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Push-pull cropping system soil legacies enhance glucosinolate production and subsequent defense against Diamondback moth (*Plutella xylostella*) larvae in Kale (*Brassica oleracea*)

Borden Opio^{1,2}, Daniel M. Mutyambai^{1,3}, Xavier Cheseto¹, Evanson R. Omuse^{1,4}, Abdul A. Jalloh¹, Naomi N. Mumo², Meredith Christine Schuman^{5,6} and Frank Chidawanyika^{1,7*}

*Correspondence:

Frank Chidawanyika
fchidawanyika@icipe.org
¹International Centre of Insect Physiology and Ecology (ICIPE), P.O. Box 30772-00100, Nairobi, Kenya
²Department of Horticulture and Food Security, Jomo Kenyatta University of Agriculture and Technology (JKUAT), P.O. Box 62000-00200, Nairobi, Kenya
³Department of Life Sciences, South-Eastern Kenya University, P.O. Box 170-90200, Kitui, Kenya
⁴Faculty of Natural and Agricultural Sciences, Department of Zoology and Entomology, University of Pretoria, Hatfield 0028, Pretoria, South Africa
⁵Department of Geography, University of Zurich, Zurich 8057, Switzerland
⁶Department of Chemistry, University of Zurich, Zurich 8057, Switzerland
⁷Department of Zoology and Entomology, University of the Free State, Bloemfontein, South Africa

Abstract

Push-pull cropping integrates repellent ‘push’ plants with attractive ‘pull’ plants to manage insect pests and other ecosystem services. Whilst field evidence showed that push-pull cropping provides protection against diamondback moth (DBM) (*Plutella xylostella*), the mechanisms remain underexplored. This study investigated the influence of soil legacies formed under the push-pull system on kale (*Brassica oleracea* var. *acephala*) defense against DBM larvae. We compared responses of DBM larvae to kale grown in medium and old push-pull-conditioned soil to those grown in control soil using behavioural assays, larval performance tests, and glucosinolate profiling. Behavioural and feeding assays revealed a significant reduction in DBM larval survival (0–24%), feeding activity (leaf area eaten: 0.31–0.68 cm²), and orientation (28–32%) on kale grown in push-pull-conditioned soil. Phytochemical analyses indicated elevated concentrations of key glucosinolates, including gluconapin, glucoiberin, sinigrin, and glucobrassicin (0.01–1.08 ng sinigrin equivalents/g) from kale grown in push-pull-conditioned soil compared to those grown in control soil (0.1–0.87 ng sinigrin equivalents/g). Notably, glucocheirolin and glucotropaeolin were uniquely detected in kale planted in old push-pull-conditioned soil. These secondary metabolites are associated with defense against insect herbivory and potential dietary benefits to humans. Our study demonstrates that soil conditioned by push-pull can enhance the kale’s defense against pest herbivory through plant-soil feedback, paving the way for the sustainable production of kales.

Keywords Agroecology, Pest resistance, Plant-soil feedback, Plant defense, Secondary metabolites, Sustainable pest management, Trophic interactions



1 Introduction

Brassicas comprise the most consumed leafy vegetable group in sub-Saharan Africa (SSA) (Wang et al. 2022). However, Brassica cultivation faces significant challenges, primarily due to infestation by diamondback moth (DBM), *Plutella xylostella* L. (Zalucki et al. 2012). *Plutella xylostella* is a widely known pest of cruciferous crops, causing substantial damage, resulting in estimated annual economic losses of approximately US\$4–5 billion globally (Furlong et al. 2013). The high fecundity and multivoltinism of DBM make it a prolific sporadic pest (Gholizadeh et al. 2009; Silva and Furlong 2012; Rahman et al. 2023). Currently, DBM management relies largely on synthetic insecticides (Jamtsho et al. 2021). However, insecticides have been associated with the development of resistant pest populations and negative environmental and health risks (Jamtsho et al. 2021). Therefore, agroecological approaches, including crop diversification such as push-pull cropping systems, offer promising alternatives. The push-pull cropping system is an innovative intercropping technique with multifaceted benefits, including pest management, soil health improvement, control of parasitic weeds such as *Striga* spp., and improved farm productivity (Khan et al. 2002; Chidawanyika et al. 2023, b, 2025; Jalloh et al. 2024). This system integrates a primary crop with repellent “push” plants such as *Desmodium* and attractant “pull” trap plants such as *Brachiaria* grass, creating a habitat that deters pests while attracting natural enemies (Mutymbai et al. 2019; Chidawanyika et al. 2023, 2025a). Initially developed for cereal crops in SSA, push-pull cropping is now being further intensified by integrating vegetables, including kale and cabbage, with improved yield and crop protection (Chidawanyika et al. 2025a, b). Through plant-soil feedback (PSF) mechanisms, this cropping system enhances soil fertility by recruiting beneficial microbes, strengthens natural defenses of plants against insect pests, and increases plant productivity, thereby promoting regional food security (Berendsen et al. 2012; Ouya et al. 2023; Waiswa et al. 2025). Push-pull cropping has also been associated with other improved soil properties, including organic content, nitrogen and phosphorous (Drinkwater et al. 2021; Ndayisaba et al. 2022).

Plant soil feedbacks are critical agroecological interactions in which plants alter soil properties, subsequently influencing the growth, health, and resilience of future plant generations (Whitaker et al. 2017; Jalloh et al. 2023; Mutymbai et al. 2024). Altering the soil microbiome through practices that support PSFs has shown potential in reducing aboveground insect pest populations and strengthening plant resistance mechanisms (Heinen et al. 2018; Mutymbai et al. 2019, 2024; Lang et al. 2024). Given the rapid evolution of insecticide resistance in DBM, sustainable alternatives such as PSF-based pest management strategies offer an environmentally benign solution (Dempsey and Klessig 2017; Mutymbai et al. 2019). This aligns with sustainable agriculture principles, ensuring long-term soil health and ecological viability for smallholder farmers in SSA.

Furthermore, PSFs stimulate the production of defense-related secondary metabolites, including volatile organic compounds (VOCs) in crops, including kales (Dempsey and Klessig 2017). Soils enriched with organic matter or conditioned by diverse cropping systems typically have elevated microbial activity, resulting in enhanced nutrient availability and the activation of hormonal signalling pathways that reinforce plant defenses (Berendsen et al. 2012; Whitaker et al. 2017; Jalloh et al. 2024). Soil bacteria, especially those in the rhizosphere, facilitate the synthesis of key plant hormones such as jasmonic acid and salicylic acid, which regulate glucosinolate biosynthesis and VOC production

(Mithen et al. 1995; Bones and Rossiter 2006). These hormones induce systemic acquired resistance (SAR), priming plants for enhanced defense against herbivores. Moreover, soil legacy effects from intercropping can suppress herbivore populations by modifying the chemical and microbiological properties of plants (Fening et al. 2020). Typically, one mechanism of PSF is the influence of soil organisms on aboveground insects, which could be mediated by glucosinolates (see Fig. 1).

Brassicas such as kale have developed complex defense mechanisms against herbivorous pests, largely mediated by synthesizing VOCs that function in pest deterrence, host signalling, and disease resistance (Sidhu 2017). One such VOCs are isothiocyanate precursors (glucosinolates), which exhibit toxic, repellent, or antifeedant properties against several herbivorous pests, including DBM (Hopkins et al. 2009; Mithen et al. 1995).

Despite field evidence of crop protection against DBM for vegetables grown under push-pull systems (Chidawanyika et al., 2025a), no studies have evaluated how soil conditioning in the system can influence kale's defense mechanisms against DBM. Understanding these belowground–aboveground interactions can inform ecologically sound pest management strategies. Therefore, this study evaluated the direct resistance of two kale varieties, *Tausi* and *Kanzira*, grown in potted soils conditioned by push-pull and monoculture cropping systems, against DBM. Specifically, we examined DBM larvae's behaviour, survival, feeding activity, and glucosinolate accumulation in kale tissues to determine how soil legacies shape plant resistance.

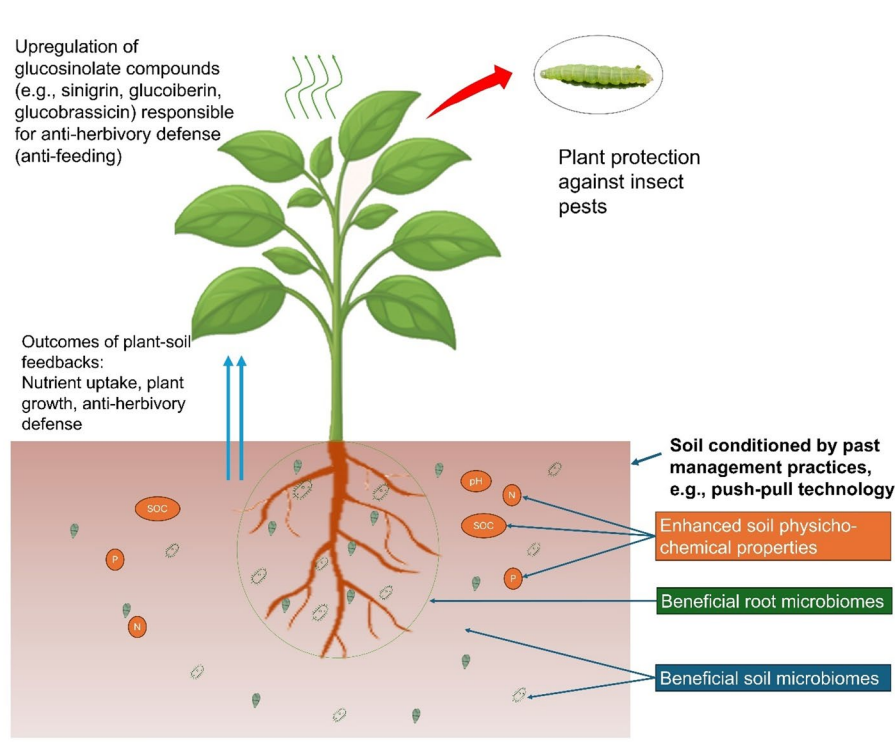


Fig. 1 Plant-soil feedback loop illustrating the influence of beneficial microbiomes and soil physicochemical properties on plant defence against aboveground insect pests when grown in soil conditioned by past management practices. Beneficial root and soil microbiomes can alter a range of host plant features, including the release of defensive chemicals such as glucosinolates

2 Materials and methods

2.1 Study site and soil collection

The study was conducted at the Thomas Odhiambo Campus, Mbita Point, Homa Bay County, Kenya (0° 25'S, 34° 12'E, 1,200 m above sea level), a field station of the International Centre of Insect Physiology and Ecology (ICIPE). Soil samples were collected from smallholder farms in five regions in western Kenya: Homa Bay, Migori, Kisumu, Vihiga, and Siaya counties (Table S1), practicing push-pull and conventional maize monoculture cropping systems that had been in place for 2 to 10 years (Mutiyambai et al. 2019; Jalloh et al. 2024). All selected smallholder farms did not use synthetic chemical inputs and practiced minimal tillage for weed control. Farms were categorized based on the duration of push-pull cropping system implementation: “old” (> 5 < 10 years) and “medium” (2–4 years). All control soils were collected from soils under maize monocropping for 5 to 10 years. The push-pull cropping and control plots measured roughly between 0.4 and 1.2 acres. Adjacent control farms growing maize alone served as control. From each farm, soil samples were collected from 10 randomly selected positions appearing at least 5 m apart to ensure representation of the entire farm. Soil from a depth of 15 cm was sampled using a soil auger during the late vegetative stage of maize, when *Desmodium* plants were fully mature and provided ground cover. The soil samples were thoroughly mixed in separate hermetic bags to form a composite sample before laboratory and greenhouse experiments. Most of the soil samples consisted of nitisols and vertisols, which are found in tropical climates with long and short rainy seasons occurring from March to May and October to December, respectively (Mutiyambai et al. 2019).

3 Source of Kale varieties and experimental design

Two varieties of kale (*Brassica oleracea* L. var. *acephala* DC) were used for this study. These varieties included *Tausi*, a tropical hybrid kale well-adapted to heat and rainfall, which was purchased from East Africa Seed Company Ltd., Nairobi, and *Kanzira*, a traditional Ethiopian mustard variety that was sourced locally from western Kenyan farmers. Both varieties are commonly grown by smallholder farmers in the western, central, and Rift Valley regions of Kenya. Pre-germinated seedlings were transplanted into 3-litre pots containing soil collected from the different push-pull and control fields. A total of 27 plants per kale variety (54 plants in total) were grown under controlled greenhouse conditions (25 °C ± 2, 65% ± 5 RH; 12 L:12D photoperiod) and watered daily with 200 mL of tap water. For the *Kanzira* variety, plants were arranged in a randomized block design (CRD) with five replicates (plants) for each soil-conditioned treatment, which constituted the first set of experiments. A similar design was adopted for the second set, which had four replications (plants) for the *Kanzira* variety. The same experimental design and number of replications were adopted for the *Tausi* variety. To avoid cross-contamination, gloves were used and regularly changed when handling soil from different farms and/or cropping systems. After three weeks of plant growth, a period sufficient for root establishment and soil adaptation and coinciding with the plant growth stage preferred for insect feeding, the plants were divided into two sets for the subsequent experiment. The first set ($n=30$ plants) was for larval feeding assays, while the second set ($n=24$ plants) was used for glucosinolate analysis.

4 Source and rearing of insects

Diamondback moth larvae were obtained from the insect mass-rearing unit at ICIPE, Mbita Point. The colony was initially established using field-collected larvae from small-holder farms in western Kenya and maintained under controlled conditions (25 ± 2 °C, $70 \pm 5\%$ RH, 12 L:12D photoperiod of natural light). Larvae were reared on a kale-based semi-synthetic diet following an established protocol (Ochieng et al. 1985; Kattagoudar et al. 2018). Adult moths were allowed to mate randomly for 24 h in mesh cages. Three-week-old *Kanzira* and *Tausi* kale plants grown in the greenhouse were provided as oviposition substrate, and larval development occurred in ventilated plastic containers ($30 \times 20 \times 15$ cm). Wet cotton wool was provided daily as a water source until pupation. Pupae were then transferred to larger oviposition cages ($60 \times 45 \times 45$ cm) before adult emergence. To maintain natural phenological and behavioural traits, the laboratory colony was periodically supplemented with field-collected insects every two to three months. The resulting second-generation insects from the laboratory-reared colony were used for all the experiments, with first-instar larvae selected for all bioassays.

5 Larval orientation, settlement, and feeding assays

To assess the preference and feeding behaviour of DBM first-instar larvae on kale plants grown in soils conditioned by push-pull and control soils, a combined orientation and feeding experiment was conducted, following previously described methodologies (Khan et al. 2007; Kemunto et al. 2022; Mutyambai et al. 2014). For orientation and settlement assays, a 3×3 cm leaf section was cut from the second-youngest leaves of three-week-old kale plants grown in each soil treatment. Two leaf discs from old push-pull and control soils versus control soils were placed on opposite sides of a 15 cm (diameter) $\times$ 1.5 cm (height) Petri dish lined with moist filter paper to maintain humidity. The same procedure was repeated with leaf discs from medium push-pull and control soils versus control soils. The adaxial side of each leaf was placed facing up. Five first-instar larvae were placed at the center of each Petri dish, which was sealed with Parafilm® and kept under controlled laboratory conditions as described above. Larval orientation and settlement were recorded after 1 and 24 h. For each soil-conditioned treatment and kale variety, the bioassays were replicated five times.

For the feeding assay, a no-choice test was used, with leaf samples (3×3 cm) placed in a 15 cm (diameter) $\times$ 1.5 cm (height) Petri dish lined with moist filter paper (Cheruiyot et al. 2018). Five DBM first-instar larvae were placed on each leaf, and the Petri dishes were sealed with Parafilm® and maintained under the same laboratory conditions. For each treatment and kale variety, five replications were prepared. After 24 and 48 h, the leaf area consumed was photographed and analyzed using ImageJ software (Schneider et al. 2012; Jalloh et al. 2023).

6 Larval survival assay

The impact of three soil-conditioned treatments and two kale varieties on DBM larval survival was evaluated under laboratory conditions. Leaf discs from 3-week-old kale plants, each with standardized dimensions as described above, were placed in 1-litre glass jars, each containing five newly hatched first-instar larvae. The lids consisted of mesh at the top for aeration. The jars' lids were reinforced with paper towels to prevent larval escape. For each soil-conditioned treatment and kale variety, five replicates were

prepared. The jars were stored at 26.5 °C (daytime), 23.5 °C (nighttime), and 65% RH to simulate semi-natural conditions. Larval mortality progress was recorded at three-day intervals up to the ninth day, prior to the expected emergence of adults.

6.1 Analysis of glucosinolate metabolites

To analyze glucosinolates, previously described methodologies by Doheny-Adams et al. (2017) and Ishida et al. (2011) were adopted. Freshly harvested three-week-old kale leaves and roots (~ 3 g each) were collected and immediately frozen in liquid nitrogen, ground into a fine powder, and stored at -80 °C. For extraction, 2 g of ground tissue was mixed with 9.95 mL of ice-cold methanol: water (80:20, v/v), vortexed for 10 s, sonicated for 10 min, and allowed to stand at room temperature for 30 min, and gently mixed at 70 rpm on a platform rocker for an additional 30 min. The mixture was then centrifuged at 4200 rpm for 10 min, and the supernatant was filtered through a 0.22 µm syringe filter (Millex GP Merck KGaA, Darmstadt, Germany) into vials and analyzed (5 µL) using Liquid Chromatography-Mass Spectrometry (LC-MS). Chromatographic separation was formed on an Agilent 1260 Infinity HPLC system (Agilent Technologies, Palo Alto, CA) coupled to an Agilent 6120 mass detector MS with a single quadrupole analyzer (Agilent Technologies, Palo Alto, CA) using a ZORBAX SB-C18, 4.6 × 250 mm, 3.5 µm column, operated at 40 °C. The elution was conducted using a gradient system with 0.01% formic acid in water (A) and acetonitrile (ACN) (B), at a flow rate of 0.7 mL/min: 0–5 min ACN, 0–20% ACN; 5–35 min, 20–95% ACN (Sasaki et al. 2012). The LC was interfaced to a quadrupole mass spectrometer operated in ESI positive mode at a mass scan range of m/z 100–2000. The dwell time for each ion was 50 ms. The mass spectrometer parameters were as follows: capillary voltage, 3.0 kV; cone voltage, 30 V; extract voltage, 5 V; RF voltage, 0.5 V; source temperature, 110 °C; nitrogen gas temperature for desolvation, 380 °C; and nitrogen gas flow for desolvation, 400 L/h. The LC-MS data were processed using Chemstation software (Agilent Technologies), and chromatograms were aligned using the R package 'Alsace' (Wehrens et al., 2015). Metabolite compounds were identified based on $[M-H]^-$ ions and characteristic adduct ions and confirmed with authentic standards when available, using sinigrin as an external calibration standard for glucosinolates. Quantification was performed based on peak areas, and concentrations were expressed as ng sinigrin equivalents/g of plant (Table S2; Figure S1) (Hooshmand and Fomsgaard 2021). Rutin (100 ng/µL in methanol) was used as a system suitability standard (Table S3, Figure S2, Figure S3). It was analysed at the beginning of the run (following the solvent and extraction blank) and reinjected after every ten samples to monitor instrument performance, verify method consistency, and ensure data quality.

6.2 Data analysis

Two-choice larval orientation and settlement data were analyzed using a generalized linear model (GLM) with treatments and assessment time as predictors for each kale variety. Further, a chi-squared test was used to determine whether the dual choice by DBM larvae (test versus control) deviates from a 50/50 split. No-choice larval feeding data were analyzed using one-way analysis of variance (ANOVA) after a square root transformation was applied to the response variable (leaf area consumed by larvae). In this analysis, treatments were fixed variables for the kale varieties. The effect of treatments on the survival of DBM larvae on each kale variety was established using a cox

model and log-rank test to analyse survival data, and the Kaplan–Meier estimator was used to generate survival curves. One-way ANOVA was used to assess the impact of soils conditioned by different cropping systems on individual glucosinolate quantifications. Pairwise comparisons were performed for significant factors using Tukey's HSD test. To visualize the impact of treatments, we performed principal component analysis (PCA) of glucosinolates in *Kanzira* and *Tausi* kale varieties. All statistical analyses were performed using R software (version 4.5.0) (R Core Team 2025).

7 Results

7.1 Orientation and settlement

Orientation and settlement of DBM larvae on leaves of the *Tausi* kale variety varied significantly among treatments ($\chi^2 = 9.41$, $df = 2$, $p = 0.009$) but not across assessment times ($\chi^2 = 0.08$, $df = 1$, $p = 0.474$), but there was significant interaction of treatment and assessment time ($\chi^2 = 7.58$, $df = 2$, $p = 0.023$). Similarly, significant variation in DBM larvae orientation and settlement on leaves of the *Kanzira* kale variety was observed among treatments ($\chi^2 = 7.83$, $df = 2$, $p < 0.019$) but not across assessment times ($\chi^2 = 0.06$, $df = 1$, $p = 0.801$), and there was no significant interaction of treatment and assessment time for *Kanzira* ($\chi^2 = 5.15$, $df = 2$, $p = 0.075$). For both kale varieties, *Kanzira* and *Tausi*, a significantly greater proportion of DBM larvae oriented toward and settled on kale plants grown in control soil compared to those grown in medium and old push-pull-conditioned soil (Fig. 2). During the first hour of observation, there was no significant difference in larval settlement between *Kanzira* plants grown in medium and old push-pull-conditioned soils, with settlement percentages ranging from 44% to 52%. However, after 24 h, the settlement of DBM larvae on both *Kanzira* and *Tausi* plants grown in medium and old push-pull-conditioned soils dropped significantly, ranging from 28% to 32%, while in control soils it remained consistently high, ranging from 68% to 72%.

7.2 Larval feeding

We observed a significant difference among treatments for both the *Kanzira* variety ($F = 42.78$, $df = 2, 12$, $p < 0.001$) and the *Tausi* variety ($F = 4.20$, $df = 2, 12$, $p = 0.041$). For *Kanzira*, the larvae consumed significantly higher leaf area on kale plants grown in control soils (1.8 cm²), followed by those grown in soils from medium push-pull (0.9 cm²), and least in old push-pull (0.3 cm²) (Fig. 3). Similarly, the leaf area of the *Tausi* variety consumed by DBM larvae was significantly higher in kale plants grown in control soils (1.5 cm²) than in those grown in soils from either old push-pull (0.7 cm²) or medium push-pull (0.7 cm²), whereas there was no difference in leaf area consumption between the medium and old push-pull treatments for *Tausi*.

7.3 Larval survival

We observed significant variations of larval survival across the treatments for both *Kanzira* variety ($\chi^2 = 23.99$, $df = 2$, $p < 0.001$) and *Tausi* variety ($\chi^2 = 8.92$, $df = 2$, $p = 0.023$). Significantly lower survival rates of DBM larvae were observed in kale planted on medium and old push-pull-conditioned soils compared to control soils for both *Kanzira* and *Tausi* kale varieties (Fig. 4a, b).

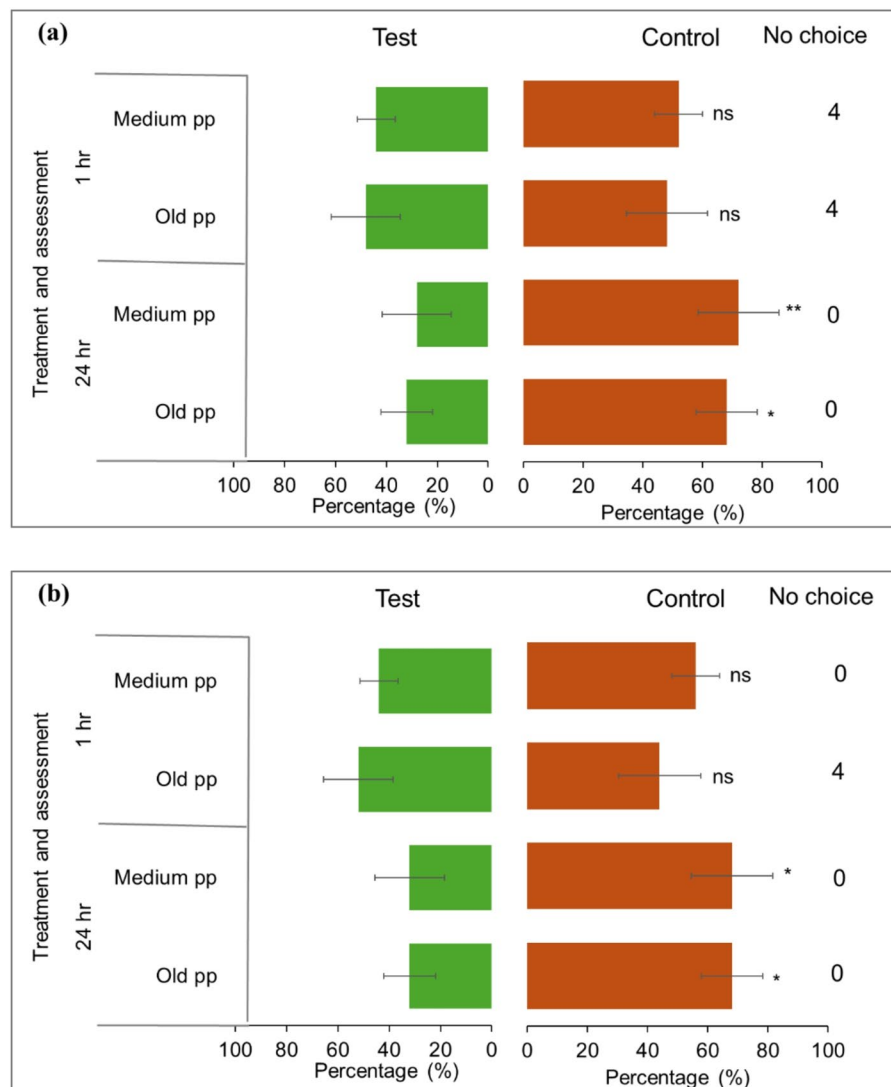


Fig. 2 Orientation and settlement of diamondback moth larvae on two kale varieties: *Kanzira* (a) and *Tausi* (b), planted in soil conditioned by medium and old push-pull cropping systems compared to the control. pp=push-pull, error bars indicate standard errors and ns=not significant. For the significant deviations, i.e., * denotes $p=0.05$, and ** denotes $p=0.001$ based on the chi-square test with $df=2$; chi-squared (χ^2) values ranged from 5.97 to 9.27

7.4 Impact of push-pull-conditioned soils on Kale glucosinolates

7.4.1 Glucosinolates in leaves and roots of *Kanzira* Kale

Kanzira kale leaves contained 11 glucosinolate compounds, classified into three main groups: aliphatic glucosinolates (such as progoitrin, sinigrin, gluconapin, glucocheirolin, glucoiberin, and epiprogoitrin), indole glucosinolates (4-hydroxyglucobrassicin, glucobrassicin, and 4-methoxyglucobrassicin), and aromatic glucosinolates (including gluconastrurtiin and sinablin) (Table 1; Figure S4). Sinigrin increased significantly from control to medium to old push-pull-conditioned soils ($F=11.60$, $df=2$, $p=0.003$). Glucoiberin was significantly higher in old push-pull-conditioned soil than in both medium push-pull-conditioned soils and control soils ($F=145.45$, $df=2$, $p<0.001$). Conversely, epiprogoitrin was significantly higher in control soil, followed by medium push-pull-conditioned soils, while undetected in old push-pull-conditioned soils ($F=14.51$, $df=2$,

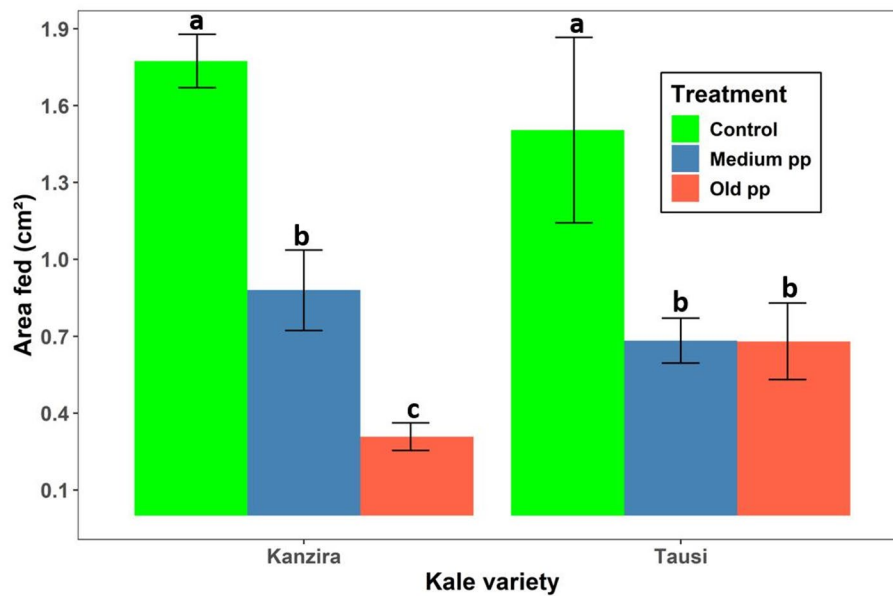


Fig. 3 Mean ($\pm$ standard error) leaf area consumed by diamondback moth larvae on kale varieties: *Kanzira* (a) and *Tausi* (b), planted in control soils and soils conditioned by medium and old push-pull (pp) cropping systems. Error bars indicate standard errors. Different small letters above the error bars indicate statistically significant differences according to Tukey's HSD test at $\alpha = 0.05$

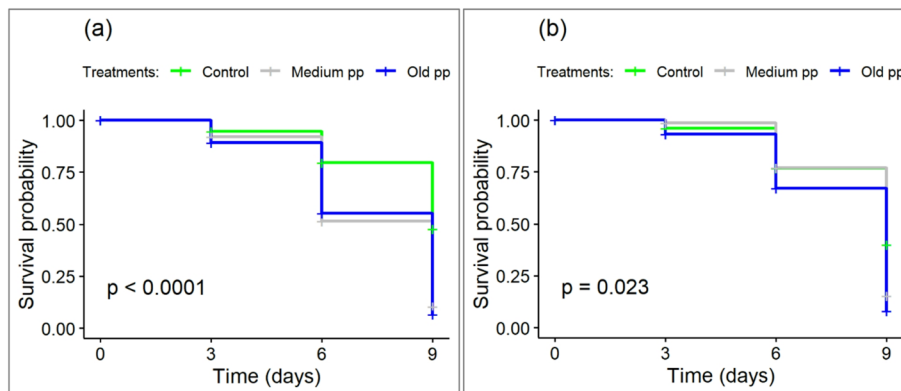


Fig. 4 Kaplan-Meier survival curves of diamondback moth (DBM) larvae fed on kale varieties; *Kanzira* (a) and *Tausi* (b), grown in control soils and soils conditioned by push-pull (pp) cropping systems. $n = 25$ DBM larvae per treatment. '+' indicates right censorship

$p = 0.002$). Glucocheirolin was uniquely found in leaves from plants grown in old push-pull-conditioned soils.

The glucosinolate composition in the roots of *Kanzira* was similar to that in the leaves, including the presence of sinigrin, progoitrin, glucotropaeolin, gluconasturtiin, gluconapin, glucocheirolin, glucobrassicin, glycolysis, 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin, and 3-methylpentylglucosinolates (Table 2; Figure S5). Among these compounds, many tended to be more concentrated in the roots of *Kanzira* plants grown in push-pull soils. 4-hydroxyglucobrassicin was significantly higher in roots from plants grown in old push-pull-conditioned soil (mean: 0.044 ng sinigrin equivalents/g) compared to those in control soils (mean: 0.035 ng sinigrin equivalents/g), with medium push-pull-conditioned soils also tending to have higher but more variable amounts.

Table 1 Glucosinolate compounds (ng sinigrin equivalents/g) in leaves of *Kanzira* Kale grown in push-pull-conditioned and control soils

Number	RT	Compounds	Soil-conditioned treatments			F-value	P-value
			Old push-pull	Medium push-pull	Control		
1	5.69	Sinablin	0.009±0.002 ^a	0.008±0.003 ^a	0.009±0.002 ^a	0.08	0.924
2	5.77	Glucoiberin	0.116±0.003 ^a	0.022±0.031 ^b	0.019±0.030 ^b	145.45	<0.001
3	6.14	Progoitrin	0.009±0.005 ^a	0.004±0.001 ^a	0.008±0.002 ^a	0.89	0.442
4	6.23	Sinigrin	1.077±0.011 ^a	0.868±0.017 ^{ab}	0.691±0.012 ^b	11.60	0.003
5	6.49	Epiprogoitrin	–	0.004±0.002 ^b	0.008±0.023 ^a	14.51	0.002
6	10.71	Gluconapin	0.010±0.004 ^a	0.009±0.003 ^a	0.010±0.002 ^a	0.002	1.00
7	16.26	4-Hydroxyglucobras-sicin	0.030±0.013 ^a	0.013±0.003 ^a	0.048±0.004 ^a	1.318	0.315
8	18.53	Glucobrassicin	0.079±0.317 ^a	0.063±0.118 ^a	0.093±0.076 ^a	1.239	0.335
9	19.05	Gluconasturtiin	0.023±0.002 ^a	0.019±0.002 ^a	0.019±0.002 ^a	1.859	0.211
10	19.50	4-Methoxyglucobras-sicin	0.157±0.029 ^a	0.073±0.001 ^b	0.169±0.002 ^a	7.974	0.010
11	24.78	Glucocheirolin	0.026±0.025	–	–	1.049	0.389

ng/g = nanogram per gram; RT = retention time. Different superscript letters within a row indicate significant differences across the different glucosinolate compounds and soil conditioning treatments ($P < 0.05$) following Tukey's honest significance test (HSD). En dash (–) indicates the compound in question could not be detected. Significant differences are denoted in bold

Table 2 Glucosinolate compounds (ng sinigrin equivalents/g) in roots of *Kanzira* Kale plants grown in push-pull-conditioned and control soils

Number	RT	Compounds	Soil-conditioned treatments			F-value	P-value
			Old push-pull	Medium push-pull	Control		
1	6.14	Progoitrin	0.005±0.002	–	–	45.79	<0.001
2	6.23	Sinigrin	0.483±0.039 ^a	0.416±0.011 ^{ab}	0.266±0.044 ^b	10.50	0.004
3	10.71	Gluconapin	0.04±0.002 ^a	0.014±0.002 ^a	0.012±0.001 ^a	3.26	0.67
4	16.26	4-Hydroxyglucobras-sicin	0.044±0.001 ^a	0.047±0.012 ^{ab}	0.035±0.009 ^b	6.61	0.017
5	18.53	Glucobrassicin	–	0.113±0.036	–	9.69	0.006
6	18.67	Glucotropaeolin	0.003±0.001	–	–	16.49	<0.001
7	19.01	3-Methylpentyl glucosinolate	0.009±0.003 ^{ab}	0.016±0.001 ^a	0.006±0.002 ^b	5.97	0.022
8	19.05	Gluconasturtiin	0.177±0.198 ^{ab}	0.299±0.036 ^a	0.092±0.035 ^b	11.17	0.004
9	19.5	4-Methoxyglucobras-sicin	0.243±0.013 ^a	0.166±0.014 ^a	0.203±0.048 ^a	1.66	0.244
10	24.78	Glucosylsin	0.012±0.002 ^a	0.014±0.003 ^a	0.009±0.002 ^a	1.30	0.319
11	24.78	Glucocheirolin	–	0.018±0.005	–	16.49	<0.001

ng/g = nanogram per gram; RT = retention time. Different superscript letters within a row indicate significant differences across the different glucosinolate compounds and soil conditioning treatments ($P < 0.05$) following Tukey's honest significance test (HSD). En dash (–) indicates the compound in question could not be detected. Significant effects are denoted in bold

Similarly, sinigrin, 3-methylpentyl glucosinolate, and gluconasturtiin all tended to be higher in roots from plants grown in push-pull-conditioned soils ($F = 5.97–11.17$, $df = 2$, $p = 0.022–0.004$). Progoitrin and glucotropaeolin were uniquely detected from the roots of plants grown in old push-pull soils. Glucocheirolin was uniquely detected in roots of plants grown in medium-push-pull conditioned soils.

7.5 Glucosinolates in leaves and roots of *tausi* Kale

The glucosinolate composition in leaves and roots of *Tausi* kale revealed distinct differences between plants grown in soils conditioned by push-pull cropping systems

(medium and old) and those grown in control soils. *Tausi* kale leaves contained eight glucosinolate compounds, which were classified into two groups: indole (4-methoxyglucobrassicin, 4-hydroxyglucobrassicin, and glucobrassicin) and aliphatic hydrocarbons (glucoiberin, sinigrin, gluconubrietin, glucoraphanin, and glucoiberin) (Table 3; Fig. S6). Among these, sinigrin and glucoiberin concentrations were significantly higher in leaves from plants grown in old push-pull-conditioned soils, followed by those in medium push-pull-conditioned soils and least in control soils ($F = 7.62$, $df = 2$, $p = 0.011$ and $F = 5.50$, $df = 2$, $p = 0.027$, respectively). 4-hydroxyglucobrassicin was highest in old push-pull-conditioned soils, followed by control soils, and lowest in medium push-pull-conditioned soils ($F = 7.69$, $df = 2$, $p = 0.011$). Glucobrassicin was detected in leaves from old push-pull-conditioned and control soils but was absent in leaves from medium-push-pull-conditioned soil. There was no clear tendency in gluconubrietin and glucoraphanin concentration across all the treatments.

Tausi roots contained eleven glucosinolate compounds comprising indole (4-methoxyglucobrassicin, 4-hydroxyglucobrassicin, and glucobrassicin) and aliphatic hydrocarbons (glucoiberin, sinigrin, glucobrassicin, glucoraphanin, sinablin, gluconatrurtin, glucoerucin, and gluconapin) (Table 4; Fig. S7). Sinigrin, glucobrassicin, 4-hydroxyglucobrassicin and 4-methoxyglucobrassicin were significantly higher in roots from plants grown in old-push-pull-conditioned soil compared to those in medium-push-pull-conditioned soil and control soils ($F = 4.74$ – 14.60 , $df = 2$, $p = 0.039$ – 0.001), while progoitrin was uniquely detected in the roots of plants grown in old-push-pull-conditioned soils, and gluconapin was detected in roots from push-pull-conditioned soils but not in roots from control soils. Conversely, glucoerucin concentrations were highest in roots from kale plants grown in control and medium push-pull-conditioned soils compared to those from old push-pull-conditioned soils ($F = 12.77$, $df = 2$, $p = 0.002$), and glucobrassicin was uniquely detected from the roots of plants grown in control soils.

7.6 Principal component analysis of glucosinolates

A PCA of the glucosinolate profiles of *Kanzira* and *Tausi* kale varieties revealed distinct clustering patterns based on soil conditioning treatments in most cases, associated with differences in glucosinolate composition across the three treatments (Fig. 5a, b, c,

Table 3 Glucosinolate compounds (ng sinigrin equivalents/g) in leaves of *Tausi* Kale grown in push-pull-conditioned and control soils

Number	RT	Compounds	Soil-conditioned treatments			F-value	P-value
			Old push-pull	Medium push-pull	Control		
1	6.14	Progoitrin	0.085 ± 0.021 ^a	0.063 ± 0.025 ^a	0.039 ± 0.013 ^a	1.25	0.332
2	6.23	Sinigrin	0.135 ± 0.031 ^a	0.085 ± 0.022 ^{ab}	0.041 ± 0.013 ^b	7.62	0.011
3	8.62	Glucoraphanin	0.171 ± 0.027 ^a	0.199 ± 0.035 ^a	0.187 ± 0.049 ^a	0.14	0.875
4	12.43	Glucoiberin	0.356 ± 0.061 ^a	0.255 ± 0.037 ^{ab}	0.181 ± 0.024 ^b	5.50	0.027
5	16.02	Glucoaubrietin	0.028 ± 0.005 ^a	0.033 ± 0.006 ^a	0.031 ± 0.008 ^a	0.18	0.849
6	16.26	4-Hydroxyglucobrassicin	0.073 ± 0.011 ^a	0.006 ± 0.001 ^c	0.039 ± 0.006 ^b	7.69	0.011
7	18.53	Glucobrassicin	0.193 ± 0.035 ^a	–	0.151 ± 0.034 ^a	8.23	0.009
8	19.5	4-Methoxyglucobrassicin	0.035 ± 0.002 ^b	0.008 ± 0.001 ^c	0.040 ± 0.003 ^a	128.31	<0.001

ng/g = nanogram per gram; RT = retention time. Different letters within a row indicate significant differences across the different glucosinolate compounds and soil conditioning treatments ($P < 0.05$) following Tukey's honest significance test (HSD). En dash (–) indicates the compound in question could not be detected. Significant effects are denoted in bold

Table 4 Glucosinolate compounds (ng sinigrin equivalents/g) in roots of *Tausi* Kale grown in push-pull-conditioned and control soils

Number	RT	Compounds	Soil-conditioned treatments			F-value	P-value
			Old push-pull	Medium push-pull	Control		
1	5.69	Sinabin	0.014±0.003 ^a	0.014±0.002 ^a	0.017±0.001 ^a	0.78	0.484
2	6.14	Progoitrin	0.078±0.011	–	–	51.62	<0.001
3	6.23	Sinigrin	0.240±0.026 ^a	0.148±0.017 ^b	0.144±0.013 ^b	11.30	0.004
4	8.62	Glucoraphanin	0.007±0.001 ^a	0.010±0.002 ^a	0.003±0.002 ^a	0.86	0.456
5	10.71	Gluconapin	0.004±0.001 ^a	0.005±0.001 ^a	–	8.79	0.008
6	16.02	Glucobertoin	–	–	0.002±0.001	8.80	0.008
7	16.7	Glucorucin	0.280±0.019 ^b	0.431±0.044 ^a	0.487±0.021 ^a	12.77	0.002
8	16.26	4-Hydroxyglucobrassicin	0.062±0.006 ^a	0.040±0.007 ^b	0.042±0.004 ^b	4.74	0.039
9	18.53	Glucobrassicin	0.039±0.005 ^a	0.024±0.003 ^b	0.026±0.002 ^b	11.66	0.003
10	19.05	Gluconasturtiin	0.107±0.022 ^a	0.114±0.006 ^a	0.114±0.013 ^a	2.09	0.179
11	19.5	4-Methoxyglucobrassicin	0.199±0.016 ^a	0.142±0.006 ^b	0.152±0.007 ^b	14.60	0.001

ng/g, nanogram per gram; RT, retention time. Different letters within a row indicate significant differences across the different glucosinolate compounds and soil conditioning treatments ($P < 0.05$) following Tukey's honest significance test (HSD). En dash (–) indicates the compound in question could not be detected. Significant effects are denoted in bold

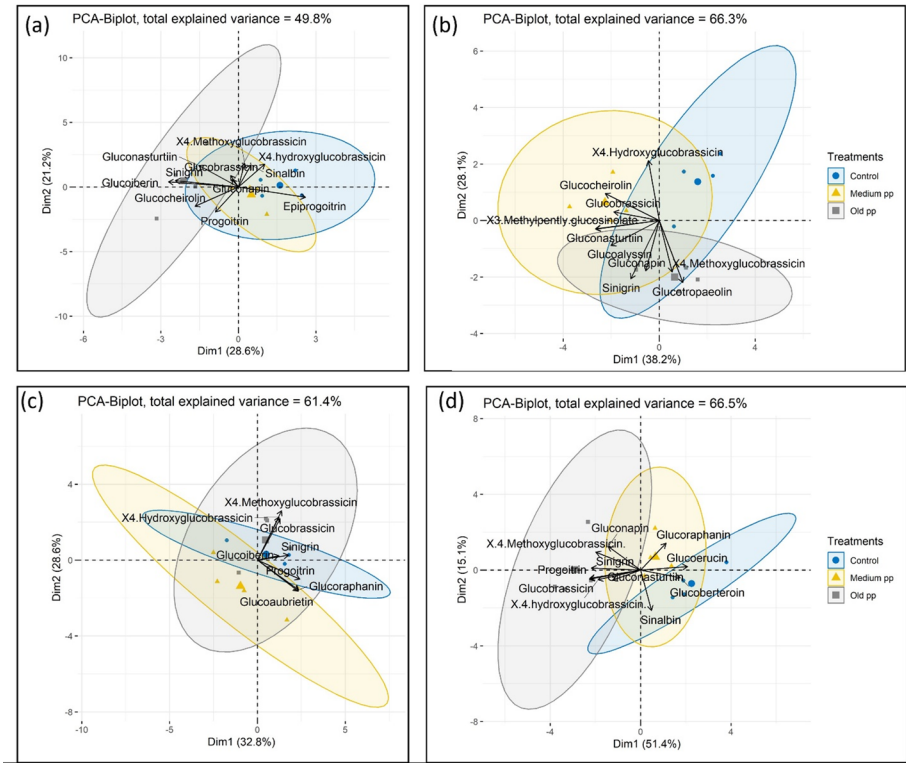


Fig. 5 Principal component biplots showing the distribution of glucosinolate compounds in *Kanzira* leaves (a), *Kanzira* roots (b), *Tausi* leaves (c), and *Tausi* roots (d) after being grown in control as well as medium and old push-pull-conditioned soils; pp=push-pull. The shaded area represents the 95% confidence interval

and d). In *Kanzira* leaves, the first two principal components, Dim1 (28.6%) and Dim2 (21.2%), explained a cumulative variation of 49.8% (Fig. 5a). Higher concentrations of gluconasturtiin, glucoiberin, glucocheirolin, and sinigrin were positively associated with leaves from plants grown in old push-pull soils. In contrast, higher concentrations of 4-hydroxyglucobrassicin, sinabin, and epiprogoitrin were associated with leaves from

control soils (Fig. 5a). In *Kanzira* roots, Dim1 (38.2%) and Dim2 (28.1%) accounted for 66.3% of the total variation (Fig. 5b). Roots from medium push-pull soils were associated with higher concentrations of glucocheirolin, glucobrassicin, 3-methylpentylglucosinolate and gluconasturtiin, whereas roots from old push-pull-conditioned soils were associated with 4-methoxyglucobrassicin, glucotropaeolin, gluconapin and sinigrin. Overall, the distributions in *Kanzira* indicate a transition in glucosinolate composition between control and old push-pull-conditioned soils, with medium push-pull soils overlapping substantially with both control and old push-pull soils; however, this transition is clearer in leaves than in roots.

For *Tausi* leaves, Dim1 (32.8%) and Dim2 (28.6%) together accounted for 61.4% of the total variance, and all three treatments showed substantial overlap (Fig. 5c). Nevertheless, leaves from old push-pull-conditioned soils were associated with higher concentrations of 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin, and glucobrassicin (Fig. 5c). *Tausi* root samples from differently conditioned soils were better separated, with PCA Dim1 (51.4%) and Dim2 (15.1%) together explaining 66.5% of the total variance (Fig. 5d). Higher concentrations of 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin, glucobrassicin, progoitrin and gluconapin were associated with roots from plants grown in old push-pull soils, while higher concentrations of glucoerucin, glucobriterion and sinabalin were associated with roots from plants grown in control soils, and roots from plants grown in medium push-pull soils were intermediate (Fig. 5d). Thus, for *Tausi*, the transition in glucosinolate composition between push-pull-conditioned and control soils was clearer in the roots.

Overall, cultivation in old push-pull-conditioned soils was consistently associated with higher concentrations of sinigrin and 4-methoxyglucobrassicin in *Kanzira* leaves and roots and with higher concentrations of glucobrassicin and 4-methoxyglucobrassicin in *Tausi* leaves and roots, with no such clear patterns defining which glucosinolates were more abundant in plants grown in control soils. This is consistent with the results of GLM on individual compounds that, however, highlight differences that were stronger or better detected among plants grown in differently conditioned soils and overall stronger differences in glucosinolate concentrations for *Tausi* kale plants grown in push-pull-conditioned soils (Tables 1, 2, 3 and 4, Figures S3-S6).

8 Discussion

Crop diversification can influence the defence of future plants via biochemical changes mediated by PSF. In the present study, soil conditioned by push-pull cropping promoted changes in the composition of key sulfur-rich defense-related glucosinolates and often was associated with higher concentrations of these compounds, which are known to play a critical role in plant defense against herbivores in the mustard family (Halkier and Gershenzon 2006; Hopkins et al. 2009; Robin et al. 2017). Upon hydrolysis by the enzyme myrosinase, these compounds produce toxic degradation products such as isothiocyanates and nitriles, which deter herbivorous pests (Winde and Wittstock 2011; Mandrich and Caputo 2020).

In this study, the tendency for kale grown in push-pull-conditioned soils to have higher glucosinolate concentrations likely contributed to reduced feeding, stunted development, and increased mortality of DBM larvae. These compounds not only impair insect development but also provide antimicrobial protection, further bolstering plant

resilience (Soler et al. 2013; Mandrich and Caputo 2020; Jia et al. 2024). Sinigrin, which acts as a feeding stimulant at moderate levels, exhibits toxicity at higher concentrations (Barve et al. 2025) and was consistently found in higher concentrations (though not always statistically significant) in kale grown in push-pull-conditioned soils. These findings align with previous studies that demonstrated the effectiveness of soil conditioned by push-pull cropping systems in managing stemborers and fall armyworm in maize plants when compared to plants grown in non-push-pull soil (Jalloh et al. 2023; Mutyambai et al. 2024). The consistency of these results across different crops underscores the broad applicability of push-pull in integrated pest management (IPM).

Varietal differences in defense inducibility in response to push-pull soil legacies were observed between the two kale varieties, especially with regard to larval feeding. The local variety, *Kanzira*, showed a more graded response to push-pull soil conditioning as well as a different detected glucosinolate composition compared to the hybrid variety, *Tausi*. Such differences in induced plant defense have been observed in different plant varieties and are often affected by domestication and breeding processes (Mutyambai et al. 2014; Whitehead et al., 2017). These differences underscore the importance of genotype selection in breeding pest-resistant cultivars for agroecological systems.

The findings of this study further emphasize the critical role of PSF in push-pull cropping systems, demonstrating their ability to bolster the defense mechanisms of kale plants. The reduced DBM infestations and feeding damage observed on kale cultivated in push-pull-conditioned soils may be associated with improved soil health and microbial diversity, which can support accumulation of glucosinolate compounds and other plant defense metabolites (Berendsen et al. 2012; Philippot et al. 2013; Siebers et al. 2018; Jalloh et al. 2024; Mutyambai et al. 2024). This study supports the notion that diversified cropping systems, such as push-pull and/or intercropping systems, positively influence plant secondary metabolism and pest resistance through enhanced soil nutrients and microbial interactions (Bakker et al. 2018; Zhang et al. 2025). While we did not directly measure soil pH, microbial biomass, or organic matter in this study, these parameters have been well-characterized in previous work on similar sites (e.g., Jalloh et al. 2024; Mutyambai et al. 2024), where push-pull soils exhibited elevated microbial diversity and functional group abundance compared to monoculture soils. Future studies should aim to integrate such edaphic variables to better isolate the microbial or chemical drivers of induced plant defenses. Also, our results support the notion that diversified cropping systems, such as push-pull and intercropping systems, positively influence plant secondary metabolism and pest resistance through enhanced soil nutrients and microbial interactions (Bakker et al. 2018; Zhang et al. 2025). In contrast, monocropping systems often lead to soil degradation, nutrient depletion, and increased pest pressures, highlighting their unsustainability in the long term (Bommarco et al. 2013; Altieri et al. 2015). These findings underscore the potential of push-pull to reduce pest pressures, enhance soil health, and strengthen plant defense mechanisms, offering a robust and sustainable pathway for agricultural resilience in pest-prone regions.

Beyond their role in pest resistance, glucosinolates such as sinigrin and gluconapin have been reported in other studies to possess antioxidant properties that modulate cellular redox balance by enhancing the activity of enzymes such as glutathione peroxidase and superoxide dismutase (Iwar et al. 2024; Maldonado et al. 2020; Becker and Juvik 2016; Luengo et al. 2018, 2020). Other glucosinolates, including benzyl glucosinolate

and glucobrassicin, have been reported as chemo-preventive agents, capable of reducing tumour growth, especially when metabolized by beneficial human gut microbiota, including *Lactobacillus plantarum* and *Lactococcus lactis* (Krul et al. 2002; Song et al. 2010; Melim et al. 2022). It is not clear if the upregulation of glucosinolates in kales grown in soils conditioned by push-pull cropping systems can also confer such human health benefits. In addition to their nutritional value, push-pull cropping systems offer long-term benefits that significantly contribute to soil health and agricultural sustainability. The intercropping of *Desmodium*, cereal crops, and *Brachiaria* grass fosters rich soil legacies by enhancing soil fertility and promoting beneficial microbial communities (Reiss and Drinkwater 2018; Dang et al. 2020). These improvements in soil quality support better plant health, leading to increased resistance against herbivorous pests and disease-causing pathogens. Notably, the higher concentrations of certain glucosinolates observed in kale grown in old push-pull-conditioned soils, and the tendency of plants grown in medium push-pull soils to be intermediate in their glucosinolate composition between those grown in control or old push-pull soils, indicate cumulative effects of these soil legacies. These results suggest that prolonged implementation of push-pull systems not only improves soil health but also enhances plant biochemical defenses, reinforcing suitability for sustainable pest management and resilient crop production.

9 Conclusion

Kale grown in push-pull-conditioned soils exhibited a significant reduction in DBM infestations and feeding activity in choice and no-choice tests, underscoring the pest-suppressive potential of this sustainable agricultural approach. By improving soil health and promoting beneficial microbial diversity, push-pull systems enhance plant-soil feedback mechanisms that bolster kale's natural defense through increased glucosinolate production. This eco-friendly approach offers a promising alternative to synthetic chemical pesticides, particularly in resource-constrained regions like East and Central Africa, where synthetic chemical inputs pose significant environmental and health risks. The increased glucosinolate levels in kale grown in push-pull-conditioned soils highlight the dual advantages of these systems, extending beyond pest management to include enhanced plant defense and potential nutritional gains. To fully harness these benefits, future studies should prioritize the development of glucosinolate-rich plant cultivars aimed at improving both resistance to herbivores and dietary value. Additionally, exploring crop diversification strategies and their impact on plant-insect-microbiome interactions could further enhance ecological and agronomic performance in these systems. It is also essential to assess the human health benefits of glucosinolate-enriched kale, particularly its antioxidant and therapeutic properties. Finally, determining the economic feasibility and scalability of push-pull systems for smallholder farmers in SSA is crucial for widespread adoption.

Supplementary Information

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Supplementary Material 1

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Author contributions

Conceptualisation: FC, DM. Data Curation: BO, FC. Formal Analysis: BO, XC, ERO, FC, AAJ. Funding Acquisition: FC. Investigation: BO, XC, FC, AAJ, DMM. Methodology: BO, AAJ, XC, DMM, MCS, FC. Project Administration: FC. Supervision: FC, DM, NM. Validation: DM, MCS, XC, FC. Visualization: BO, ERO, AAJ. Writing – Original Draft Preparation: BO, ERO, AAJ, FC. Writing – Review & Editing: All authors.

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Data availability

The datasets generated during and/or analyzed during the current study are available and archived on Zenodo repository ([<https://zenodo.org/records/17072066>]).

Declarations

Ethics approval and Consent to participate

Not applicable. This study was conducted exclusively with plant biological material (kale: *Brassica oleracea* var. *acephala*) in accordance with applicable ethical and legal guidelines. The tested kale varieties are commercially available, and applicable guidelines for their cultivation was followed. The requirement for permission to collect plant material in the study area was not applicable. All stages of the work followed current research ethics standards and guidelines, ensuring compliance with the standards required for conducting scientific activities with biological material. The plant material was not collected from any other specific areas apart from the study site; detailed information is provided in the form of coordinates in the manuscript.

Consent for publication

Not Applicable.

Plant reproducibility

Not Applicable, as the tested plant is commercially available for cultivation.

Competing Interests

The authors declare no conflict of interest, whether financial or non-financial.

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