Push-pull cropping system soil legacy alter maize metabolism and fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) resistance through tritrophic interactions

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Abstract

Background and aims: Crop cultivation practices and soil legacies are intrinsically linked and are hypothesized to influence direct and indirect plant defences against phytophagous insects. In this study, we tested how soils conditioned by push-pull (maize (*Zea mays*)-*Desmodium* spp.- *Brachiaria* spp. intercrop) or maize monoculture (non-push-pull) affect maize phytochemistry and subsequent resistance to fall armyworm (*Spodoptera frugiperda* Smith, Lepidoptera: Noctuidae). We hypothesised that conditioning soil with push-pull positively impacted maize growth, metabolism, and subsequent direct and indirect resistance to an invasive herbivore pest.

Methods: Maize was grown in soils collected from push-pull and maize monoculture fields. We compared maize growth, herbivore larval feeding, production of defense secondary metabolites on maize grown in soils conditioned by push-pull and non-push-pull cropping. As a proxy for indirect defence effects, we also measured behavioural responses of egg-larval parasitoid *Chelonus bifoveolatus* Szpligeti (Hymenoptera: Braconidae) to maize volatiles from plants planted in soils conditioned by each cropping system.

Results: Maize plants grown in soil conditioned by push-pull had a higher biomass accumulation and plant height. Higher quantities and more diverse volatile and non-volatiles metabolites were observed in maize grown in push-pull soil in comparison to those grown in maize monoculture soil. Behavioural assays showed that *S. frugiperda* neonate fed more on leaf tissue from maize plants planted in soil conditioned by maize monoculture than those planted in push-pull conditioned soil. Parasitoid wasps were more attracted to volatiles from maize planted in push-pull conditioned soils than those planted in non-push-pull soils.

Conclusion: Our results indicate that conditioning soil with polyculture push-pull enhances maize growth, alters phytochemistry and subsequent direct and indirect resistance to *S. frugiperda*.

Keywords: Ecological interactions; Fall armyworm; Maize; Push-pull cropping system; Soil legacy

Introduction

Plant-soil feedbacks encompass reciprocal multifaceted interactions among plants and their related microbiota which collectively determine nutrient availability, physical, chemical, and biological properties of soil (Klironomos 2002; Kulmatiski et al. 2008). Such interactions subsequently influence growth and resistance of plants to herbivores, pathogens, and shape plant diversity and ecosystem dynamics (Klironomos 2002). Despite their potential to enhance agricultural productivity through plant growth promotion and crop protection, these interactions have been understudied and underutilized in agroecosystems (Mariotte et al. 2018; Koyama et al. 2022). Plant-mediated mechanisms which influence nutrient accessibility, microbial communities, and organic matter composition are also known to alter plant phytochemistry, herbivore resistance and so influence the outcome of species interactions in natural ecosystems (Meyer 2000). However, studies in agroecosystems regarding knowledge of specific factors driving plant–soil interaction and the potential impacts on pest control are limited (Koyama et al. 2022; De Long et al. 2023). A good example in agroecosystem has been shown where all wheat diseases can be suppressed by the allelopathic compounds of canola (Angus et al. 1994). Thus, wheat benefit immensely when grown in soil conditioned by canola as opposed to monocropping.

Plant-soil feedbacks negatively or positively influence growth of plants and it has been hypothesized that certain agricultural practices can impact outcomes of these interactions (Mariotte et al. 2018). Two fundamental approaches in which plant-soil feedbacks could be managed towards increased crop protection and yield are through soil-protective strategies and inter- and cover cropping practices (Mutyambai et al. 2019; Davidson-lowe et al. 2021). Functional intercropping (cropping strategies designed to perform certain functions like pest management, soil fertility enrichment among others) can increase farm biodiversity and, in turn, provide ecosystem services, including optimized effects of plant-soil feedbacks (Wang et al. 2021; Huss et al. 2022). However, not all intercropping strategies are equal in providing these ecological services (Waha et al. 2020). One well-optimized intercropping system is push-pull technology (PPT) (www.push-pull.net).

Push-pull is a functional intercropping technology designed to control major insect pests and weeds while enhancing soil fertility and biodiversity conservation (Khan et al. 2011). Kenyan push-pull technology intercrops maize (*Zea mays* L., Poales: Poaceae) with pest resistance-mediating *Desmodium* spp. (Fabales: Fabaceae) (push plant) and surrounding plots with trap crops including Napier grass (*Pennisetum purpureum* Schumach.) (Poales: Poaceae) or brachiaria (*Brachiaria* spp.) (Poales: Poaceae) (pull plant) which attract herbivore pests (Khan et al. 2011). In addition to controlling several stem-boring lepidopteran pests, *Desmodium* spp. induce abortive germination and thus effectively control parasitic *Striga* weeds. Further, the companion plants facilitate livestock integration and production through high value fodder provision. Soil health is also improved through nitrogen fixation, moisture conservation and organic matter accumulation (Kremen and Miles 2012).

Originally designed to control stem-boring pests, Midega et al. 2018 also demonstrated the ability of PPT to control fall armyworm (*Spodoptera frugiperda* Smith, Lepidoptera: Noctuidae), a new invasive lepidopteran pest to Africa. Initially, studies had suggested that pest repellency was mediated by VOC signalling of push and pull plants but there are recent findings that propose additional mechanisms. For example, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) moths do avoid maize grown in soils conditioned by push-pull technology for oviposition, suggesting soil legacy-mediated effects associated with the intercrop species (*Desmodiun* spp.) that affect crop plant metabolism and pest resistance (Mutyambai et al. 2019). Similarly, push-pull cropping has been shown to reduce soil-borne mycotoxins in maize crops, likely also mediated by soil legacy effects (Njeru et al. 2020). However, the mechanism and thus the general applicability to a broader spectrum of pest species of such soil conditioning effects is unknown. Such knowledge is particularly valuable when dealing with new and emerging pests, such as *S. frugiperda* in East Africa.

Spodoptera frugiperda, is very destructive to many crop plants including maize. The pest is native to Americas and in recent years, it has invaded and established in Africa (Goergen et al. 2016), Asia (Nagoshi et al. 2020) and Australia (Qi et al. 2021) threatening food security and livelihoods. Caterpillars of this pest feed and inflict substantial damage on vegetative (leaf whorls) and reproductive parts (corn ears and tassels) in maize and other grain crops resulting to high crop loss (Montezano et al. 2018; De Almeida Sarmento et al. 2002). After its first detection in East Africa, chemical control using insecticides has been the focus. However, continuous use of synthetic insecticides has adverse effects on biodiversity including pollinators and biological control agents and increases risk of developing insecticide resistance. Integrated pest management approaches including intercropping and use of biological control agents like parasitoids are being developed to manage this pest. For example, egg-larval parasitoids such as *Chelonus bifoveolatus,* Szpligeti (Hymenoptera: Braconidae) have been found to effectively parasitize *S. frugiperda* pests in the areas it has invaded in Africa (Ahissou et al. 2021).

This study investigated soil legacy effects of PPT intercropping on *Zea mays*-*S. frugiperda*-natural enemy (*C. bifoveolatus*) tritrophic interactions. We examined the effects of conditioning soil with push-pull cropping on maize growth, phytochemistry and consequentially on direct (pest performance) and indirect resistance (natural enemy, *C. bifoveolatus* attraction) against *S. frugiperda.* We hypothesised that conditioning soil with push-pull positively impacted maize growth, metabolism and subsequent direct and indirect resistance to an invasive herbivore pest.

Material and methods

Description of study area

Both laboratory and semi-field greenhouse studies were conducted at International Centre of Insect Physiology and Ecology (*icipe*) located in Nairobi, Kenya, at an elevation of 1616 m above sea level, 1.2921° S and 36.8219° E. Soil samples for plant growth were collected from six different smallholder farms, each farm having PPT and maize monoculture plots, in Kwale county (coastal Kenya), Homabay and Kisii counties (western Kenya). Push-pull technology and maize monoculture cropping system had been practiced in the sampled farms for 3-10 years. Ten soil samples were randomly collected for each conventional maize monoculture and push-pull cultivation with the maize crop at the V6-V9 vegetative growth stage from each farm. For PPT plots, samples were collected in the middle between *Desmodium* and maize rows while for maize monoculture plots, soil was collected midway between maize rows. For each treatment per farm, soil samples were pooled together into a khaki bag for later planting into pots. No fieldwork permission and ethical approval was required.

Plants

Two maize seeds (SC Duma 43 variety), sourced from Seed Co Ltd., Nairobi, were sown in a 5-L plastic pot within 48 h of soil collection. Four replicates for each push-pull and monoculture field treatment from each of the six farms (total of 48 pots) were collected. Maize plants were grown 50 cm apart in a screenhouse at *icipe* under the following conditions: a photoperiod of L12:D12 and average diurnal temperature of 25 °C while at night the temperature was on average 19 °C. Pots were sterilized with 70% ethanol before planting. The weakest of the two maize seedling was thinned out 2 days after germination. The pots were watered daily with 0.2 L of water for three weeks when they were used in subsequent experiments. This is the age at which maize is especially susceptible to *S. frugiperda* attack (De Lange et al. 2020).

To determine plant growth, maize plant height of 48 seedlings planted in soil conditioned by PPT and maize monoculture was measured every week for three weeks after germination. The height measurements were taken from maize seedling baseline at the soil up to the fully expanded uppermost leaf's arch. Fresh shoot and root biomass was obtained from 24 plants after three weeks of growth through destructive sampling by cutting the shoot at the plant baseline and washing off the soil from the roots with excess water drained off the roots using paper towels. To obtain dry weight, fresh shoot and root biomass was dried in an oven set at 80 °C for 48 hours and weighed afterwards.

Insects

Fall armyworm neonates were raised in a maize-based diet prepared following methodologies described by Onyango and Ochieng'-Odero (1994) at *icipe*, Nairobi, Kenya. The larvae rearing was maintained under optimal conditions of L12:D12 photoperiod, 75 ± 5% relative humidity, and a temperature of 27 ± 2 °C until pupation. Pupae were placed on 110 mm diameter lids containing moisture in cages measuring 40 × 20 × 20 cm for adult eclosion where the emerging adults were held in 80 × 50 × 70 cm reproduction cages. These cages were provided with fresh intact maize plants as oviposition substrate. Deposited eggs were placed in plastic containers until they hatched.

Chelonus bifoveolatus parasitoids were collected from the field as cocoons and reared on *S. frugiperda* eggs in 80 × 50 × 70 cm cages. Honey and wet cotton were provided in the cages for wasp feeding and drinking *ad libtum*. One-day-old *S. frugiperda* neonates were used in larval feeding experiments while two-day old naïve gravid female *C. bifoveolatus* wasps were used in subsequent bioassays. Insects used in all experiments had been reared in the laboratory for at least two generations. Field collected insects were mixed with laboratory-reared ones at three-months interval for phenological and genetic characteristics maintenance.

Maize volatile organic compounds (VOCs) collection and analysis

Volatile organic compounds were collected after three weeks of growth from plants planted in PPT and maize monoculture conditioned soil through headspace volatile sampling following methodologies described by Agelopoulos et al. 1999. We enclosed maize seedlings in Polyethyleneterephthalate (PET) bags (dimensions: ~ 12.5 mm thickness, 3.2 L,) (Thomas Plant Ltd., Birmingham, UK). The PET bags were heated at 150 °C for one hour prior to their use. Air was pulled through ORBO-32 charcoal traps (Supelco, Bellefonte, PA, USA) by use of a 12-V GAST vacuum pump (Gast Manufacturing Inclusive, Benton Harbor, MI, USA) at 200 ml min-1 for 12 hours starting with the first hour of photophase. Headspace samples collected from empty PET bags served as background control. Elution of trapped VOCs by traps was done using 400 µl of dichloromethane (Sigma-Aldrich, St. Louis, MO, USA). Chemical analysis of the eluted VOCs was done using a gas chromatograph-mass spectroscophy (GC-MS) (Agilent 7890A autosampler) (Agilent Technologies Inc., Santa Clara, CA, USA). A 30 m, 0.25 ID low bleed GC capillary column (5%-phenyl)-methylpolysiloxane (HP5 MS) (J&W, Folsom, CA, USA) was fitted to GC-MS with helium as carrier gas. Chemical compound identification was done using ChemStation B.02.02 acquisition software. Generation and quantification of mass spectrum peak of each maize semiochemical compound was achieved following methodology outlined in Mutyambai et al. 2019.

Non-volatile maize secondary metabolites analysis

Analysis of non-volatile secondary metabolites was focused on maize defence metabolites such as phenolics and benzoxazinoids. Using a hole punch, leaf tissue (~ 0.30 g on average) was cut from maize seedlings grown in soil conditioned by both PPT and maize monoculture. The leaf tissue was immediately transferred into FastPrep® vials (2 ml with screw top) half-filled with 50% high pressure liquid chromatography-grade methanol extraction buffer. To avoid sample degradation, we stored them in a -20 °C refrigerator awaiting further processing. Leaf extraction involved adding 0.9 g Zirconia/Silica 2.3 mm grinding beads (BioSpec®) in each vial containing leaf tissue. Homogenization of the leaf for 60s at six metre per second in a tissue homogenizer and the supernatant was collected into 1.5 ml HPLC vials and analysed using HPLC following methodologies described in Mutyambai et al. 2019. Identification of maize metabolites was done using ultraviolet spectra and confirmed by injecting synthetic standards of the detected metabolites. Signal intensities were used to quantify the maize metabolites detected following methodologies described in Mutyambai et al. 2019.

Parasitoid assays

The response of gravid female *C. bifoveolatus* to maize VOCs from soil conditioned by push-pull technology and maize monoculture and solvent controls was measured using a four-arm olfactometer described by Pettersson 1970. 10 µL of headspace maize volatiles from maize grown in soil conditioned by push-pull technology and maize monoculture were held by the two opposite arms. Dichloromethane (solvent) which served as control was held in the other two arms. We applied each test stimuli separately onto a 4 × 25 mm piece of filter paper put at each arm of the olfactometer. One female parasitic wasp without any prior exposure to host was placed at the centre of the olfactometer and allowed to make choice on the four arms. A suction pump pulled the air towards the central chamber through the arms at a speed of 26 mL min^{-1}. The parasitic wasp was allowed 12 minutes to make a choice of the four arms. Olfa software (F. Nazzi, Udine, Italy) was used to record time spent in each arm. Twelve different *C. bifoveolatus* individuals were tested separately.

Larval feeding assessment

To assess leaf feeding as a measure of direct resistance to *S. frugiperda* on maize planted in push-pull technology and maize monoculture conditioned soil, no-choice feeding assay was carried out. Fifteen-millimetre diameter round-shaped maize leaf pieces were excised from the uppermost maize leaf of each maize seedling. Two cut leaf pieces from each maize seedling were each placed into a 30 ml transparent cup which had been filled halfway with agar (Technical Agar #3) to prevent desiccation. Ten first instar *S. frugiperda* were put into the cup holding the leaf piece and allowed 24 hours to feed. Thereafter, the leaf area consumed was measured by taking a photograph of the leaf disc and analysing it using ImageJ software (National Institutes of Health, Bethesda, USA) (Schneider et al. 2012). 24 replications were carried out both for soil conditioned by PPT and maize monoculture.

Data analysis

Data was tested for normality using Shapiro-Wilk test. Parametric tests were used when data was normally distributed and non-parametric tests were used for analysis of non-normally distributed data. Data on maize plant growth taken for the three weeks was analysed using Repeated-Measures analysis of variance (rANOVA). Data on biomass and leaf tissue larval feeding of maize plant planted in maize monoculture and push-pull conditioned soil was analysed using student's t test (unpaired two sample). One-way ANOVA was used to analyse four-arm olfactometer data on response of gravid *C. bifoveolatus* to different test stimuli. Being a composite data, time spent in each arm by female *C. bifoveolatus* was converted into proportions of the total 12 minutes that the wasp was allowed to make choice among the four arms before the analysis. To test the impact of conditioning soil by different cropping systems on maize secondary maize metabolism, we used Permutational multivariate analysis of variance (PERMANOVA; 999 permutations; using *adonis2* function in *vegan* package) as described by Anderson 2001. To account for random effect in PERMANOVA test, we treated smallholder farms where soil was collected as strata. *Vegan* package was also used to determine Shannon diversity of total VOC bouquets emitted from plants in the two different treatments. Pairwise permutation test from the *rcompanion* package was used to compare selected maize secondary metabolites from PPT and maize monoculture conditioned soils. R Development Core Team (2020) was used in all data analyses and α set at 0.05.

Results

Maize growth parameters

We observed differences in terms of plant height and fresh biomass in maize seedlings planted in PPT and maize monocultures conditioned soil. Maize plants grew faster in PPT conditioned soil compared to those planted in Mono conditioned soil during second and third week after germination ($F_{5,47} = 1.81$, $P = 0.002$, Fig. 1). This difference was not apparent during the first week after germination ($t = 0.36$, $P = 0.72$, Fig. 1). Moreover, we observed higher root and shoot maize fresh weight in those plants planted in PPT conditioned soil in comparison to those planted in soil conditioned by maize monoculture cropping ($t = -3.74$, $P < 0.001$; $t = -4.39$, $P < 0.001$ respectively, Fig. 2). However, we did not find any significant differences in dry biomass for both shoot and root of maize plants in push-pull and maize monoculture conditioned soil ($t = 1.43$, $P = 0.08$; $t = 0.72$, $P = 0.24$ respectively).

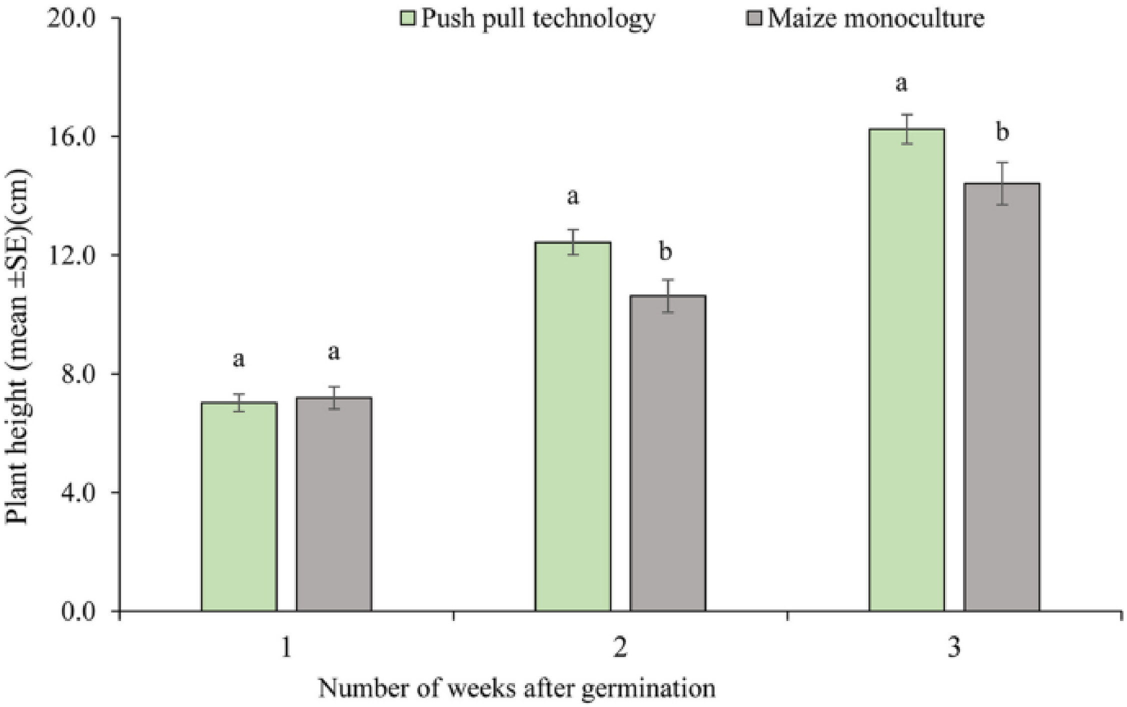

Fig. 1. Mean (±SE) maize (*Zea mays* L.) height measured at 1-, 2- and 3-weeks post germination in maize monoculture and push-pull technology conditioned soil. Bar graphs with different letters indicate statistical difference in each week ($P < 0.05$)

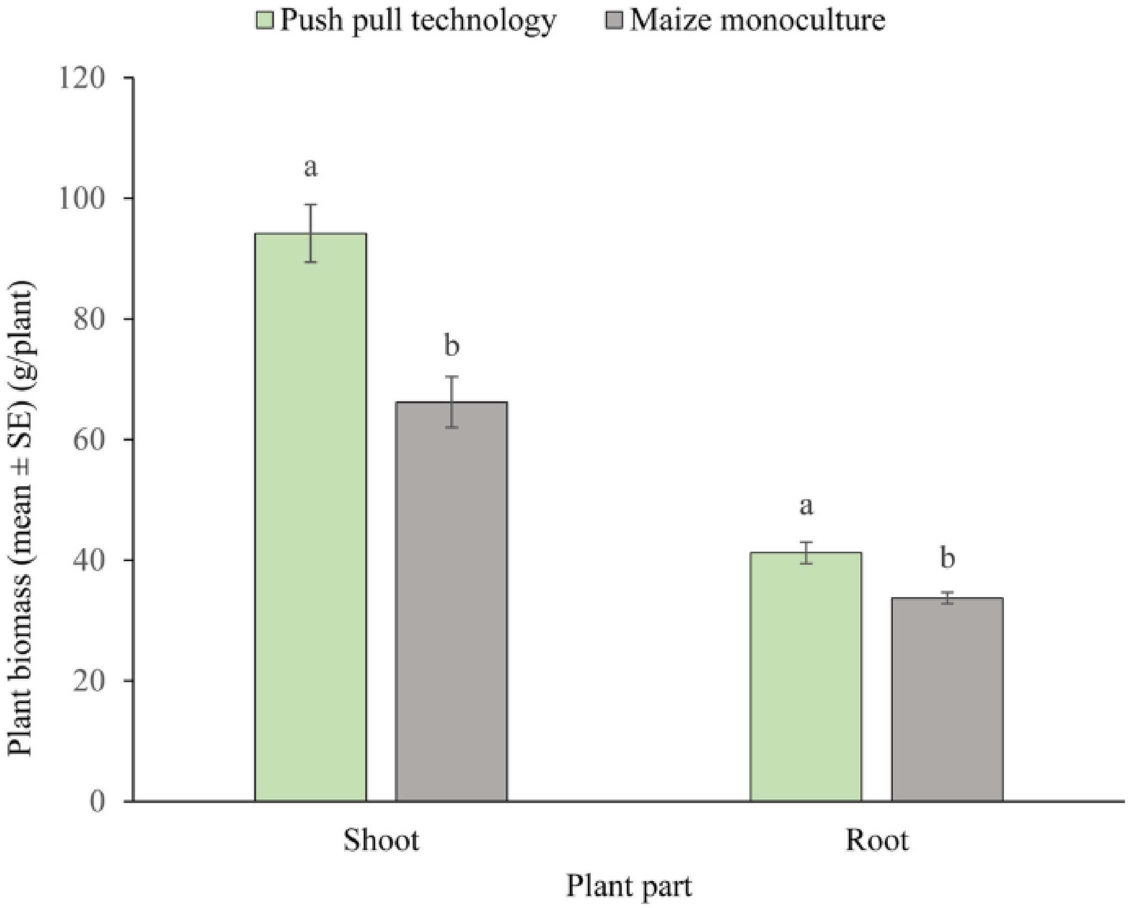

Fig. 2. Mean (±SE) maize (*Zea mays* L.) plant shoot and root fresh biomass three weeks after germination planted in maize monoculture- and push-pull technology conditioned soil. Bar graphs with different letters indicate statistical difference in biomass ($P < 0.05$)

Maize volatile emissions

There were quantitative and qualitative differences in volatile bouquets between plants planted in PPT and maize monoculture soil ($F_{1,46} = 3.106$; $P = 0.009$, Fig. 3). Monoterpene volatile organic compounds such as β-pinene and limonene; homoterpenes including (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) and (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT); sesquiterpenes like α-humulene, β-caryophyllene, germacrene D, and β-farnesene, and three additional unidentified compounds were produced in larger amounts by maize planted in PPT conditioned soil (Fig. 3). Further, maize seedlings planted in PPT conditioned soil emitted higher quantities as well as more diverse VOCs in comparison to those planted in maize monoculture conditioned soil (Shannon diversity index; $F_{1,46} = 20.148$, $P = <0.001$).

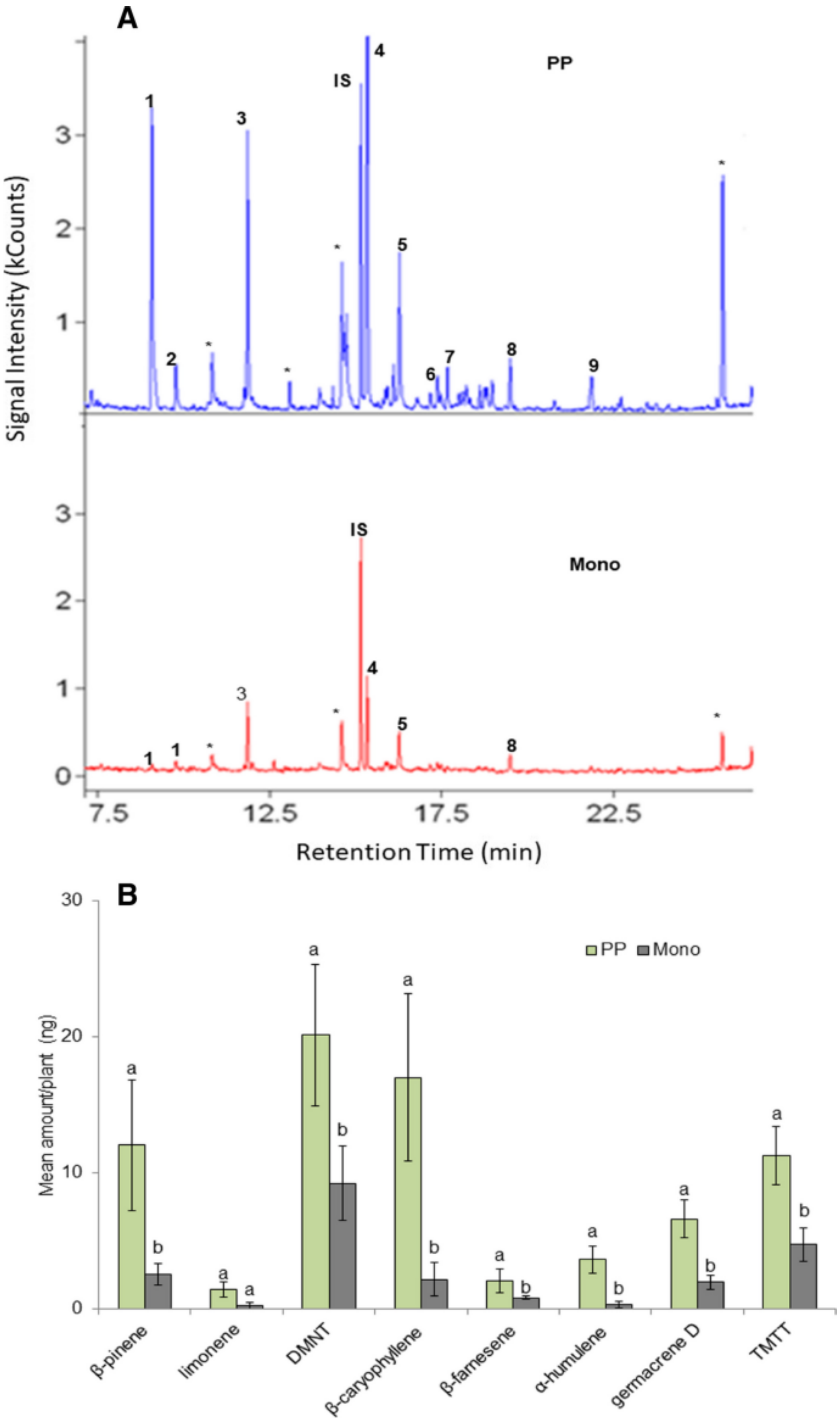

Fig. 3. Volatile organic compounds emitted by maize plants planted in push-pull technology (PP) and maize monoculture (Mono) conditioned soil. **A** maize representative gas-chromatogram profile from maize monoculture and push-pull technology conditioned soil. Key volatile compounds are designated by numbers as follows: 1. β-pinene, 2. limonene, 3. (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), 4. 1,6-Octadien-3-ol,3,7 dimethyl-, 5. β-caryophyllene, 6. β-farnesene, 7. α-humulene, 8. germacrene D, 9. (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT). IS = internal standard (tetralin), * indicates volatile compounds which were not confirmed by co-injection with synthetic standards. **B** Mean (±SE) quantities of major volatiles emitted per maize plant planted in push-pull technology and maize monoculture conditioned soil

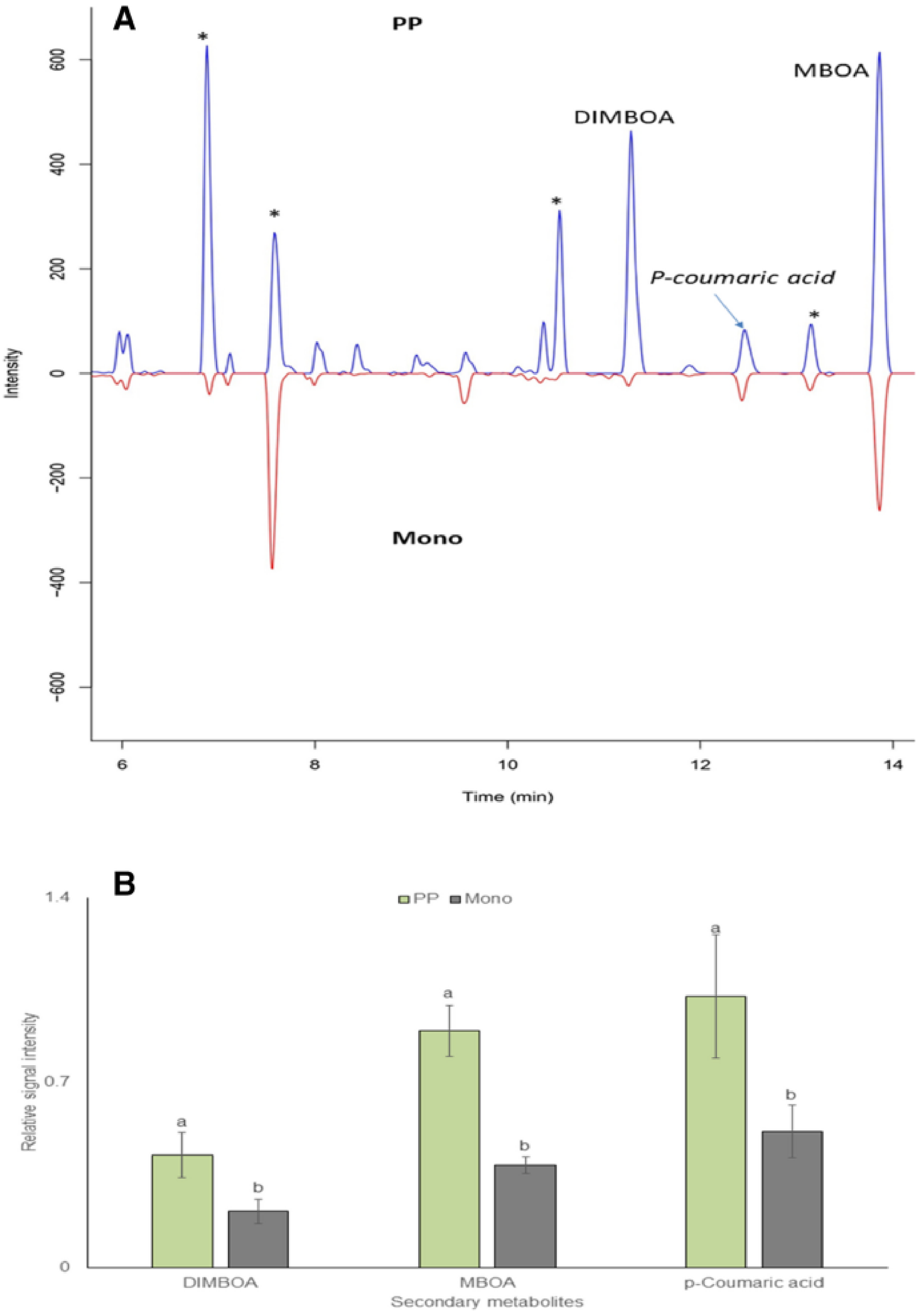

Fig. 4. Profiles and quantities of non-volatile maize metabolites from plants planted in maize monoculture (Mono) and push-pull technology (PP) conditioned soil: **A** representative metabolites profile from maize planted in push-pull technology (PP) and maize monoculture conditioned soil. * indicate secondary metabolites that were not confirmed using authentic standards. **B** Mean (±SEM) quantities of major resistance-mediating maize metabolites from soil conditioned by maize monoculture (Mono) and push-pull technology (PP). Bar graphs with different letters indicate statistical difference in metabolite quantity (*$P < 0.05$*)

Maize non-volatile defence metabolites

We observed higher production of maize metabolites including MBOA (6-methoxy-benzoxazolin-2-one), DIMBOA (2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one), and phenolic compounds including *p*-coumaric acid in plants grown in PPT soil than those grown in monoculture soils ($F_{1,44} = 3.638$, $P = 0.015$, Fig. 4). As with VOCs, higher diversity of non-volatile defence metabolites was observed in maize planted in push-pull technology conditioned soil in comparison to those grown in maize monoculture conditioned soil ($F_{1,44} = 2.758$, $P = 0.03$).

Behavioural response of parasitoids to maize volatiles

Chelomus bifoveolatus females spent more time on maize VOCs from push-pull conditioned soil (ANOVA, $F_{2,33} = 9.962$, $P = < 0.001$, Fig. 5) over solvent controls and VOCs from plants in maize monoculture soil (Tukey's post hoc, $P = 0.004$; $P = < 0.001$, respectively). Parasitoid females did not differentiate between VOCs from plants in monoculture soil and the solvent control ($P = 0.75$).

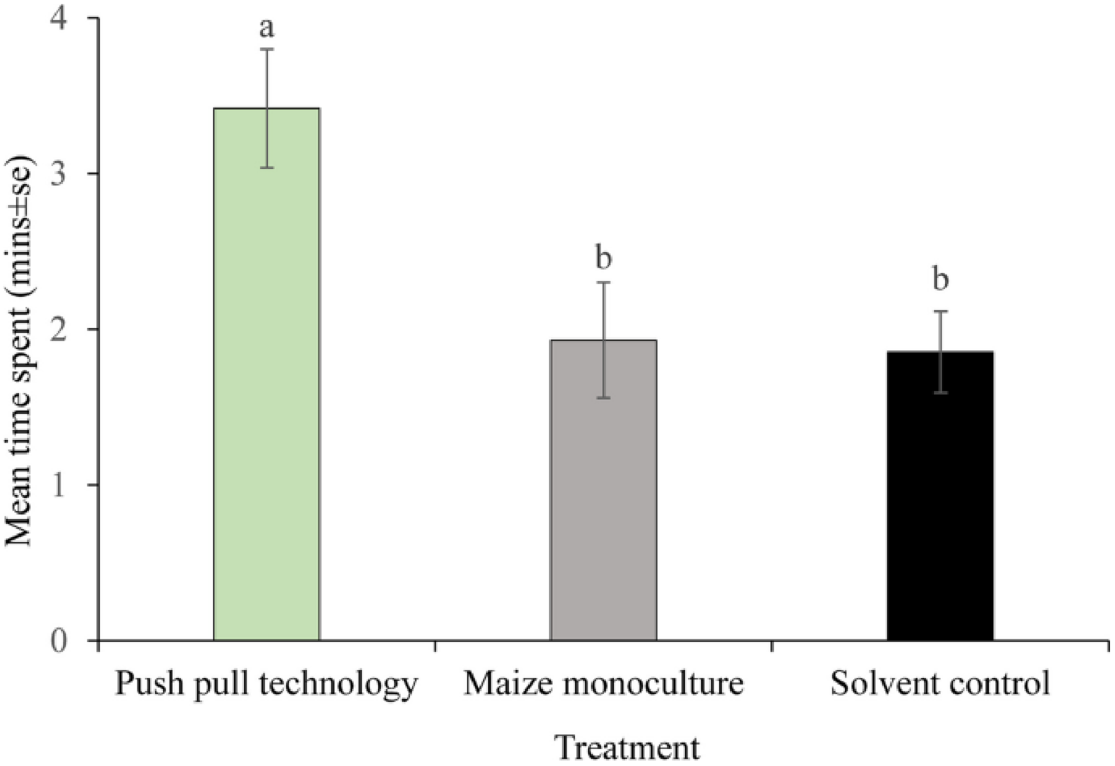

Fig. 5. Mean time (±SEM) spent by female *Chelonus bifoveolatus* parasitoids in four-arm olfactometer with volatile blends collected from maize plants planted in maize monoculture and push-pull technology conditioned soil as well as solvent control observed for 12-minutes. Bar graphs with different letters indicate statistical difference in time spent by *C. bifoveolatus* with each volatile blend ($P < 0.05$)

Larval feeding assay

Spodoptera frugiperda neonates consumed higher amounts of maize leaf tissue from monoculture conditioned soil (21.63 ± 2.09 mm^2) in comparison to maize leaf tissue from push-pull technology conditioned (13.59 ± 1.26 mm^2), ($t = 3.072$, $df = 37.799$, $P = 0.002$).

Discussion

Our findings show that push-pull technology soil legacies positively impact maize growth, secondary metabolism, as well as direct and indirect resistance to *S. frugiperda*, a new pest in Africa. Remarkably, plant-soil feedback effects on plant metabolism do not only increase direct resistance to *S. frugiperda* larvae, but do also mediate the attraction of parasitoids, suggesting indirect defences as an additional mechanism mediated by push-pull functional intercropping. Our data suggest a plant-soil feedback-mediated alteration of crop plant secondary metabolism that affects direct and indirect defence trait expression.

These results significantly expand on earlier findings that demonstrated associational positive effects on maize growth and secondary metabolism mediated by push-pull technology conditioned soil (Mutyambai et al. 2019). Contrary to Mutyambai et al. 2019 where resident insect pest (*Chilo partellus*) was used, in this study soil conditioning with push-pull was tested against an invasive insect pest (*Spodoptera frugiperda*). In addition, Mutyambai et al. 2019 did not investigate indirect defense mechanism using parasitic wasp's response to volatile organic compounds produced by maize plants grown in push-pull and maize monoculture conditioned soil. This study therefore brings new insights on how conditioning soil with push-pull cropping system contributes to the cropping system's resilience against an invasive pest both directly and indirectly by changing the main crop's (maize) metabolism. Push-pull cultivation as well as some other soil-preserving methods have been found to restore soil function and fertility (Drinkwater et al. 2021). Such increased soil health may in part explain increased plant vigor, but resulting differences also explain differences in the overall impacts of push-pull technology depending on soil type. For example, direct resistance differences between plants raised in soil conditioned by maize monoculture and push-pull technology have been shown to be higher in relatively poor black cotton (vertisol) soils compared to nutrient-rich red volcanic soils (nitisols) (Mutyambai et al. 2019). Most of the soil health effects in the Kenyan push-pull system are due to the facts that, *Desmodium* spp.*,* the most commonly used intercrop, is (A) a legume, fixing nitrogen, (B) grown as a permaculture and trimmed seasonally, which prevents soil erosion, and (C) at least part of the *Desmodium* spp. root and shoot tissues usually remain in and on the ground after harvest and so contribute to elevated organic matter in the soil. In contrast, conventional monocultures, specifically those of maize, lack all of these attributes and usually negatively impact soil properties (Drinkwater et al. 1998; Niassy et al. 2022). What is surprising, however, is that this improvement of plant growth is accompanied by an increase in maize plants' expression of direct and indirect resistance in push-pull soils.

In general, plant defence theory predicts a trade-off between defence and growth, which is commonly found both in species and genotype comparisons (genetic pattern) and when plants allocate resources differentially in response to environmental stresses (phenotypic plasticity) (Cope et al. 2021). Here we demonstrate that maize grown in push-pull soil not only grows better but also alters plant secondary metabolite production and with it, the herbivore's behaviour on the plant and foraging behaviour of the herbivore's parasitoid.

Exposure of maize plants to push-pull soil increased direct resistance relative to plants in conventional maize monoculture soils. These reductions in *S. frugiperda* consumption rates are ecologically and economically relevant because tissue removal has negative effects on both photosynthetic activity and yield (Davidson-lowe et al. 2021) and the magnitude of herbivore damage can also be correlated with increased infections by pathogens (Gossner et al. 2021).

What drives this increased resistance to herbivores in maize planted in soil conditioned by push-pull technology? In Poaceae including maize, benzoxazinoids such as 6-methoxy-benzoxazolin-2-one (MBOA) and 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA) have been identified as potent defence compounds that mediate resistance against insect pests and pathogens (Dudareva et al. 2006). In this study, benzoxazinoid metabolites including DIMBOA and MBOA as well as a phenolic compound, *p*-coumaric acid among other metabolites were elevated in maize seedlings planted in soil conditioned by push-pull intercropping technology. This elevated production of these known maize defence metabolites in maize planted in soil conditioned by push-pull technology is strongly correlated with the reduced *S. frugiperda* larval feeding observed in our study. This reduced herbivore performance in otherwise better performing plants represents a paradox. In general, insect herbivores tend to perform poorly in nutrient-deficient plants and better on well fertilized plants, such as those in the push-pull system (Chen et al. 2010). Ren et al. 2013 observed that *S. exigua* fed more on nitrogen-fertilized plants as opposed to nutrient-deficient one. These differences are associated with a reduced defensive secondary metabolite production in plants fertilized with inorganic mineral fertilizer (Ibrahim et al. 2013). Here, we suggest three basic mechanisms that could allow well fertilized plants in push-pull soils also to have higher defence-related secondary metabolism. (A) Phytohormone-like compounds secreted by the intercrop species could directly induce crop plant responses (Xu et al. 2018; Erb et al. 2012) (B) Intercrop root exudates can alter soil chemistry that can affect crop plant metabolism and nutrient uptake (Canarini et al. 2019). Finally (C) the intercrop species alone or the interaction between crop and intercrop may significantly alter soil microbial communities, which, in turn, induce differential metabolic responses in plants interacting in the respective soil environment (Howard et al. 2020). These three hypotheses are by no means mutually exclusive and their coactive function towards an increased defence metabolism may be the reason why we see this strong direct pest resistance enhancement with *Desmodium* spp. intercropping.

Interestingly, this increase in direct resistance-mediating non-volatile secondary metabolites is also apparent in the production of VOCs with yet additional effects on ecological interactions. We found far-reaching differences in VOCs emissions both quantitatively and qualitatively in maize planted in soil conditioned by PPT relative to the ones planted in soil conditioned by maize monoculture (Fig. 2). In contrast to non-volatile defensive metabolites, soil nitrogen availability has been found to be positively correlated with diverse and higher terpene volatile emissions (Blanch et al. 2007). Thus, the more diverse VOC profiles and higher emissions of maize plants in soils conditioned by PPT are likely related to the enriched soil fertility, associated with *Desmodium* spp. intercropping (Ndayisaba et al. 2021).

There are widespread ecological ramifications of plant-soil feedback impacts on VOCs emissions and their subsequent influence on multitrophic level species interactions (Bennett and Klironomos 2019). VOCs, including terpenes we found elevated in maize grown in PPT conditioned soils are known to be host-finding cues for parasitic wasps which are natural enemies of herbivore pests (Dicke 1994). Such a VOC-mediated attraction of parasitoids and predators can function as an indirect defence if the attracted natural enemies can significantly reduce herbivore pressure on the VOC-emitting plants (Bruce et al. 2010). In our study, emission of terpenoid VOCs, in particular, was significantly elevated in plants grown in soil conditioned by PPT in comparison to maize planted in soil conditioned by maize monoculture. Most of these compounds have been found to be herbivory-induced in previous studies (Mutyambai et al. 2015, 2016), while in this study they were constitutively emitted in higher amounts from plants grown in push-pull soils even without herbivory. Like inducible VOC bouquets in herbivore-attacked plants, so did the bouquets emitted from push-pull maize plants attract parasitoids, such as *C. bifoveolatus*. This implies the possibility that plants in push-pull soil may constitutively attract natural enemies of herbivores into the crop plant population and so mediate constitutive indirect resistance. Previous studies have shown that intercropping systems are associated with higher natural enemy numbers of herbivores and play a role in reducing herbivore numbers in these systems through predation and increased parasitism (Otieno et al. 2019; Khan et al. 1997). In our study, we suggest intercrop-induced VOC emission as a new mechanism for increased pest control in intercropping systems in general and push-pull in particular.

Conclusion

In conclusion, our current study shows that polyculture cropping systems like push-pull soil legacies in an agroecosystem influence plant growth and alter plant chemistry. The altered phytochemistry plays an important role in tritrophic interactions beneficial for crop protection against an invasive pest herbivore by reducing larval feeding and recruiting egg-larval parasitoids of the pest. The link between the soil legacies and the associated plant metabolism in general represents a new mechanism of associational resistance from functional neighbourhood and in particular shows an additional mechanism through which push-pull cropping technology is controlling the invasive fall armyworm where this cropping system is practiced. Conducting further studies that refine effects of conditioning soil with different polyculture cropping systems and their impact on soil microbiota and gene expression will contribute to a deeper understanding of the mechanisms governing plant growth and herbivore resistance in these systems. While this study employed field soils, it's important to note that experiments were conducted within controlled laboratory and semi-field settings. We recommend subsequent detailed spatial and temporal field studies testing when the polyculture cropping soil legacy effects manifest and how long can they be retained in conditioned soil in absence of the companion crops. These studies will play a pivotal role in understanding the broader effectiveness of this cropping system in crop production and management of herbivore pests through its soil legacy effects. Nevertheless, in a real-world agricultural context, these findings could be further exemplified, as intercropped plants in push-pull cropping system continually contribute nutrients to the soil while simultaneously producing non-volatile and volatile organic compounds that repel insect pests but attract the pest's parasitoids.

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Conflict of interest

All the authors declare that they have no conflict of interest.

Contributions

DMM, AK, SS conceived and designed the study; DMM, JMM, BNN and AAJ collected data; DMM, JMM, FC, AAJ and SN analysed data; DMM, JMM, AK and TD, led the drafting of the manuscript; SM, ZK and SS supervised the work and provided resources. All authors critically reviewed and approved the final version.

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