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Top-down suppression of arthropod herbivory in intercropped maize and organic farms evidenced from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope analyses

Nickson Erick Otieno^{1,2} · James Stephen Pryke¹ · Mike Butler³ · Shayne Martin Jacobs¹

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Abstract

Maize is a globally important cereal crop and a staple in sub-Saharan Africa, where it is predominantly grown by small-scale farmers. Its production, however, is undermined by numerous herbivorous arthropods, and agrochemicals used for controlling such pests are increasingly unaffordable. Farmers therefore require cheaper, effective and environmentally sustainable pest management alternatives. This study explored the hypothesis that boosting habitat heterogeneity through organic farming and intercropping enhances arthropod predator-herbivore feeding linkages that naturally suppress herbivory across non-Bt maize fields. To test this, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope analyses were conducted to establish feeding pathways from maize, legume intercrops, and marginal vegetation, through arthropod herbivores and predators across 15 small-scale maize fields in western Kenya. Farming and cropping systems' roles in trophic linkages were also assessed. Feeding connections and plant food source contributions to arthropod consumer diets were subsequently determined using Bayesian mixing models, and predator relative efficiencies also evaluated. The results showed significantly stronger predator-herbivore trophic linkages within intercropped than monoculture fields, while farming system showed no effect. Herbivores showed stronger connections to crops than noncrops, suggesting higher vulnerability for crops. For predators, earwigs derived most basal carbon from maize; wasps and predatory beetles, from legumes; ants, from both maize and legumes; and spiders, from both crops and noncrops. Ants and earwigs are important in maize herbivore regulation, particularly for intercropping; wasps and predatory beetles for regulating legume herbivores; and spiders for whole-field herbivore regulation. Most studies have focused on single species at single-trophic levels, but here we demonstrate, for the first time in sub-Saharan Africa, application of stable isotope analyses to characterize multitrophic feeding interactions that indicate effective agronomic practices for fostering top-down arthropod herbivore suppression in non-Bt maize fields. The results are useful in prescribing field practices with low-impact habitat management for sustainable small-scale agriculture rather than pesticide-based arthropod herbivore control.

Keywords Ecosystem service · Food-web · Climate-smart agriculture · Sustainable development

1 Introduction

Global warming due to climate change, is expected to support a greater abundance and wider geographical distribution of herbivorous arthropods and to exert increased ecological stress on crops (Thomson et al. 2012). The problem is anticipated to be particularly serious in tropical regions where agriculture is mainly undertaken by small-scale farmers with limited economic capacity to sustain chemical-based control of cropland herbivorous arthropods (FAO 2014). In Africa, this will cause further crop yield loss and an increase in agricultural production costs due to higher crop management expenses and these impacts will further threaten food security

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(FAO 2014). Increased agrochemical use, such as within conventional farming systems, also threatens to reduce biological diversity, which affects stable agroecosystems and sustainable farming. There is a need for more affordable but sustainable pest control strategies.

Biological control of herbivorous arthropods through habitat management practices offers one of the most sustainable solutions for low-income farmers to reduce maize yield loss from pest damage (Landis et al. 2000). Existing evidence shows that structural configuration of agricultural fields has significant influence on corresponding compositional and functional assemblages as well as the distribution of various arthropod communities. Many predatory insects and parasitoids, in particular, provide the ecological service of herbivore suppression (Tilman et al. 1997; Wolts et al. 2012). Wolts et al. (2012) observed that structurally more complex habitats or heterogeneous mosaics at the crop-field spatial level, with semi-natural patches, can provide increased niche options. These can attract a wide variety of herbivorous arthropod natural enemies that utilize them as foraging, nesting, burrowing, roosting, or refuge resources. Thus, one of the benefits of intercropping cereals with legume crops is that the intercrops enhance habitat complexity, functional diversity, and trophic interconnections among arthropods (Landis et al. 2000; Wolts et al. 2012). For example, some leguminous intercrops are known to attract predatory insects that prey directly on maize-consuming arthropods such as the stem borer *Busseola fusca* Fuller (Lepidoptera, Noctuidae) (Cook et al. 2007). Furthermore, habitat heterogeneity arising from mixing crops with diverse photosynthetic pathways, including legume cover crops, may also help reduce intra-guild predation among herbivore natural enemies. Specifically, high plant diversity may provide more herbivore prey options to the intra-guild predators and thus facilitate the focus of their attention to herbivore regulation rather than to intra-guild predatory competition (Tixier et al. 2012).

However, Winqvist et al. (2011) showed that the benefits of habitat complexity for biocontrol of crop arthropod consumers are more likely to be realized if complexity exists at larger spatial scales than the crop-field level. Furthermore, according to Tilman et al. (1997), habitat management practices that change functional rather than compositional diversity are likely to have more tangible impacts on ecosystem processes such as herbivorous arthropod population turnovers. One field-scale habitat management technique that has been successfully applied in the tropics to promote natural suppression of maize-consuming arthropods is the “push-pull” method, described by Khan et al. (2001). This method involves intercropping maize with legume plants which produce volatile chemicals to “repel” *B. fusca* from the crop. Concurrently, *B. fusca* is “attracted” by some species of field-margin grasses that facilitate interference with larval development to maturity, thus reducing its net impact on maize. The “push-pull”

method may potentially be a viable strategy for also biocontrolling other arthropod pest species, and at spatial scales beyond single crop fields.

However, direct field observations, trials, and monitoring that are necessary for quantifying such trophic and biochemical linkages are often costly and only feasible for one or a small range of herbivore species. Stable isotope analysis (SIA) offers a more robust, comprehensive, and less sampling-intensive alternative for identifying trophic linkages within a wider range of herbivorous consumers, their plant food source options, and their predators across plot, field, or landscape spatial units (Post 2002; Phillips et al. 2005). When used with mixing models, SIA helps in inferring identity of food sources as well as contribution of each food source to the consumers’ diets for a specified period (Phillips et al. 2005). Consumers are typically enriched in $\delta^{15}\text{N}$ by $\sim 3.4\text{‰}$ relative to their food source, and therefore fractionation of $\delta^{15}\text{N}$ is used to determine consumers’ relative trophic level. On the other hand, fractionation of $\delta^{13}\text{C}$ causes only $\sim 1.0\text{‰}$ enrichment per trophic level. Due to such low fractionation, $\delta^{13}\text{C}$ analysis allows determination of the range of basal food resource options with different $\delta^{13}\text{C}$ signatures (Post 2002). Furthermore, $\delta^{13}\text{C}$ is instrumental in distinguishing the photosynthetic pathway of the basal plant food source (whether C_3 , C_4 or Crassulacean Acid Metabolism) because each of these categories has a distinct range of $\delta^{13}\text{C}$ values (Ostrom et al. 1997). By contrast, a consumer’s $\delta^{15}\text{N}$ signature is always higher than that of its food source, and this makes it possible to use $\delta^{15}\text{N}$ to infer consumer trophic positions. Therefore, combining the analyses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures with Bayesian statistics constitutes a robust method for identifying consumers, trophic positions, food source options, and their relative contribution to multiple consumer diets at multiple trophic scales.

Organic farming systems are typically consistent with maintenance of farm fields in structurally more complex and natural forms than conventional farming (Norton et al. 2009). However, it is not always clear how such benefits are influenced by variations in cropping systems within the “organic” framework, or the impacts to arthropod predator-prey trophic interactions. We examined how habitat management affects interactions between several maize field predatory arthropods and their herbivorous arthropod prey. Specifically, this study used $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope analyses to assess how farming systems (organic versus conventional) and cropping methods (intercropping versus monoculture) influence trophic connections between predatory and herbivorous arthropods in small-scale non-Bt maize fields. The goal of the research was to identify farming system and cropping method options that optimize trophic linkages between predatory and herbivorous arthropods to increase natural top-down suppression of potential pests. We hypothesized that higher structural habitat complexity under organic farming and intercropping would boost

the predator-herbivore feeding linkage due to enhanced prevalence of predators, and thus increase the overall suppression of potential pests in maize fields. Maize is a popular staple crop in sub-Saharan Africa with considerable strategic significance for the continent's food security. However, small-scale farmers, its main producers, are faced with numerous challenges due to losses incurred from damage by many vertebrate and invertebrate pests (Fig. 1).

2 Materials and methods

2.1 Study area and study farm selection

The study was conducted in the Kakamega County in western Kenya ($00^{\circ} 11' 09''$ – $00^{\circ} 26' 08''$ N and $34^{\circ} 44' 30''$ – $34^{\circ} 51' 26''$ E), across 15 small-scale maize fields ranging from 0.6 to 9.0 ha in extent. The fields were selected within a mid-elevation landscape characterized by a high human population (density of approximately 547 per km^2) with largely subsistence rural agriculture, involving mainly staple crops (e.g., maize, beans, potatoes, and vegetables), and livestock rearing. The bimodally distributed rainfall ranges from 1 200 to 2 000 mm annually, with long-rain and short-rain periods occurring between March–May and August–November, respectively. Out of the 15 selected study farms: three were monoculture organic, five were intercropped organic, four were

monoculture conventional, and the remaining three were intercropped conventional fields. Inter-farm distances were maintained at 500–600 m to ensure regional similarity in general abiotic characteristics, while also ensuring sampling independence by minimizing effects of interfarm dispersal of mobile arthropods.

Organic or non-chemical-based farming is practiced by approximately 10% of farmers in the study region. On the farms sampled in this study, organic farming is defined as non-use of pesticides or herbicides; application of farmyard enrichments instead of inorganic fertilizers for soil improvement; mixed or rotational cropping; multiple cover-cropping; occasional field fallowing; and maintaining live indigenous hedgerows and woodlots. Conversely, conventional or chemical-based farming entailed the use of synthetic fertilizers, pesticides, and other agrochemicals to control pests, weeds, and crop diseases; no crop rotation; monocropping and often, no uncultivated margins. Samples were collected six times between 2016 and 2017, i.e., once at each of three crop stages in the short-rain season and once at each of three crop stages in the long-rain season. The crop stages were early crop (from germination to first weeding), mid-crop (from second weeding through flowering to corn-ear formation), and at mature-crop (from corn hardening to harvesting). Four samples each of three plant types were collected per farm for isotope analyses, i.e., maize was the target C_4 plant type; legumes (intercropped with maize e.g., beans and other pulse crops) were the target

Fig. 1 Images showing comparative effects of arthropod pests on maize crops. **a** Healthy, unaffected maize. **b** A stunted maize stand due to attack by arthropod pests. **c** A maize cob destroyed by arthropod pests.



legume crop C₃ plant type, and noncrop field-margin plants (e.g., the dominant hedgerow species) were the target noncrop C₃ plant type). In the field, the plant samples were sealed in paper envelopes, labeled, and sent to the laboratory for isotope analyses.

2.2 Arthropod and plant sampling

Arthropod samples were collected using both standard sweep nets (using the standardized 100 sweeps along transects in each farm) and pitfall traps, which comprised the standard 70 mm diameter and 120-mm high plastic cups inserted upright and flush with the ground surface (Sekamatte et al. 2003). The pitfall traps were filled to one-third volume with 25% sodium chloride solution for preservation and maintenance of isotopic integrity, and conical plastic rain shields were propped above each trap. Four replicates were randomly placed along a diagonal line running across each maize field, making a total of 60 pitfall traps. Along the diagonal lines, the traps were spaced at distance intervals that depended on maize field size and they were collected after 3 days. Samples were discarded, and traps were reset if flooding occurred from heavy rainfall. Arthropods were also sampled on maize plants using a search-and-pick procedure on leaves, stems, flowers, and cobs using forceps; all maize plants along every tenth row, totaling ten rows per farm, were sampled. At trap collection, samples were transferred to airtight bags and frozen. Samples were later identified to species and morphospecies and pooled into two trophic guilds as either primary consumers (herbivores) or secondary consumers (predators). Pooled predators were further subdivided into six taxonomic groups: spiders, predatory beetles, predatory bugs, ants, wasps, and earwigs. Similarly, herbivorous arthropods were subdivided into phytophagous beetles, phytophagous bugs, caterpillars (lepidopteran larvae), and grasshoppers and crickets. The sampled plant groups were maize (C₄ plant), legume crops (C₃ crops), and noncrop marginal plants (noncrop C₃). All samples were oven-dried at 60 °C to constant mass before being ground to fine powder using a mortar and pestle. Arthropods from each taxonomic and feeding group were ground whole, a 5-mg subsample was collected for each group, and the milled samples for three replicates per farm were transferred into tinfoil capsules for isotope analyses.

2.3 Isotopic analyses

The plant and arthropod samples were analyzed at the Environmental Isotope Laboratory of the iThemba Laboratory for the Accelerator Based Sciences (LABS) in Johannesburg, South Africa, to test for signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes. The analyses were accomplished on a Flash HT Plus elemental analyzer coupled to a Delta V Advantage isotope ratio mass spectrometer by a ConFloIV

interface combusting at 1 020 °C (ThermoFisher, Bremen, Germany). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were expressed as fractions of international reference standards Vienna Pee Dee Belemnite and air, respectively (Post 2002). The difference (δ) in isotopic ratio between the sample and standards was calculated as: $\delta^X = [(R_{\text{sample}}/R_{\text{standard}} - 1)] \times 1000$; where δ^X is the parts per thousand difference (‰) between the X isotope in the sample and that in the standard; R_{sample} is the ratio of the heavier to the lighter isotope of the element carbon or nitrogen, and R_{standard} is the ratio of the heavier to the lighter isotope in the standard (Post 2002).

2.4 Data analyses

A Bayesian mixing model method was used for analysis of the stable isotope data, specifically within the *siar* v. 4 package Library in R (Erhardt and Bedhart 2013; R Core Team 2017). First, a baseline isoscape of basal food source signatures was established, as outlined by Layman et al. (2011). Subsequently, a multisource mixing model technique enabled the establishment of trophic linkages between the consumer groups and the basal isoscape, through the consumers' respective intermediary food sources or prey. This was achieved by incorporating the appropriate trophic enrichment factors. Trophic enrichment factors represent average (\pm SD) incremental turnover values by which stable isotopic signatures change from food source to consumer tissues up the food chain during the process of metabolic fractionation (Post 2002). By constructing a multi-isotope (e.g., $\delta^{15}\text{N} - \delta^{13}\text{C}$) biplot, the Bayesian mixing model technique creates an isospace polygon of the food source options for evaluating the proportion of food sources to consumer diets. The suitability of Bayesian statistics here is that when used with the mixing model procedure, it reduces additive errors in final probability inference as it combines the two steps of identification and proportion estimation for multiple food sources to consumer diets, apart from facilitating food source ranking. The trophic enrichment factors were averages that were applied for herbivorous arthropod consumers from a review by Spence and Rosenheim (2005), and for predatory arthropod consumers, we applied values from Caut et al. (2009) on a review for terrestrial arthropods. To minimize potential statistical distortions, the models were run for each trophic step separately rather than all trophic steps simultaneously (Christensen and Moore 2009). Finally, the relative contribution of maize (RCM) and the other plant food sources to the diets of consumers (herbivores and predators) were assessed (Erhardt and Bedhart 2013), allowing determination of relative importance of maize in arthropod consumer diets (Phillips et al. 2005).

The mixing models were run again to more accurately examine predator efficiencies in maize herbivore suppression, but this time, predatory arthropod taxa were the consumers, and arthropod herbivores were the food source options. We

quantified relative contributions of the range of herbivorous arthropod consumer groups to predator diets, which provided a surrogate for prey preferences by predators. This enabled ranking of maize-carbon pathways to predators and helped determine predator groups which were more beneficial to improvement of herbivore regulation. We treated effects of farming systems and cropping methods as first-order predictors and their interaction as second-order predictors for the contribution of maize to consumer's diets. This was performed using generalized linear mixed models (GLMM) in R within the *nlme* package (R Core Team 2017). We fitted the models using the binomial error distribution with the logit-link function (Bolker et al. 2009). Fixed factors were farming system and cropping method whereas farm location was used as a random factor. These models were rerun for each feeding guild separately. Therefore, from these analyses, we managed to (1) determine feeding connections along a pathway from plant food sources through herbivores to predators to evaluate relative contribution of each food source to consumer diets; (2) compare the strength of these connections between organic and conventional maize farming systems, as well as between maize intercropped with legume crops versus monoculture maize fields; and (3) rank predator groups in terms of relative potential efficiency as biocontrol agents against herbivorous consumers. Although certain predators may prey on some non-herbivore prey, in this study, we assumed that intrapredator trophic relations had negligible or inconsequential influence on the key predator-prey and herbivore-plant relations we targeted.

3 Results and discussion

3.1 Taxonomic variation in isotopic signatures

Although there was no significant difference in relative importance of maize specifically in herbivore diets ($F = 2.282$; $p = 0.131$; $df = 15$), the majority of arthropod consumers showed stronger trophic linkages to crops than to field-margin noncrops (Fig. 2). This suggests that occasional fallowing of crop fields might contribute to disruption of in-field reproductive cycles for some herbivores, or help in limiting the threshold of their population growth. Only spiders showed substantial and nearly equal proportions of diet carbon derived from both crops and noncrops, followed by phytophagous beetles, earwigs, and predatory beetles (Fig. 2). All the other groups derived less than 30% of their carbon from noncrops. These results have two possible explanations. First, for herbivorous consumers, noncrops have comparatively lower nutritional value, possibly as an evolutionary adaptation to minimize herbivory upon themselves (Jander and Howe 2008). As a consequence, herbivores that fed on noncrop plants may also have a correspondingly lower nutritional

appeal to predators. Therefore, such semi-natural areas are probably important to non-spider predators primarily as refuges or breeding grounds, not as foraging grounds. This finding concurs with observations by Gaigher et al. (2015) and Cotes et al. (2018) who reported the occurrence of parasitoids, in particular, at higher abundances and diversities in remnant vegetation surrounding agricultural field crops. Alternatively, such non-spider predators fed predominantly on crop-borne herbivore prey because these prey occur on crops at higher densities, thus easier to locate.

Spiders, on the other hand, are mobile generalist feeders. Their generalist nature (i.e., access to crop food items is not fundamental) was demonstrated by a bet-hedging strategy, which is often characterized by them inhabiting multihabitat edges (Clough et al. 2005), and by deriving carbon from both crops and noncrops. For example, Schmidt et al. (2005) reported enhanced spider densities within a plot containing a mixture of crop and noncrop plants; and noted that their ability to shuttle between the two habitat types may make them efficient at controlling aphid abundance across whole-field or whole-farm agricultural landscapes. Therefore, the ability of spiders to derive basal food source carbon from both crops and noncrops suggests the importance of using semi-natural field margins and hedgerows to facilitate herbivore suppression by these predators in both crop fields and semi-natural field edges (Schmidt et al. 2005; Cotes et al. 2018).

The contribution of maize to diets differed significantly for predators ($F = 0.14$; $p = 0.041$, $df = 230$). Earwigs (Dermaptera) had the highest proportion of maize carbon in their diets followed by ants, which however, incorporated a comparatively higher proportion of carbon from legumes; whereas predatory beetles (Coleoptera) had the lowest trophic linkage to maize (Table 1; Fig. 2). Earwigs' strong trophic linkage to maize as a basal carbon source shows that they are beneficial contributors to regulation of maize herbivore consumers. In the case of ants, apart from deriving more carbon from both maize and legumes, they are wide-ranging foragers that are generally ubiquitous in almost all microhabitats across the farmland, from the arboreal to the ground surface and subterranean. Thus, they are able to exploit prey resources in all these microhabitats (Landis et al. 2000). Ants are also active throughout maize and legume crop growth stages at typically higher densities than are earwigs. In addition, they are highly mobile, and thus potentially more efficient at reducing herbivore prey densities over wider areas than the less-mobile earwigs (Landis et al. 2000). Earwigs are therefore potentially less suitable in multi-crop protection against herbivory when compared with ants, a fact that may be justified in four other ways. First, as shown above, earwigs rely more on maize carbon and only marginally on legumes or other plant types. Second, being generalist feeders with some species being omnivorous (Dib et al. 2011), earwigs may be less significant as natural enemies even against maize

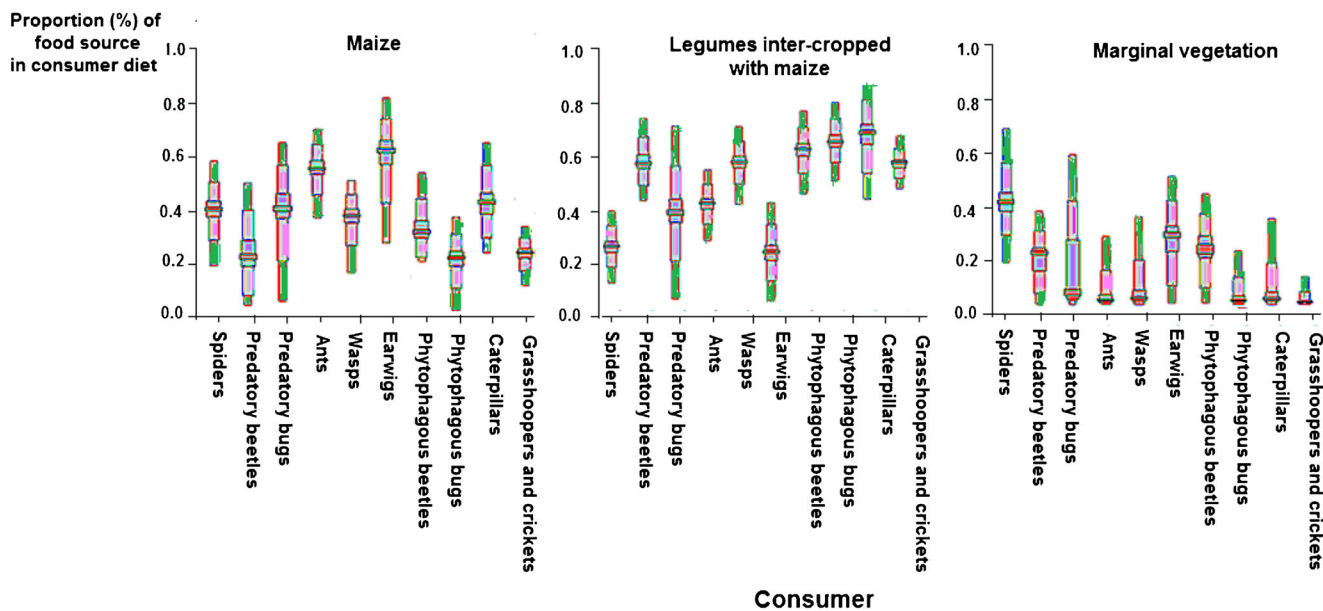


Fig. 2 Proportion of the three plant food sources in the diets of predatory and herbivorous consumers. The plot bars indicate 5% (green bands), 25% (pink bands), 75 % (blue bands), and 95% (red bands) confidence estimates for the percent contributions to consumer diets.

herbivores as compared with other non-omnivorous predators. Third, the high maize carbon in earwig tissue might emanate from the contribution of decomposing soil-borne maize residues to their diets. This is because earwigs are also known to be supplemental detritus feeders and may derive maize carbon without direct contact with maize herbivores. Finally, earwigs, unlike ants or spiders, have more localized distributions and are not abundant during all maize growth stages. They may be absent at some critical stages when the crop is at its most vulnerable to arthropod herbivory.

Compared to maize, legume crop carbon was most strongly linked to wasps (Hymenoptera) as predators, followed by predatory beetles (Coleoptera, dominated by ground beetles

and ladybirds); and ants; whereas earwigs had the weakest predatory linkage to legume crops (Fig. 2). However, wasps also incorporated maize carbon in their diet, although their trophic linkage to maize was weaker than that of ants, and only stronger than that of predatory bugs (Fig. 2). Therefore, based on the relative proportion of food source carbon in their diets, wasps and predatory beetles would be the efficient agents in suppressing arthropod consumers of legume crops.

Although caterpillars (Lepidoptera) and phytophagous beetles incorporated the highest proportion of maize carbon of all the herbivores, they generally showed a preference for legumes rather than maize crop as a food source. Notably, caterpillars incorporated the highest combined proportion of

Table 1 Ranking of herbivorous and predatory arthropod consumers across farming systems and cropping methods based on relative contribution of maize (RCM) to their diets. Relative contribution of maize is determined as the proportion contribution of maize out of all three food source options

| Consumers | Organic farming RCM | Conventional farming RCM | Intercropping RCM | Maize monoculture RCM | Mean RCM |
|-------------------------------|---------------------|--------------------------|-------------------|-----------------------|----------|
| Spiders (P) | 0.42 | 0.34 | 0.35 | 0.29 | 0.35 |
| Predatory beetles (P) | 0.33 | 0.36 | 0.32 | 0.31 | 0.33 |
| Predatory bugs (P) | 0.40 | 0.34 | 0.40 | 0.36 | 0.38 |
| Ants (P) | 0.46 | 0.42 | 0.46 | 0.36 | 0.43 |
| Wasps (P) | 0.34 | 0.36 | 0.38 | 0.43 | 0.38 |
| Earwigs (P) | 0.38 | 0.60 | 0.42 | 0.38 | 0.43 |
| Phytophagous beetles (H) | 0.22 | 0.08 | 0.18 | 0.34 | 0.21 |
| Phytophagous bugs (H) | 0.32 | 0.40 | 0.22 | 0.32 | 0.32 |
| Caterpillars (H) | 0.22 | 0.46 | 0.32 | 0.42 | 0.36 |
| Grasshoppers and Crickets (H) | 0.28 | 0.36 | 0.32 | 0.32 | 0.31 |

P, predator; H, herbivore

carbon from both maize and legumes (Table 1, Fig. 2), whereas phytophagous beetles were the only herbivores to derive any significant amount of carbon from noncrop marginal zones (Fig. 2). These results have two implications. First, although caterpillars had the highest trophic connection to maize of all herbivores, by nevertheless deriving proportionately more carbon from legumes than from maize, they appear to pose a lower damage risk to maize than to legumes. Second, the stronger inclination of all the herbivore groups towards a diet of legume plants than towards maize itself (Fig. 2) suggests that intercropping maize with legumes may buffer maize against damage by such consumers. Cook et al. (2007) demonstrated that intercropping maize with some *Desmodium* sp varieties considerably reduces infestation of maize by the stem borer *B. fusca*. Similarly, a review of many studies by Sekamatta et al. (2003) showed that intercropping maize with *Phaseolus* beans, soybeans, or groundnuts can reduce maize damage by termites (Latreille, Blattodea) and even increase yields in the maize crop.

3.2 Trophic connections in response to different farming and cropping systems

Predators showed significantly stronger feeding connections to maize food sources in intercropped fields than in monocultures ($\chi^2 = 1.25$; $p = 0.021$), whereas herbivores were dietically more inclined to consume maize in monocultures ($\chi^2 = 2.48$; $p = 0.013$) (Table 2). However, consumer feeding connection to maize did not significantly differ for either guild between organic and conventional farming systems. Similarly, there was only minor evidence to suggest that intercropped organic farming may promote enhanced predator-herbivore trophic connections over the monoculture conventional alternative (Table 2). This suggests that intercropping maize with legume crops may be more important than organic farming alone for promoting predator-prey trophic linkages. This may partly owe to the fact that legumes typically have a more ground-cover growth form, which provides enhanced structural complexity attractive to many arthropod natural enemies including ants and ground beetles. By contrast, monoculture

maize fields offer a structurally simpler, homogenous habitat with less ground cover (Norton et al. 2009) that may serve to promote feeding linkages between maize and potential pest herbivores. The results further showed that contributions of herbivore prey to predator diets were neither influenced by farming system ($\chi^2 = 2.125$; $p = 0.027$) nor by cropping method ($\chi^2 = 0.124$; $p = 0.124$) suggesting that although such habitat management methods considerably influence predator-prey and herbivore-crop trophic connections as was demonstrated earlier on, they have limited effect on actual herbivory reduction rates.

3.3 Relative roles of predator groups in herbivore regulation

Tests linking plant food source carbon to predators from their herbivorous prey options showed that spiders incorporated plant carbon from every herbivore group except phytophagous beetles, whereas predatory beetles received carbon mainly from phytophagous beetles and lepidopteran consumers of legumes (Figs. 2 and 3). Similarly, ants derived carbon from four herbivorous prey groups but almost none from phytophagous bugs. The limitation of spiders in regulating phytophagous beetles is unclear. However, the diminished feeding linkage of ants to phytophagous bugs can be explained by the often-reported ant-aphid symbiotic relationship. In the relationships, ants derive honeydew produced by the bugs and in exchange protect the bugs from other predators (Buckley 1991). Therefore, biocontrol measures for reducing the impact of aphids across maize fields should neither incorporate ants nor prioritize conservation of predatory beetles, especially ladybirds, because these also showed limited potential impact on aphids. This is supported by observations from studies in a citrus grove by Pinol et al. (2009). Kindlmann et al. (2005) added that inefficiency of ladybirds to control aphids can be attributed to the generation time ratio hypothesis which states that long-lived predatory arthropods such as coccinellids are poor regulators for short-lived prey such as leaf aphids. Similarly, phytophagous bugs were less important prey to wasps despite no symbiotic linkage between the two groups.

Table 2 Generalized linear mixed model test results of the effect of farming systems and cropping methods on relative contribution of maize (RCM) to arthropod consumer diets. The significant value for the

test was $p < 0.05$ and significant values are shown in italic. Contrasted farming mix scenario refers to intercropped organic (non-chemical-based) versus monoculture conventional (chemical-based) farms

| Consumer type | Variables (Farming type) | Coefficient estimate | df | <i>p</i> |
|---------------|----------------------------------|----------------------|----|--------------|
| Predators | Farming system | 0.390 | 4 | 0.204 |
| | Cropping method | 2.113 | 4 | <i>0.013</i> |
| | Contrasted farming mix scenarios | - 0.066 | 8 | 0.424 |
| Herbivores | Farming system | 0.103 | 4 | 0.322 |
| | Cropping method | 1.981 | 4 | <i>0.021</i> |
| | Contrasted farming mix scenarios | 0.294 | 4 | 0.184 |

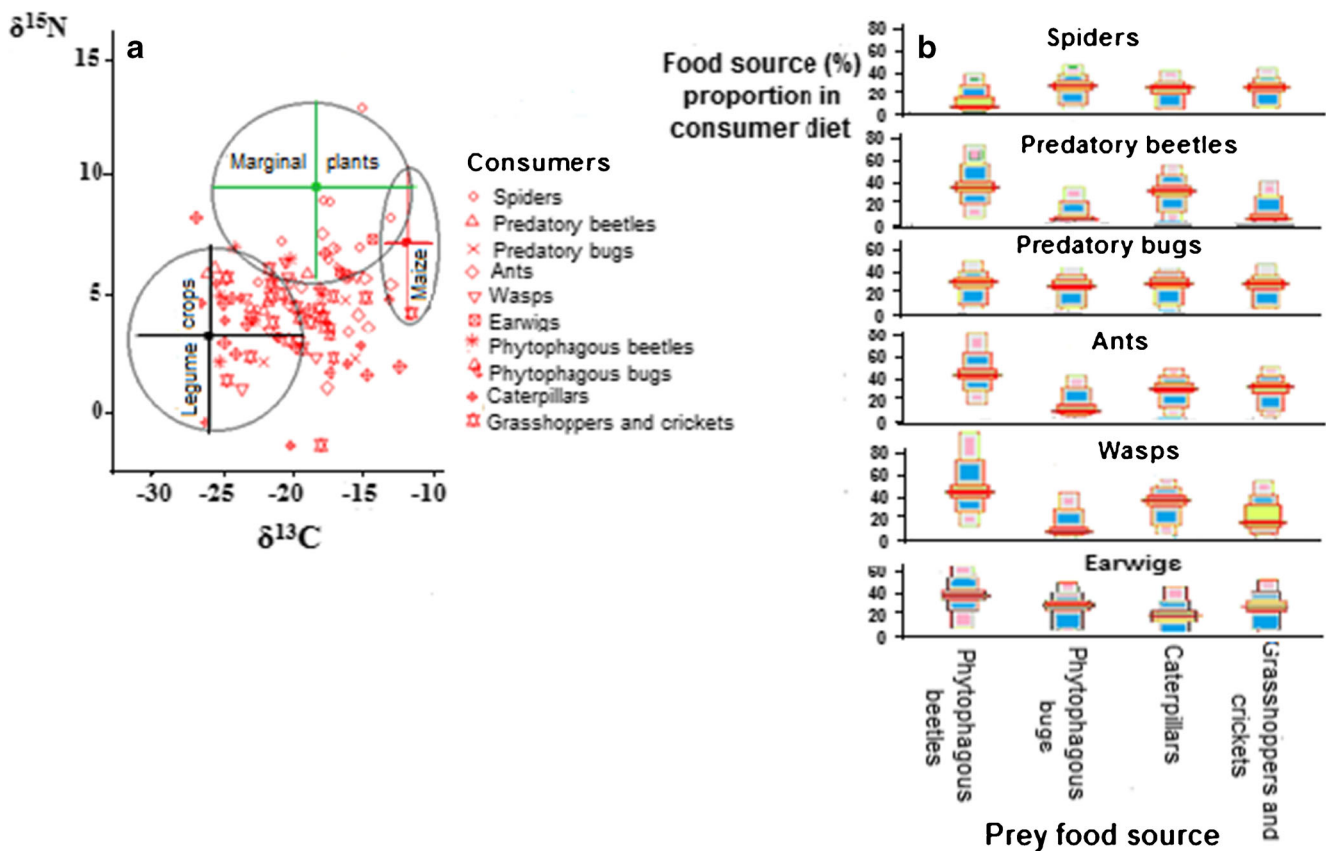


Fig. 3 **a** Biplot of mean \pm standard deviations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures in herbivorous and predatory arthropod consumers. **b** Proportion by source plot of relative contribution of herbivore groups to

predatory arthropod diets. The plot bars indicate 5% (pink bands), 25% (blue bands), 75% (yellow bands), and 95% (red bands) confidence estimates for the percent contributions to consumer diets.

Nevertheless, phytophagous bugs significantly mediated carbon transfer to spiders, predatory bugs, and earwigs, though the latter derived more of its carbon from phytophagous beetles and only approximately 10% from caterpillars (Fig. 3). Given that caterpillars were the most prominent herbivorous maize consumers (Fig. 2), this finding, therefore, supports our earlier suggestion that earwigs are potentially less efficient than ants at reducing damage to maize from herbivory. The only predators incorporating maize carbon from all the herbivorous consumer groups were predatory bugs; they derived carbon through each of the four herbivore groups though they achieved this in much smaller proportions when compared with the other predators (Fig. 3). In this regard, predatory bugs would therefore be important for maintaining a general baseline top-down regulation of multiple herbivores, albeit at quantitatively small scales, to compliment the role of the other major predator groups such as spiders and ants.

Overall, field management strategies aimed at minimizing impact of herbivores on maize should focus on conservation of earwigs and ants as they had the largest proportion of maize carbon, whereas reducing herbivory on legume crops alone should involve boosting the presence of wasps and predatory beetles. Minimizing overall crop damage by caterpillars alone should prioritize efforts to retain spiders, predatory bugs, and

ants, because these predators each incorporated substantial caterpillar-mediated carbon in their diets. At the plot level, the best results would be achieved by adopting the practice of intercropping maize with any variety of legume crops. Farmwide, however, suppression of both crop and noncrop herbivores should target agronomic practices that enhance presence of spiders, predatory beetles, and predatory bugs. Therefore, this study has illustrated, for the first time in a rural sub-Saharan Africa setting, how stable isotope analysis can be applied as a technique for systematically characterizing feeding linkages between predatory arthropods, herbivores and their crop food sources, and the consequent implications for reduction of arthropod crop damage.

4 Conclusion

The results of this study have demonstrated four main points. First, planting maize with intercrops such as legumes, rather than as a monoculture, may potentially facilitate reduction in arthropod herbivory on both crops by enhancing trophic connections between arthropod herbivores and their predatory natural enemies. Second, most predatory groups showed a preference or affinity for herbivorous prey that feed on crops rather than on

noncrops; this helps narrow down the range of habitat management options needed to optimize arthropod herbivore regulation. Third, the overall effectiveness of pest suppression in maize fields would require management techniques aiming to promote the presence of multiple species of natural enemies rather than any single species or taxonomic group. This is because the most sustainable measure for herbivorous arthropod biocontrol depends on the collective role of predatory agents, particularly to prevent pest outbreaks (Wolts et al. 2012). Fourth, we have used stable isotope analyses to determine strengths of predatory arthropod feeding linkages to the various herbivore groups, as well as ranked potential predators for relative biocontrol efficiencies at different functional or spatial scales. Therefore, through stable isotope analyses, the present study has provided evidence that multiple cropping in maize fields can improve feeding interactions between arthropod predators and their herbivore prey, and this can potentially enhance suppression of arthropod herbivory, particularly if a diverse array of predators are maintained. The strategy constitutes a cost-effective approach that is potentially significant in integrated pest management for sustainable maize production.

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Data availability The datasets generated during and/or analysed during the current study are available in the FigShare repository [10.6084/m9.figshare.8294861].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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