total of 24 cages. Each cage was covered with white fine net (0.2 mm mesh diameter) with two zips on one side for ease of access. A cage contained either one of the four field margin plant species (*C. album*, *B. pilosa*, *G. parviflora* and *T. minuta*) in a 20 L planting pot and retained up to flowering stage or was a control without a plant, all plant-containing cages also provided a water source for parasitoids. Control (positive and negative, respectively) cages contained either 10% sucrose solution only or only water with no companion plant; one negative and one positive control was placed in each plot. The sucrose and water were supplied using 50 mL glass bottles with wicks. A sentinel plant infested with 80 clean aphids (*A. fabae*) from a laboratory culture was then introduced in each cage. Six, 2-day old *A. colemani* parasitoids were released into the cages at a ratio of two males to four females and allowed to colonize freely for 30 days. *A. colemani* was selected because it was the most abundant primary parasitoid identified to control bean aphids in lablab from another experiment in the same study system (Mkenda et al., 2019).

## 2.4 | Data collection

### 2.4.1 | Natural enemies' temporal distribution, diversity, abundance and bean aphid parasitization

To determine enhanced support of individual margin species plants to aphid natural enemies, fluorescent dyes were used to trace margin visitation by the natural enemies and their movement back to the crop. The movement of insects from the field margin vegetation into the experimental plots was monitored by applying four different fluorescent dyes (pink, yellow, green and blue) to the flowers of the planted margin species (Karp et al., 2018; Mkenda et al., 2019; Wratten et al., 2003). One dye was assigned to a different plant species as follows; yellow for *C. album*, green for *B. pilosa*, pink for *G. parviflora* and blue for *T. minuta*. Insects visiting flowers would passively pick up the dye; thus, it was possible to ascertain whether insects captured on the crop visited the field margin flowers. The dyes were prepared according to the manufacturer's instructions, mixing 100 g of powder with 1 L of water to make a thick paste that was applied to all open flowers on the plot, with a stiff medium–hard brush. A total of four transparent Perspex sticky traps (Plaskolite Inc) measuring $10 \times 25$ cm and coated on both sides with Tanglefoot (The Tanglefoot Company) were placed in each plot. The traps placement was done at a distance of 5 m from the field margin each facing a side of the plot. The sticky traps were suspended by a string at 30 cm above the lablab crop canopy to capture flying natural enemies. The choice of transparent traps was chosen to minimize bias for colour influences on insect movement and avoid artificially inducing movement into the crop. Similarly, to capture crawling natural enemies, pan traps made of transparent round plastic containers measuring 20 cm diameter by 5 cm depth filled with a premixed liquid solution containing 250 mL of water, 5 g of salt to preserve the natural enemies and 5 mL of odourless liquid detergent to break the surface tension, were placed at ground level just below the sticky traps and left in the field for 48 h. Traps were deployed at three lablab crop stages (early vegetative, late vegetative and podding growth stages). Dyes were applied to all flowers immediately before trap placement.

Insects on sticky traps were taken to the laboratory for identification. The pan-trapped insects were retrieved by sieving and washing with clean water and transferring them to 50 mL plastic falcon tubes filled with 25 mL of 70% ethanol using a camel-hair brush size 1/8″ width and 1/2″ hair length. Observation for traces of the different dyes on the sampled insect bodies was done under UV light in a dark room using a dissecting microscope illuminated. Only natural enemies with traces of dye on their body were recorded as having visited/been supported by the planted margin species. All the collected specimens, regardless of whether dye was present, were viewed under a dissecting microscope (Leica ZOOM 2000 Inc.) at $200 \times$ magnification counted and identified to family level using taxonomic keys (Sadof et al., 2014).

Aphid parasitization was assessed using sentinel plants, which were potted lablab seedlings and infested with 60 bean aphids

(*A. fabae*) each. Sentinel plants were placed in the same experimental plots immediately (same day) after removal of transparent sticky traps and pan traps (48 h after the fluorescent dye application). Every plot (lablab monocrop or maize-lablab intercrop) received two sentinel plants: one placed at the centre of the crop (5 m from the outermost row) and the at margin vegetation (0.5 m from the outermost row). The plants were left in the field plots for 7 days to allow aphid parasitoids to lay eggs. Upon retrieval, the sentinel plants were covered with a fine net mesh during transport, then placed in mesh cages. The number of live parasitoids, dead parasitoids and aphid mummies was recorded every 3 days for 30 days. It was not feasible to count the non-parasitised aphids, as aphids have a very high reproduction rate and can be cryptic in large cages, thus we focused on standardizing the number of aphids at the start and then recording the number of mummies arising.

### 2.4.2 | Bean aphids

Data on aphid damage severity and percent incidence were collected in the same field trial described in Section 2.2. Damage severity was collected from 10 randomly selected plants from the inner five rows in each replicate. The severity of damage was determined by visually observing and scoring the level of damage on the selected plants. A scoring scale of 1–5 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.

### 2.4.3 | 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.). At 13% moisture content, grains from each treatment were weighed separately using a portable digital scale (Manufacturer: Comglobal Solutions) and converted to kg ha$^{-1}$ using the following formula:

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

## 2.5 | Data analysis

To evaluate field margin vegetation and cropping systems in terms of conservation and enhancement of bean aphid natural enemies, a species diversity index was calculated. The unit for measurements was the number of natural enemies in each broad taxonomic group caught at vegetative, flowering and podding growth stages of lablab per field margin vegetation and cropping system. The diversity of natural enemies was determined using Shannon–Weaver index of diversity (*H*; Pielou, 1966).

$$H = -\Sigma p_i \ln(p_i)$$

Where *H* is the Shannon's diversity index, $p_i$ is the proportion of the sample consisting of individuals in the *i*th family, and ln is the natural log of the proportion of individuals found from the *i*th family.

Aphid natural enemy counts obtained from traps were transformed using natural $\log_{10}(x+1)$ transformation and subjected to repeated measure analysis of variance using a general linear mixed model, with main factors: cropping system, margin plant species and a repeated measure of crop growth stage. The effects of cropping system and margin vegetation on natural enemy abundance were analysed using a two-sample *t*-test. The effects of margin vegetation and cropping system on aphid damage and yield were analysed using a two-way Analysis of Variance (ANOVA).

In the cage trials and emergence of parasitoids from the sentinel plants, data on counts of emerged parasitoids, mummies, live and dead parasitoids were analysed using repeated measures analysis of variance. The analysis included data across the whole time series, while the effect of time/change over time was not a key question here, the repeated measures analysis takes the effect of time into account. Mean differences between the treatments on mummies, live and dead parasitoids, means were separated using Tukey's Honestly Significant Difference (HSD) test at $p < 0.05$. The statistical analyses were done using XLSTAT version 2019.2.2.59614 (Addinsoft, 2019), XLSTAT statistical and data analysis solution (https://www.xlstat.com).

## 3 | RESULTS

### 3.1 | Abundance, diversity and temporal distribution of bean aphid natural enemies

A total of 2029 natural enemy individuals were collected, with higher number captured on sticky traps (1122) as compared to pan traps (907). The collected invertebrate taxa were grouped into two main categories of natural enemies either as a predator or parasitoid. Based on these categories a total of 10 natural enemy families were identified, composed of crawling and flying predators and parasitoids, from six insect orders: Hymenoptera (Braconidae, Ichneumonidae and Formicidae) (we decided to include ants as potential natural

enemies, as ants can provide pest control services in tropical cropping systems; Milligan et al., 2016; Wielgoss et al., 2014), Hemiptera (Nabidae and Geocoridae), Diptera (Tachinidae and Syrphidae), Coleoptera (Coccinellidae and Carabidae) and Neuroptera: (Chrysopidae) (Table 1). In relative percent abundance Tachinidae was the most abundant family (comprising 32.4% of insects on sticky traps and 10.5% in pan traps) followed by the Ichneumonidae (11.7% pan and 10.5% sticky), and lowest abundance (0.1% pan and 5.4% sticky) was observed in the Chrysopidae (Table 1). The most abundant family captured by sticky traps was the Tachinidae (364 individuals) and the least abundant was the Carabidae. A different observation was made on captures by pan traps where Ichneumonidae was the most (106) and Chrysopidae was the least (1) often captured (Table 1).

Generally, the presence of field margin vegetation did not predict increased natural enemy taxonomic richness (ANOVA: $F_{1,18}=1.01$; $p=0.32$), abundance (ANOVA: $F_{1,18}=1.12$; $p=0.30$) or diversity (ANOVA: $F_{1,18}=0.01$; $p=0.91$). Cropping system showed a significant difference only for taxonomic richness index for monocrop (8.33) versus intercrop (7.08) (ANOVA: $F_{1,18}=4.63$; $p=0.04$) with no significant differences between monocrops and intercrops in terms of natural enemy abundance (ANOVA: $F_{1,18}=3.69$, $p=0.07$) and diversity (ANOVA: $F_{1,18}=2.85$; $p=0.11$). Natural enemy taxonomic richness (ANOVA: $F_{2,18}=3.48$; $p=0.05$) and abundance (ANOVA: $F_{2,18}=6.06$; $p<0.001$) changed over the course of the cropping season but there was no significant change in diversity (ANOVA: $F_{2,18}=2.33$; $p=0.13$). The number of insect families captured ranged between 4 and 10, which was influenced by field margin vegetation, cropping system and lablab growth stage. The Shannon–Weaver index ranged between 2.06 and 0.62. The highest diversity index of 2.06 was recorded on lablab intercrop at flowering stage in the presence of field margin vegetation for the sticky trap. The lowest diversity index of 0.62 was recorded on lablab monocrop at flowering stage in the absence of field margin vegetation (Appendix S2).

## 3.2 | Bean aphid natural enemy abundance as influenced by cropping system, margin vegetation and lablab growth stage

The trapping method significantly influenced the abundance of natural enemies recorded (ANOVA: $F_{1,712}=3.916$; $p=0.048$), with no significant effect of field margin vegetation (ANOVA: $F_{1,712}=0.701$; $p=0.403$) and cropping system (ANOVA: $F_{1,712}=2.038$; $p=0.154$) (Table 2). In relation to cropping system, lablab monocrop had a higher number of natural enemies in the presence of margin plants ($5.55\pm2.57$) as compared to lablab-maize intercrop ($3.25\pm1.25$) for sticky trap and pan traps (Table 3). The presence or absence of margin, however, significantly influenced the number of some taxa of aphid natural enemies: Braconidae were significantly ($p=0.0012$) more abundant ($0.24\pm0.02$) in plots with a margin and Nabidae were significantly ($p=0.02$) more abundant ($0.24\pm0.01$) in plots with no margin vegetation borders (Figure 1). In the temporal distribution of the natural enemy families across the season, generally all the 10 families were present in the fields during all the three crop stages. No family had significantly higher densities across more than one crop stage. The mean abundance of most of the natural enemies was highest during the podding stage followed by the vegetative stage and was lowest at the flowering stage (Appendix S3).

## 3.3 | Dye experiment results – Interactions with margin plants

Fluorescent dye traces were observed on insects both in plots with and without margin plant species around the crop borders. Generally, out of 2029 of natural enemy captured, 201 had dye traces and 1828 had no dye traces representing 10% and 90%, respectively. Out of the 201 fluorescent dye marked natural enemies, *Galinsoga parviflora* had the highest proportion (6.31%) and lowest in *Tagetes*

**TABLE 1** Relative abundance of natural enemies and other insect as captured by pan and sticky traps.

| | | | Pan traps | | Sticky traps | |
|---|---|---|---|---|---|---|
| Category | Order | Family | Counts | Relative abundance (%) | Counts | Relative abundance (%) |
| Parasitoid | Hymenoptera | Braconidae | 38 | 4.2 | 81 | 7.2 |
| Parasitoid | | Ichneumonidae | 106 | 11.7 | 118 | 10.5 |
| Other | | Formicidae | 463 | 51.0 | 138 | 12.3 |
| Predator | Hemiptera | Nabidae | 30 | 3.3 | 111 | 9.9 |
| Predator | | Geocoridae | 42 | 4.6 | 57 | 5.1 |
| Parasitoid | Diptera | Tachinidae | 93 | 10.3 | 364 | 32.4 |
| Predator | | Syriphidae | 52 | 5.7 | 94 | 8.4 |
| Predator | Coleoptera | Coccinellidae | 51 | 5.6 | 58 | 5.2 |
| Predator | | Carabidae | 31 | 3.4 | 40 | 3.6 |
| Predator | Neuroptera | Chrysopidae | 1 | 0.1 | 61 | 5.4 |
| Total | | | 907 | 100 | 1122 | 100 |

**TABLE 2** Analysis of variance for abundance of natural enemies captured by two different trapping methods within lablab bean crops planted as either a monocrop or intercrop (with maize) where field margin vegetation was either present or absent around the field plot.

| Source of variation | df | NEs abundance |
|---|---|---|
| Trapping method | 1 | 3.916 |
| | | 0.048 |
| Margin vegetation | 1 | 0.701 |
| | | 0.403 |
| Cropping system | 1 | 2.038 |
| | | 0.154 |
| Trapping method×Margin vegetation | 1 | 0.495 |
| | | 0.482 |
| Trapping method×Cropping system | 1 | 1.470 |
| | | 0.226 |
| Margin vegetation×Cropping system | 1 | 0.006 |
| | | 0.939 |
| Trapping method×Margin vegetation×Cropping system | 1 | 0.006 |
| | | 0.941 |
| $R^2$ | | 0.012 |
| $F$ | | 1.233 |
| $Pr>F$ | | 0.282 |

**TABLE 3** Mean abundance of natural enemies captured by two different trapping methods within lablab bean crops planted as either a monocrop or intercrop with maize where field margin vegetation was either present or absent around the field plot.

| Trap type | Field margin vegetation | Cropping system | Natural enemies |
|---|---|---|---|
| Pan | Absent | Intercrop | 2.1 b |
| | | Monocrop | 4.167 ab |
| | Present | Intercrop | 3.267 ab |
| | | Monocrop | 5.550 a |
| Sticky | Absent | Intercrop | 2.033 b |
| | | Monocrop | 2.408 b |
| | Present | Intercrop | 2.250 b |
| | | Monocrop | 2.658 b |

*Note*: Values in the same column followed by the same letter are not different from each other at the 95% confidence interval using the Tukey's Honestly Significant Difference (HSD) test.

*minuta* (0.20%) (Figure 2). However, the natural enemy taxa showed their preference in the field margin plants by carrying dye originating from the four different margin species (Figure 3), indicating potentially that different taxa have preferences for different plant species. However, all the families had evidence of visiting and interacting with the planted margin vegetation at least once, even though with
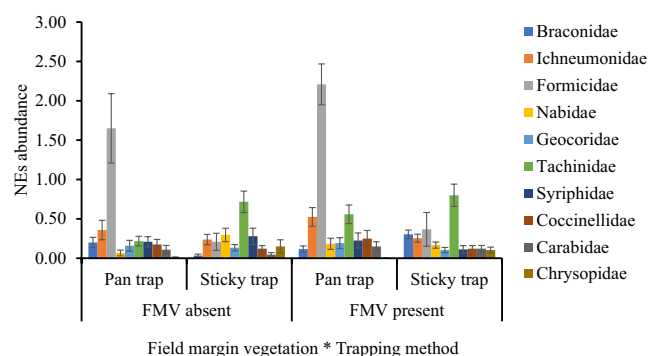


**FIGURE 1** Abundance (Mean±SE) of bean aphid natural enemies as influenced by field margin vegetation (present or absent).
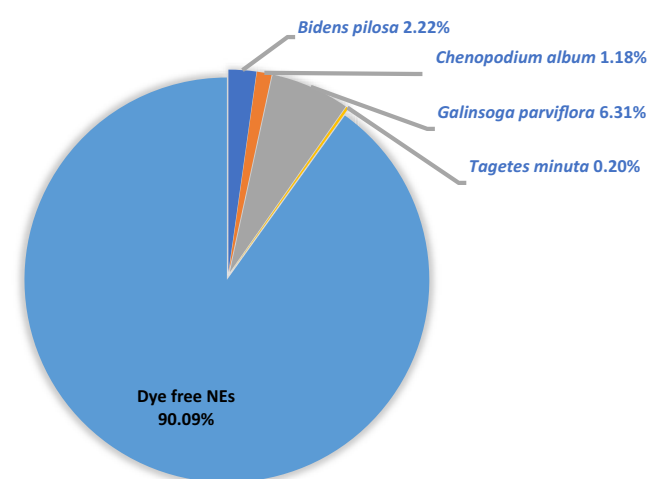


**FIGURE 2** Proportion of natural enemies with and without florescent dye captured. Florescent dye was applied to individual margin species that supported natural enemies where; yellow=*Chenopodium alba*, green=*Bidens pilosa*, pink=*Galinsoga parviflora* and blue=*Tagetes minuta*.

varied numbers and frequency. The most visited plant species was *G. parviflora*, which was dominated by Tachinidae for both pan traps (average 0.58) and sticky traps (average 0.60). *B. pilosa* and *G. parviflora* had the highest number of visitations with at least 3 families (Figure 3). Ichneumonidae most often visited *B. pilosa* compared to *G. parviflora*. *T. minuta* was the least visited plant species with low visit numbers by Syrphidae (Figure 3). In general, bean aphid natural enemies and other insects were noted to have interacted most often with *B. pilosa* followed by *G. parviflora* and *C. album* with very few having visiting *T. minuta* (Figure 3).

## 3.4 | Bean aphid severity and incidence

The ANOVA showed that cropping system was significant for aphid incidence ($p=0.053$ and $p=0.006$) and severity ($p=0.0001$ and

**FIGURE 3** Abundance (Mean) of bean aphid natural enemies supported by individual margin species as captured pan traps and sticky traps aided with florescent dye. Florescent dye was applied to individual margin species that supported natural enemies where; yellow = *Chenopodium alba*, green = *Bidens pilosa*, pink = *Galinsoga parviflora* and blue = *Tagetes minuta*.
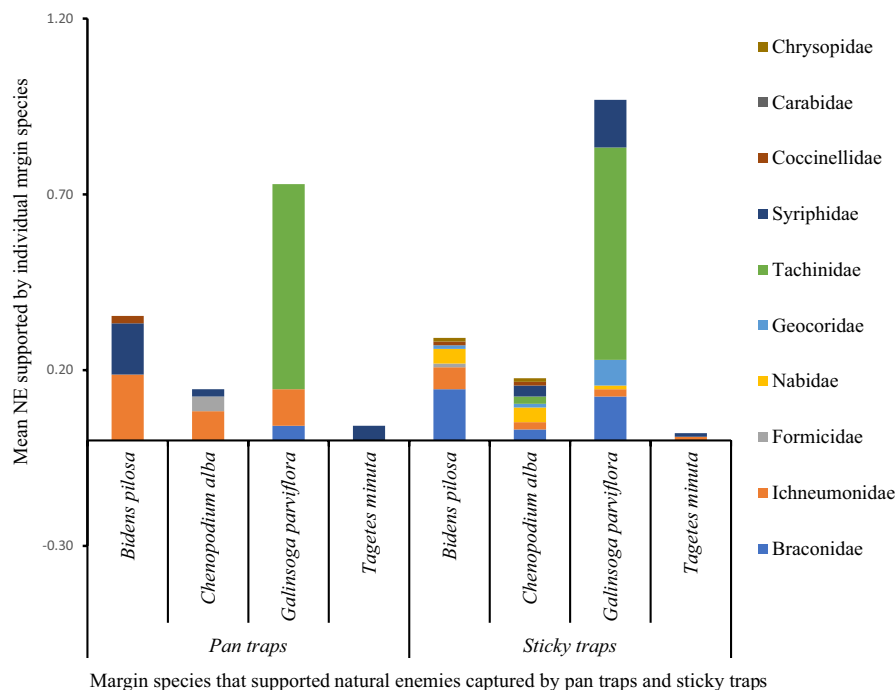
**TABLE 4** Analysis of variance for aphid incidence and severity on lablab bean crops planted as either a monocrop or intercrop (with maize) where field margin vegetation was either present or absent around the field plot.

| Source of variation | df | Aphid incidence | Aphid severity |
|---|---|---|---|
| Cropping system | 1 | 3.900 | 21.804 |
| | | 0.053 | <0.0001 |
| Field margin vegetation | 1 | 8.100 | 4.135 |
| | | 0.006 | 0.047 |
| Replication | 3 | 1.866 | 0.713 |
| | | 0.146 | 0.548 |
| Cropping system × Field margin vegetation | 1 | 0.282 | 4.447 |
| | | 0.597 | 0.039 |
| $R^2$ | | 0.239 | 0.363 |
| $F$ | | 2.980 | 5.421 |
| Pr > $F$ | | 0.013 | 0.000 |



**FIGURE 4** Mean (±SE) of aphid incidence and severity as influenced by maize-lablab cropping system and field margin vegetation. Columns bearing the same letters are not significantly different using Tukey's Honestly Significant Difference (HSD) at ($p < 0.05$).

## 3.5 | Bean aphid parasitization

A total of 403 parasitoids emerged from the sentinel plants in the netted cages for the 31 days. Cropping system did not have a significant effect on parasitoid emergence numbers (ANOVA: $F_{1,36} = 2.02$; $p = 0.164$), although the monocrop had higher numbers of emerged parasitoids (218) compared to the intercrop (185). Field margin vegetation significantly influenced the number of emerged parasitoids (ANOVA: $F_{1,36} = 43.45$; $p < 0.0001$) (Table 5). Sentinel plants that were placed in plots with field margin vegetation had more total number of emerged parasitoids (278) compared to plots without field margin vegetation (125). The two-way interaction of field margin vegetation and cropping system showed no significant difference for the emerged parasitoids (ANOVA: $F_{1,36} = 0.59$; $p = 0.625$). However, mean comparison showed slight

$p = 0.047$). The interactive effect between cropping system and field margin was only significant ($p = 0.039$) on aphid severity (Table 4). Generally, in the absence of field margins high aphid percent incidence (number of plants showing attack) were observed for both monocrop (86.5%) and intercrop (73.3%) (Figure 4). Cropping system had a great influence on the severity of aphid infestation both in the presence and absence field margin vegetation. Lablab monocrop had high aphid severity both in the absence (25.0%) and presence (21.56%) of field margin vegetation. However, maize-lablab intercrop aphid severity was not influenced by field margin vegetation (Figure 4).
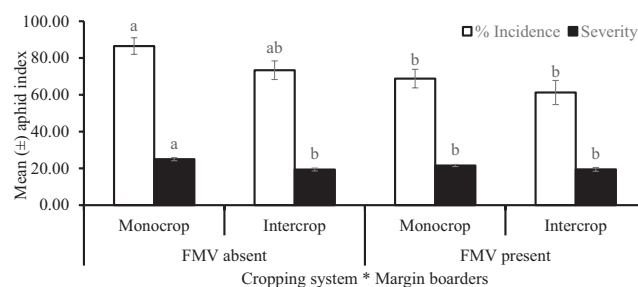
**TABLE 5** Analysis of variance for emerged parasitoids from lablab bean sentinel plants planted as either a monocrop or intercrop (with maize) where field margin vegetation was either present or absent around the field plot.

| | | Emerged parasitoids | |
|---|---|---|---|
| Source of variation | df | F | Pr > F |
| Field margin vegetation | 1 | 43.45 | <0.0001 |
| Cropping system | 1 | 2.02 | 0.164 |
| Crop growth stage | 2 | 15.59 | <0.0001 |
| Field margin × Cropping system | 3 | 0.59 | 0.625 |
| Field margin × Crop growth stage | 4 | 0.40 | 0.808 |

variations on the number of emerged parasitoids. The highest number of emerged parasitoids were observed in monocrop with and without field margin vegetation at podding (0.51±0.02) and vegetative (0.48±0.04) lablab growth stages, respectively, over 31 days (Table 6).

## 3.6 | Lablab grain yield

The presence or absence of field margin vegetation had a significant effect on lablab grain yield (ANOVA: $F_{1,12} = 9.008$; $p = 0.011$). Higher grain yield was observed in plots surrounded with field margin vegetation (298.9 kg ha$^{-1}$) compared to plots, which had no field margin vegetation (209.5 kg ha$^{-1}$). Cropping system had a significant impact on grain yield (ANOVA: $F_{1,12} = 5.179$; $p = 0.042$) where higher grain yield was recorded in lablab monocrop (288.1 kg ha$^{-1}$) compared to intercrop (220.3 kg ha$^{-1}$). Integration of field margin vegetation and lablab monocrop had the highest grain yield (351.6 kg ha$^{-1}$) compared to either intervention alone (Figure 5).

## 3.7 | The effect of field margin species on *Aphidius colemani* parasitoids and control of bean aphid in cage trials

The presence of any companion plant resulted in a lower emergence of *A. colemani* compared to 10% sucrose (positive control) except for *B. pilosa*, which did not differ from the controls in the number of live parasitoids. Analysis of variance indicated that the treatment/plant species significantly influenced the number of mummies observed on bean plants in the cages (ANOVA: $F_{5,24} = 11.131$; $p < 0.0001$) (Figure 6). Among the plant species *B. pilosa* supported the highest number of *A. colemani* parasitoids (3.40 per cage), which was not different from water and 10% sucrose. *T. minuta* was the least effective in supporting live parasitoids, which was not different from *G. parviflora*. In supporting aphid parasitization *B. pilosa* cages exhibited the highest number of mummies (8.28 per cage) with least number in *T. minuta* cages compared to 10% sucrose and water (Figure 6).

**TABLE 6** Mean abundance of emerged parasitoids from lablab bean sentinel plants planted as either a monocrop or intercrop (with maize) where field margin vegetation was either present or absent around the field plot.

| Field margin vegetation | Cropping system | Crop stage | Parasitoids emerged |
|---|---|---|---|
| Absent | Intercrop | Vegetative | 0.290 bcd |
| | | Flowering | 0.089 fg |
| | | Podding | 0.081 g |
| | Monocrop | Vegetative | 0.266 cde |
| | | Flowering | 0.161 defg |
| | | Podding | 0.121 efg |
| Present | Intercrop | Vegetative | 0.484 a |
| | | Flowering | 0.315 bc |
| | | Podding | 0.234 cdef |
| | Monocrop | Vegetative | 0.427 ab |
| | | Flowering | 0.274 cd |
| | | Podding | 0.508 a |

*Note*: Values in the same column followed by the same letter are not different from each other at the 95% confidence interval using the Tukey's Honestly Significant Difference (HSD) test.

## 4 | DISCUSSION

Our study demonstrated some beneficial effects of field margin vegetation on natural enemies with reduced aphid infestation in lablab field plots and higher yield. The diversity and abundance of natural enemies were, however, limited to a few specific taxa. The strongest positive effect was seen for Braconidae, which are likely to be highly important in aphid control on lablab. Intercropping appeared to provide no benefit for pest management of lablab in relation to promoting natural enemy populations. Through tracking fluorescent dye applied to margin flowers, we found evidence of various natural enemies moving from margins into the lablab crop, indicating that the presence of margin can provide resources for natural enemies, which spillover into the crop. However, in cage trials we saw unclear benefits of provision of non-crop nectar resources in supporting parasitoid performance. The observations can be related to the foraging behaviour of the parasitoids, which varied with respect to supplemental food sources given (Abd El-Kareim et al., 2007; Harvey & Fortuna, 2012; Kehrli & Bacher, 2008). In other studies, Wäckers and Fadamiro (2005) and Winkler et al. (2006) reported also that several parasitoids required a floral nectar source during their adult stages. Lablab is a neglected crop in terms of pest management studies, but is growing in importance as a 'future crop' (Minde et al., 2021). Therefore, understanding the natural enemy interactions within and around it is useful in developing sustainable agricultural intensification strategies. The grain yield was greatly impacted by the presence of field margin vegetation around cropping field. This difference in yield could be attributed to margin vegetation facilitation natural enemies abundance and low number of insect pests (Middleton et al., 2021; Mkenda et al., 2015).

In this study we showed that presence of plant-rich field margins positively influenced Braconidae abundance. This finding is in agreement with the 'Enemy Hypothesis', which predicts a positive correlation between plant species richness and natural enemy abundance (Bianchi et al., 2006; Root, 1973; Zhao et al., 2013). However, as these were station trials run over a single season, there was limited time for populations to build up and the founder populations would have been influenced by the composition of the wider landscape; on permanent smallholder fields the importance of non-crop habitat may be greater and affect more taxa this accords with studies such as (Mkenda et al., 2019; Mwani et al., 2021).

Understanding the pattern of natural enemies' movement into crops is critical in developing conservation biocontrol strategies (Costamagna et al., 2015; Schellhorn et al., 2014). This study observed visitation by natural enemies to the flowers of specific margin species before moving into the crop. The presence of florescent dye traces confirmed that natural enemies that could help protect the crop had previously visited margin plant species. This indicates that the natural enemies use the margin vegetation, and specifically the flowers, for enhanced support. *Bidens pilosa* and *Galinsoga parviflora* were the most visited plant species by the natural enemies. These plant species possess open flowers with highly accessible pollen and nectar, brightly coloured petals, which are known to attract numerous insect families (Amaral et al., 2013; Kishinevsky et al., 2017). The finding is in line with results of the cage experiment where *B. pilosa* and *G. parviflora* supported emergence of high number of live parasitoids. Presence of nectar and pollen in these plants have been documented to significantly increase the activity, longevity and fecundity of predators and parasitoids (Bianchi & Wäckers, 2008; Ndakidemi et al., 2022; Parajulee et al., 1994). Conversely, *Tagetes minuta* showed little evidence of use by natural enemies captured from the crop, in contrast to other studies recording beneficial insect interactions with this plant (Souza et al., 2019). Most families captured in our study were in similar insect orders to those captured in other researcher studies and included: Hymenoptera, Diptera, Coleoptera,

Heteroptera and Neuroptera as these taxa are considered to have greater species richness of natural enemies (Vattala et al., 2006).

Other studies from both tropical and temperate regions report increased abundance of a range of natural enemies such as parasitoids and spiders in non-crop land, adjacent vegetation or shelterbelts or farm holdings in agricultural farmlands (Perović et al., 2010; Schmidt et al., 2003). However, in this study field margin had a significant impact on a limited number of families such as Braconidae and Nabidae (Figure 1). This observation can be attributed to the fact that most of the insect families are highly mobile and experimental plots were relatively close to each other, meaning this observation might be most pronounced in the less mobile groups where immediate surroundings are most important. Plants with floral resources such as *Ageratum conyzoides* (a plant that is introduced to East Africa but very widespread on farmland), which grow as weeds around farmlands have been documented to host several beneficial insects, which can freely move into crops to suppress pests (Amaral et al., 2013; Amoabeng et al., 2020; Mkenda et al., 2019). In many cases, this is due to their provision of nectar and pollen, including outside the crop flowering period.

In our study, *Bidens pilosa*, a plant, which possesses easily accessible flowers with bright yellow centers, was the most preferred non-crop plant. The largest number of natural enemy individuals were observed to bear dye originating from *B. pilosa* compared to other margin plants; in addition, dye traces were found across a large number of insect families. *Galinsoga parviflora* was by also highly visited by natural enemies in the field; these plants show similar growth characteristics and stature (common, widely/naturally occurring, low growing with generalized flowers) around farmlands in Kenya.

In a review of intercropping verses monocropping systems, reduced abundance of insect herbivores have been reported in intercrop systems, with higher yield from the mixture compared to monocrops (Malézieux et al., 2009). In the present study, the cropping system showed some influence on natural enemy diversity and abundance, but contrary to expectations the lablab monocrop
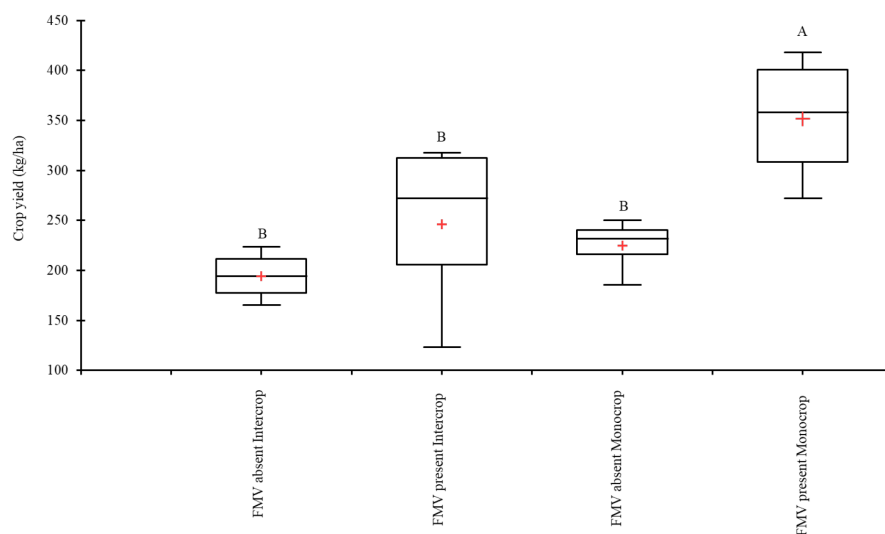


**FIGURE 5** Lablab grain yield from lablab monocrop and intercrop (with maize) when cropped with and without field margin vegetation. Letters above each box plot are from a Tukey's Honestly Significant Difference (HSD) test showing differences in mean values at the 95% confidence interval.
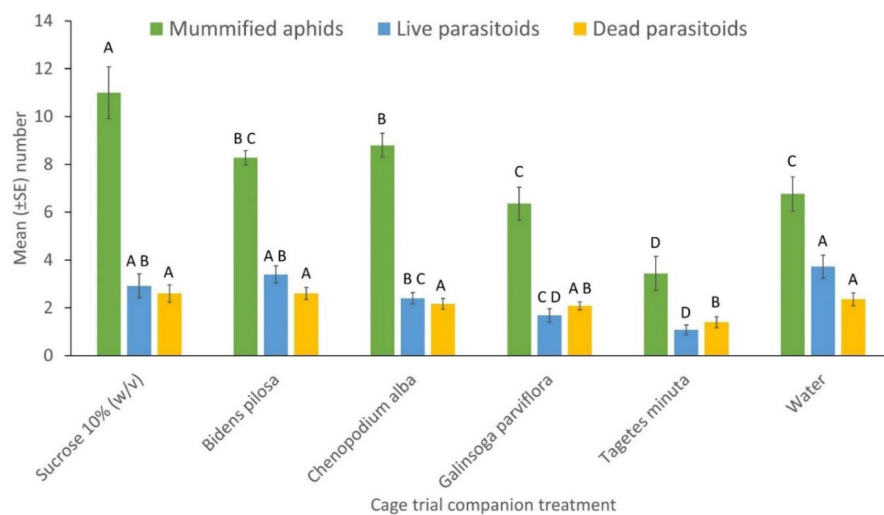
**FIGURE 6** Field margin plant species on supporting *Aphidius colemani* emergence. Bars represent the total number of mummies, live and dead parasitoids counted throughout the study period. Columns bearing the same letters are not significantly different using Tukey's Honestly Significant Difference (HSD) at ($p < 0.05$).

mostly saw more natural enemies. This could have been enhanced by the ease with which natural enemies use olfactory, visual and acoustic cues to locate the bean aphids in lablab monocrop, or the homogeneity assisting with movement through the plots. The low natural enemy occurrence in the intercrop could be associated with the 'Resource Concentration Hypothesis' (Root, 1973), which hypothesizes that 'the probability of herbivores finding their host plant, remaining on that plant, and reproducing on it is higher in monocultures than in mixtures of several species, where the resource is diluted among other resources'.

Field margins established close to crops can enable more rapid arrival of natural enemies as nearby reservoirs for fast movement into the crop. The study findings showed that fluctuations in the populations of natural enemies is a function of time, this was influenced and reflected in the crop stage and season progression. Braconidae species were captured most in plots with no margin; one possibility is that, as small and light insects, they travelled from afar and arrived late compared with some of the larger and heavier insects. Colonization and population build-ups were high at the beginning of the season (vegetation stage) as the natural enemies came into the ecosystem. However, populations reduced as resources started declining at flowering and were very high at podding stage as resources increased exponentially. Although phenology and food quality of host may significantly influence the population development of the natural enemies, the general observation was that the population control and maintenance is a combined effect of biotic and abiotic factors. At the flowering stage of the crop, which coincided with the flowering stage of the field margins; the natural enemy visits reduced into the crop and most stayed at the margins. This could be associated with high availability of food resources such as nectar and pollen in the margin plants and reduced pest prey inside the crop because of increased competition for the available resources (Lu et al., 2014). Synchronization of the life cycle and environmental requirements of the pests and natural enemies is, therefore, important in determining an effective and sustainable biological control.

While the station trials showed that natural enemies were using the weedy margin species, our cage trials did not show much

evidence of benefit of companion plants in supporting parasitoids. None of the plants outperformed where only water and aphid on sentinel plants was provided. *Tagetes minuta* was the worst performing treatment overall in terms of supporting parasitoid performance. The finding that *T. minuta* was a poor companion plant as well as being infrequently used in the field implies that in Kenyan lablab systems this plant should not be prioritized as a margin plant (Amoabeng et al., 2020; Kawuki et al., 2005).

Our cage trial results indicate that perhaps the parasitoids in this experiment were not primarily limited by access to food/energetic resources (either because they were able to feed on aphid honeydew as a sugar energy source, or perhaps because the crop itself provides sufficient nectar; likely via extrafloral nectaries) (Marazzi et al., 2019). Benefits in the field may be more nuanced, such as shelter from weather conditions or refugia during non-cropping periods. One consideration is that some crops can, themselves, be beneficial resources for natural enemies during their flowering period. With a limited flowering period other resources (i.e. long-flowering wild plants) may be required outside this time in order to ensure sustainable natural enemy populations year-round. Weeds and trees may play a particular role, especially in regions where the growing season is interrupted by either cold or dry periods.

Lablab is an orphan crop legume with high potential to support food security in Africa under the threat of climate change. However, it is understudied in terms of its role in a sustainable agricultural system and particularly in terms of pest management. This study demonstrated that plant diverse field margins have the potential to increase natural enemy abundance on this crop, particularly of parasitoids. Natural enemies in the field use field margin plants before moving into the crop. Conversely, maize intercrop may not assist in supporting natural enemy abundance and in fact, can be detrimental. Inclusion of field margin vegetation has potential in conservation of natural enemy diversity and abundance to promote integrated natural pest management and if adopted by smallholder can reduce overreliance of synthetic insecticides. However, the extent of individual field margin plant species' support to specific natural enemy

species requires more detailed studies in order to provide precise and locally relevant recommendations.

## AUTHOR CONTRIBUTIONS

**Janet N. Obanyi:** Data curation; formal analysis; investigation; writing – original draft. **Joshua O. Ogendo:** Data curation; investigation; project administration; supervision; writing – review and editing. **Richard M. S. Mulwa:** Data curation; investigation; methodology. **Jane G. Nyaanga:** Methodology; project administration; resources; writing – review and editing. **Erick K. Cheruiyot:** Methodology; project administration; resources; writing – review and editing. **Philip K. Bett:** Methodology; resources; writing – review and editing. **Steven R. Belmain:** Conceptualization; data curation; formal analysis; funding acquisition; methodology; resources; supervision; writing – original draft; writing – review and editing. **Sarah E. J. Arnold:** Conceptualization; data curation; formal analysis; investigation; supervision; validation; writing – review and editing. **Victoria C. Nash-Woolley:** Conceptualization; investigation; methodology; validation; writing – review and editing. **Philip C. Stevenson**: Conceptualization; funding acquisition, methodology, writing – review and editing.

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## CONFLICT OF INTEREST STATEMENT

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.

## DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from https://zenodo.org/record/6963255#.YuZ9bmNBzIU. Data Citation: Obanyi et al. (2022).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.