Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2022:35
Faculty of Landscape Architecture, Horticulture
and Crop Production Science

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Below and aboveground mechanisms for ecosystem services

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Alnarp

SLU

SWEDISH UNIVERSITY
OF AGRICULTURAL
SCIENCES

DOCTORAL THESIS

Alnarp 2022

Acta Universitatis Agriculturae Sueciae
2020:35

Cover: Illustration by G. Mbunda)

ISSN 1652-6880
ISBN (print version) 978-91-7760-945-2
ISBN (electronic version) 978-91-7760-946-9
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Alnarp
Print: SLU Service/Repro, Alnarp 2022

Revisiting push-pull technology: Below and aboveground mechanisms for ecosystem services

Abstract

Push-pull technology is an effective and ecological solution to suppressing major Lepidopteran pests of cereals, in particular stem-borers (*Busseola fusca*, *Chilo partellus*), the fall armyworm (*Spodoptera frugiperda*) and the parasitic weed *Striga*. The technology exploits plant-insect interactions in intercropping practices to manage the pests, increasing productivity while maintaining ecosystem resilience. In this study, we show that long-term (14 – 18 years) push-pull farming cause shifts in soil microbial communities, increasing the diversity of fungal taxa than bacteria. Further, the shift in the structure of soil microbial populations seems to require time to establish as observed by the weak impact of *Desmodium* species cultivated for just two years on soil microbial structure. However, even under the short-term cultivation period, individual taxa enrichment associated to the *Desmodium* were observed. On the other hand, whole soil microorganisms as well as rhizobia appeared to have little impact on the constitutive release of volatile emissions by *Desmodium*. Whether the plants grew on live or autoclaved soil, *Desmodium* did not release volatiles implicated in repelling lepidopteran pests, which is in stark contrast to previous reports. Upon herbivory of *Desmodium* by *Spodoptera frugiperda* larvae, a marginal increase in volatile emissions was observed. In line with this observation and contrary to expectation, intact *Desmodium* spp. did not deter oviposition by gravid *S. frugiperda*. In feeding bioassays, neonate *S. frugiperda* larvae strongly preferred *Desmodium* spp. to maize diet, but did not grow well nor did they survive on it. Older larvae were frequently immobilised on the stems, often dying in position after a few days. Deeper investigation showed that stems and leaves of *Desmodium* were covered with a dense web of straight and hooked silicon-rich trichomes of varying lengths that prevented larval movement, piercing their cuticle in the struggle. In this light, we propose that in a push-pull setting, *Desmodium* acts as a mechanical barrier and trap crop instead of a volatiles-dependent "push" crop as previously purported. In addition, intercropping practices have been shown to reduce insect pest populations through diverse mechanisms such as barrier effect and resource concentration. Push-pull technology shows that ecological approaches to pest management and increasing productivity can be effective. A clear understanding of the mechanisms of action of such approaches is critical for further improvements as well as translation into other agro-ecological practices.

Keywords: Push-pull technology, *Desmodium* spp., soil microbiome, intercropping microbiome, stem-borers, trichomes, ecosystem services

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Omdefiniering push-pull-teknik: Mekanismer under och ovan jord för ekosystemtjänster

Abstrakt

Push-pull teknologi är en effektiv ekologi-baserad lösning för att hantera allvarliga skadegörare på spannmål, detta särskilt för stamborrande insekter (*Busseola fusca, Chilo partellus)* och *Spodoptera frugiperda* samt det parasitiska ogräset *Striga.* I denna studie, rapporterar vi för första gången hur push-pull under en längre tid (14-18 år) påverkar det mikrobiella livet i jorden. Vi undersöker också den kortsiktiga påverkan från monokulturell odling av olika *Desmodium* spp. på mikrobiella samhällen. Som tillägg, så tittade vi huruvida skadeinsekternas valde att lägga ägg på endera maize eller *Desmodium* spp. samt vad larver föredrog som föda och hur väl de överlevde. Vi visar att odling i push-pull system och samodling av *Desmodium* i spannmål orsakar förändringar i jordens mikrobiom, med en ökad diversitet i svamptaxa snarare än bakterier. Dessa förändringar i jordens mikrobiella samhällen verkar vara tidsberoende, endast en liten förändring kunde ses när *Desmodium* arter var odlade i endast två år. Dock, kunde redan efter en kortare periods odling av *Desmodium* en ökning av individuella taxa skönjas. Vi kunde, i motsats till tidigare studier, endast se en minimal konstitutiv emission av de flyktiga ämnen som har rapporterats vara inblandade i bortstötandet av skadeinsekter från *Desmodium*, detta oberoende på om växterna odlades på levande eller autoklaverad jord. De fanns bara marginella emissioner av flyktiga ämnen i närvaron av växtätande larver, vi kunde dock observera en svag påverkan beroende på den mikrobiella kompositionen i jorden. I linje med denna observation och i motsats till förväntningar så blev inte *Spodoptera frugiperda*, en förödande skadegörare på spannmål, avskräckt från att lägga ägg på *Desmodium* när den fick valet. I födoförsök så föredrog nyfödda *S. frugiperda* larver snarare *Desmodium spp.* framför majs, men utvecklades då inte eller dog. Vid födoförsök på hela *Desmodium* plantor så fastnade larverna och blev immobiliserade på stammarna, oftast fastnade de och dog efter ett par dagar. En undersökning av stammar och blad på *Desmodium* spp. visade att de var täckta av raka och krokiga kiselberikade trikomer i varierande längder som förhindrade larvens rörelser och till och med genomborrade deras kämpande kroppar. I ljuset av dessa uppgifter, så är det troligt att *Desmodium* spp. snarare agerar som en mekanisk barriär och fångstgröda, snarare än en samodlad gröda som avskräcker baserat på de flyktiga ämnen den avger.

Nyckelord: Push-pull teknik, *Desmodium* spp., jordens mikrobiom, samodling, stamborrare, trikomer

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Quote

“Life would be wonderful if we only knew what to do with it.”

~ Greta Garbo

Dedication

To my two my two mothers, this is for you.

To my grandfather, Justine Kilawe, you are my hero.

To my family, I owe you who I have become.

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. **Aneth David Mwakilili**[#a], Kilaza Samson Mwaikono, Sebastian Larsson Herrera, Charles Midega, Francis Magingo, Beatrix Alsanius, Teun Dekker, Sylvester Leonard Lyantagaye (2021) Long-term maize - *Desmodium* intercropping shifts structure and composition of soil microbiome with stronger impact on fungal communities. *Plant and soil*. 467 (1):437 - 450

II. **Aneth Bella David**, Kilaza Samson Mwaikono, Charles Midega, Francis Magingo, Beatrix Alsanius, Laurie Drinkwater, Teun Dekker, Sylvester Leonard Lyantagaye (2022) Shifts in structure and diversity of soil microbial populations under cultivation of *Desmodium* species. Manuscript

III. Anna Laura Erdei*, **Aneth Bella David***, Eleni Savvidou, Vaida Džemedžionaitė, Advaith Chakravarthy, Bela Peter Molnar, Teun Dekker (2022) In push pull technology, *Desmodium* does not repel moths, but intercepts and kills their offspring. *bioRxiv*. doi: https://doi.org/10.1101/2022.03.08.482778

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The contribution of Aneth Bella David to the papers included in this thesis was as follows:

I. Designed the study with co-authors, performed data collection and analysis, produced the first draft of the manuscript and contributed to the final version with co-authors.

II. Designed the study with co-authors, performed data collection and analysis, produced the first draft of the manuscript and contributed to the final version with co-authors.

III. Designed the study with co-authors, performed data collection and contributed to manuscript writing with co-authors.

Aneth Bella David also contributed to studies presented in the following articles that are not included in this thesis:

1. Sarvenaz Sarabipour, Aziz Khan, Yu Fen Samantha Seah, **Aneth D. Mwakilili**, Fiona N. Mumoki, Pablo J. Sáez, Benjamin Schwessinger, Humberto J. Debat and Tomislav Mestrovic (2021) Changing scientific meetings for the better. *Nature Human Behaviour.* 5: 296–300. doi: 10.1038/s41562-021-01067-y
2. Raphael Zozimus Sangeda, **Aneth David Mwakilili**, Upendo Masamu, Siana Nkya, Liberata Alexander Mwita, Deogracious Protas Massawe, Sylvester Leonard Lyantagaye and Julie Makani (2021) A baseline evaluation of bioinformatics capacity in Tanzania reveals areas for training. *Frontiers in Education*. 6:665313. doi: 10.3389/feduc.2021.665313
3. Erick Sinza, **Aneth Mwakilili**, Cyprian Mpinda and Sylvester Lyantagaye (2021) Cellulase-producing bacteria isolated from mufindi paper mill industrial effluent, Iringa, Tanzania. *Tanzania Journal of Science*. 47:1
4. Mohamed Zahir Alimohamed, **Aneth David Mwakilili**, Kenneth Mbwanji, Zainab Karim Manji, Frida Kaywanga, Kilaza Samson Mwaikono, Ismael Adolf, Julie Makani, Ben Hamel, Collen Masimirembwa, Deus Simon Ishengoma and Siana Nkya (2020) Inauguration of the Tanzania Society of Human Genetics: biomedical research in Tanzania with emphasis on human genetics and genomics. The *American Journal of Tropical Medicine and Hygiene*. 04(2):474-477. doi: 10.4269/ajtmh.20-0861.
5. Oleg N. Reva, Safronova A. Larisa, **Aneth David Mwakilili**, Donatha Tibuhwa˒ Sylvester Lyantagaye, Wai Yin Chan, Stefanie Lutz, Christian H. Ahrens, Joachim Vater and Rainer Borriss (2020). Complete genome sequence and epigenetic profile of *Bacillus velezensis* UCMB5140 used for plant and crop protection in comparison with other plant-associated *Bacillus* strains. *Appl Microbiol Biotechnol* 104: 7643–7656 doi: 10.1007/s00253-020-10767-w
6. Oleg N. Reva˒ Dirk Z. H. Swanevelder, Liberata A. Mwita, **Aneth David Mwakilili**, Dillon Muzondiwa, Monique Joubert, Wai Yin Chan, Stefanie Lutz, Christian H. Ahrens, Lylia V. Avdeeva, Maksim A. Kharkhota, Donatha Tibuhwa˒ Sylvester Lyantagaye, Joachim Vater, Rainer Borriss and Johan Meijer (2019) Genetic, epigenetic and phenotypic diversity of four *Bacillus velezensis* strains used for plant protection or as probiotics. *Frontiers in Microbiology*. doi: 10.3389/fmicb.2019.02610. 10:2610

List of figures

Abbreviations

DMNT	(3E)-4,8-dimethylnona-1,3,7-triene
FAW	Fall armyworms
HIPV	Herbivore-induced plant volatiles
ICIPE	International Centre for Insect Physiology and Ecology
SEM	Scanning electron microscope
SSA	Sub-Saharan Africa

1. Background

Agricultural intensification brought about by the green revolution involves cultivation of large swathes of single or few crops with high genetic similarity, creating uniform agricultural landscapes (Snapp et al. 2010; Hufnagel et al. 2020; John & Babu 2021). Adoption of agricultural intensification was a response to growing populations worldwide in the 1950's and the resulting growing demand for food. The movement was characterised by the mechanisation of agriculture and the intensive use of agrochemical-inputs (especially mineral fertilisers) to boost soil capacity to support plants and indiscriminate application of chemical pesticides to curb both pests and pathogens (FAO 2017).

The green revolution achieved sizable success in improving food production. However, shortly afterwards the externalities of the practices on the environment and ecosystem health became apparent. The consequences of overreliance on agro-chemical inputs (fertilisers and pesticides) are reflected in polluted soils and water bodies (both underground and surface water) as well as negative impacts on humans, animals, birds, non-target insects and natural enemies, which in turn endanger the stability of agro-ecosystems. In addition, mechanisation applications such as tilling exposed soil top layers to wash off and degradation by natural elements like rainwater and wind, causing widespread decline in soil quality (Myers 1999; Pingali 2012; Eliazer Nelson et al. 2019; John & Babu 2021). It is now widely agreed that intensified farming is an unsustainable way to grow food with major repercussions on biodiversity and stability of ecosystems (IPBES 2019; IPCC 2021).

In addition, the success of the green revolution was not universal. For example, the green revolution did not take hold in most of the African continent for various political, economic and geographical reasons.

Ignorance of the local environmental context and existing food production systems that were markedly different from those in Europe, the Americas and Asia contributed to failure of the revolution in the continent (Otsuka & Muraoka 2017; Bjornlund et al. 2020), leaving pervasive food security issues to this day. Besides, food security challenges cannot be solved by doubling up on intensification, which often depends on clearing more land, a finite resource, and increased reliance on chemical inputs with their negative ecosystem corollaries.

In recent years the focus has shifted towards agricultural diversification as an antidote to the destructive intensification. Diversification refers to agronomic principles, processes and practices that aim at improving productivity of cropping systems while maintaining their stability and resilience in the long run and enhancing delivery of ecosystem services (Wezel et al. 2014; Hufnagel et al. 2020). In contrast to agricultural intensification, diversification practices have been demonstrated to be especially beneficial to smallholder farmers. The practices have been shown to reduce economic risk while improving socio-economic benefits. Some of the benefits that have been reported include soil conservation, improvement of biodiversity and systems resilience as well as pest control. Importantly, yields obtained from diversification farming approaches are similar or higher than those of conventional intensive monocultures (Hufnagel et al. 2020; Tamburini et al. 2020; Rodriguez et al. 2021). Thus, diversification has come at the forefront as an alternative approach to food production that is sustainable and environmentally friendly, which in the long run helps with mitigation of climate change, a pressing challenge of modern society.

Diversification covers a range of agronomic practices including intercropping, crop rotation, multiple cropping, variety mixtures and agroforestry. The practises may be categorised by temporal scales (crop rotation), spatial scales (intercropping), crops genetics (mixed variety cropping), landscape level (field, etc), management practises (agronomic measures such as reduced tillage, varying seeding time) and production systems (organic farming, conservation agriculture, etc) (Hufnagel et al. 2020; Rodriguez et al. 2021). In practice it is common for several of cropping and management approaches to be combined to yield an optimal food production system.

Push-pull technology is a crop diversification farming practice that employs a mixed cropping system to manage pests of cereals, suppress weeds and improve soil fertility in one stroke (Hassanali et al. 2008). Smallholder farmers in eastern and southern Africa have adopted the technology to control major pests of maize and sorghum; stem-borers (*Chilo partellus*, *Busseola fusca*) and fall armyworms (*Spodoptera frugiperda*) (Midega et al. 2018), which often infect cereal crops in unison and if left uncontrolled, can cause between 80 - 100% loss of crops (Abate et al. 2000; Kfir et al. 2002; Togola et al. 2020; Nyamutukwa et al. 2022).

In push-pull farming, a perennial leguminous intercrop in the genus *Desmodium* grown between rows of maize “pushes” the devastating pests away from the main crop, while a grass trap crop “pulls” them towards itself and halts their development (Khan et al. 2010). *Desmodium* spp. is the key component of the system, repelling the insect pests reportedly through constitutive release of volatiles, while providing a host of additional benefits, including suppression of the *Striga* spp. parasitic weeds (Hassanali et al. 2008). The aboveground components of the technology have been well studied and their mechanisms of action well documented especially by scientists at the International Centre for Insect Physiology and Ecology (ICIPE). The socio-economic benefits of the technology are also well established (Kassie et al. 2018; Muriithi et al. 2018; D’Annolfo et al. 2021). However, an understanding of belowground communities, especially soil microorganisms and their contribution to functioning of the system and provision of ecosystem services is missing, yet significant given their critical importance in contribution to plant health and ecosystem services.

In this thesis we investigated the impact of long-term push-pull farming on soil microbial communities. In addition, we looked at how different *Desmodium* species affected composition and diversity of soil microbial communities. The findings on the impact of push-pull farming and *Desmodium* intercropping on soil microbiome are discussed in terms of potential functioning and stability of push-pull technology as well as the prospect for harnessing similar benefits in other cropping systems. We then determined the role of whole soil microbiomes and rhizobia inoculations on volatile emission patterns from *Desmodium* spp. intercrops in greenhouse studies. Further, we analysed volatile emission patterns from *Desmodium* plants in the field. Observations from greenhouse experiments prompted follow-up studies on oviposition preference and feeding choice of the fall armyworms *Spodoptera frugiperda* between *Desmodium* spp. and maize (*Zea mays*) as well as characterisation of trichomes of *Desmodium* spp. and their role in mechanical defense against insect pests.

2. Introduction

2.1 The problem: insect pests, stem-borers and fall armyworms

Insect pests can cause immense damage on agricultural crops leading to economic losses and even social disasters, from loss of incomes to famine and conflicts. The impact is even more severe in developing regions of the world such as the Sub-Saharan Africa (SSA) where 70% of agriculture is subsistence and done by smallholder farmers (FAO 2017).

Maize and sorghum are the main staples of SSA, with about 300 million people in SSA dependent on maize as source of food and livelihood (Macauley 2015). Deleterious lepidopteran stem-borers are among the major threats to the cereal crops productivity and yields alongside declining soil fertility and variable weather patterns. Out of the 21 economically important damaging lepidopterans, the two of major concern are larvae of stalk borers *Busseola fusca* (Noctuidae) and *Chilo partellus* (Crambidae) (Kfir et al. 2002) with *Spodoptera frugiperda* (Noctuidae) joining the camp in recent years.

Lepidopteran insect pests can cause between 10 and 100% losses in cereal yield if left uncontrolled (Abate et al. 2000; Wale et al. 2006; Togola et al. 2020) with *C. partellus* alone causing between 357 and 450 million US dollars' worth of losses to smallholder farmers in East Africa annually (Pratt et al. 2017). As for the recent invasive pest, *S. frugiperda*, between 11 - 65 % maize yield losses were attributed to this pest alone across Sub-Saharan African countries during the 2017/18 and 2018/19 growing seasons (Nyamutukwa et al. 2022), not accounting for the massive impact of this species on other crops.

Damage of these pests to cereal crops is caused by their larvae stage that after feeding on foliage of the plants, some species tunnel inside the stems (Figure 1). This causes a wide range of problems including destruction of growing points, reduction of surface area for photosynthesis, early leaf senescence, stem breakage, plant stunting, lodging and direct damage to ears. The damage also interferes with movement of nutrients and metabolites, impacting grain formation (Wale et al. 2006; Mailafiya 2012; Kammo et al. 2019; Togola et al. 2020).

Figure 1. Larvae of *Spodoptera* spp. feeding on maize plants observed in farmers' fields in Tarime, Tanzania (September 2021). The larvae feed on the leaves before tunneling inside stems where they continue to cause damage until they reach the pupae stage. Pictures by Aneth Bella David (2021)

B. fusca is native to Africa while *C. partellus* and *S. frugiperda* were introduced from other places. *C. partellus*, which causes more damage to cereal crops than *B. fusca* and other stem-borers, was introduced from Asia between 1920's - 1930's and is now found across Eastern and Southern Africa, from Ethiopia to South Africa (Kfir et al. 2002). The pest has also demonstrated high colonisation fitness in eastern and southern Africa to a point of replacing indigenous stem-borers, a factor contributed by its short generation time compared to *B. fusca* (Kfir 1997) and the ability of its larvae to disperse further than other stem-borers (Kfir et al. 2002).

S. frugiperda on the other hand arrived in Africa more recently from the Americas, first being spotted in 2016 in West African countries but quickly spreading to more than 44 Sub-Saharan African countries by 2021 (Rwomushana 2020; Nyamutukwa et al. 2022). The pest is polyphagous but with a preference for plants in the Poaceae family, grasses that include cereal crops of economic and food security importance (Kammo et al. 2019; Rwomushana 2020). Estimates of losses from cereal damage by *S.*

frugiperda are variable but hard to miss. For example, Day et al. (2017) reported between US$2,531 and US$6,312 million annual losses on maize alone in just 12 African countries while the figure from Rwomushana et al. (2018) is at the range of US$1.1 - 4.7 billion. In the field, it is common to find both stem-borers and FAW infesting the same plots (Nyamutukwa et al. 2022).

A number of methods for stem-borers and FAW management have been proposed, including pesticide application, biological control, cultural control and habitat management approaches. Overall pesticide application for pest management in smallholder African agriculture remains low compared to the rest of the world, especially on food crops, due to economic, social and health concerns (Abate et al. 2000; Kfir et al. 2002; Schreinemachers & Tipraqsa 2012). However, recent reports show that farmers have no effective pest management choices other than pesticides and usage is rising in some countries (Oben et al. 2015; Sharma et al. 2019; Tambo et al. 2020; Nyamutukwa et al. 2022) and even promoted by governments to combat the pests and increase yields (Ogendo et al. 2015; Day et al. 2017; Rwomushana et al. 2018; Nyamutukwa et al. 2022). Pesticide usage for stem-borer management has, however, not reached a critical point to suppress pest populations and increase cereal yield. Besides, development of resistance is a common occurrence with sustained pesticide usage as is the case of FAW in the Americas (Day et al. 2017). Development of resistance is even more likely in the African continent with poorly regulated pesticide markets and low knowledge of application among farmers in addition to hazards to human health and environmental damage (Stadlinger et al. 2011; Sharma et al. 2019; Nyamutukwa et al. 2022). Development of resistance, combined with the environmental and health externalities, makes pesticide application a non-sustainable option.

Potential for use of botanical pesticides to control stem-borers and armyworms such as those from the neem plant (*Azadirachta indica*), *Schinnus molle* and *Phytolacca dodecandra* (Kammo et al. 2019; Sisay et al. 2019), *Tephrosia vogelii* and *Tagetes minuta* (Abate et al. 2000; Ogendo et al. 2015) has been demonstrated but uptake and wide dissemination remains slow. This is partly due to lack of investment to produce standardised effective formulations and poor regulatory frameworks in the SSA region. Further, adoption of genetically modified cereal crop varieties that can withstand stem-borer and FAW attack such as the Bt-maize has been slow in African countries, with commercialisation only in South Africa and trials in Kenya but not more countries (Calatayud et al. 2014;

Nyamutukwa et al. 2022). Apart from poor political will and the weak or non-existent regulatory frameworks for genetically modified crops in most African countries, reports of development of resistance against the Cry protein toxins expressed by Bt-maize in both America and South Africa, often within a short time after introduction, have waned appetite for adoption.

Biocontrol measures such as the use of predators, pheromone trapping as well as by nematodes and viruses have not been viable or successful options in SSA. The use of parasitoids especially those belonging to orders Hymenoptera or Diptera to suppress stem-borers has met limited success and slow rate of establishment (Abate et al. 2000). For example *Cotesia flavipes*, a parasitoid imported from Pakistan for biological control of stem-borers showed an effectiveness of just up to 55% in *C. partellus* population reduction (Kfir et al. 2002). Several reasons are hypothesised for the low effectiveness, including poor habitat stability and climatic differences. Parasitoids lack suitable habitat between farming seasons since cereal crops usually stay in the field for only a few months before being harvested. The dry spells between farming seasons, typical of the SSA weather, also reduce chances of the predators finding alternative hosts between growing seasons (Philippon et al. 2015), leading to poor establishment and thus effectiveness against target insect pests. In addition, biological control of *S. frugiperda* as a new pest has not been established in SSA, although potential exists since the presence of known natural enemies of the pest has been documented and field level parasitism has been observed (Sisay et al. 2018; Nyamutukwa et al. 2022).

Several management practices have been recommended to suppress cereal insect pest populations, such as destruction of crop residues, changing planting dates and till manipulation. However, they too show low effectiveness because they depend on diverse factors to be effective such as weather patterns and timing (January et al. 2020; Togola et al. 2020). Another set of cultural management methods includes practices such as handpicking larvae and crushing of eggs and larvae of insect pests (Nyamutukwa et al. 2022). Although cultural management practices may be relevant and economical methods of stem-borer and FAW control available for resource-poor farmers in Africa, they are also labour intensive and often incompatible with rainfall patterns (such as changing planting dates), and therefore by themselves affecting crop yields.

Alternative management options for controlling lepidopteran pests centre on habitat management approaches. These include adding diverse companion plants to the main cereal, crop such as leguminous intercrops, a practice that is common in SSA. The choice of intercrops depends on the balance between gains from insect pest control, maintenance and labour costs as well as interspecific competitions that may lower yield (Abate et al. 2000; Kfir et al. 2002; Mousavi & Eskandari 2011; Matusso et al. 2014; Bedoussac et al. 2018). Edible legumes such as winter peas (*Pisum sativum*), common beans (*Phaseolus vulgaris*), groundnuts (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*), commonly intercropped with cereals in SSA, show complementarity with cereal crops and have all been demonstrated to lower pest prevalence to a certain extent (Karel 1993; Nampala et al. 2002; Ndzana et al. 2014; Ju et al. 2019).

Noteworthy among habitat management approaches is the push-pull technology that involves cereal intercropping with non-edible leguminous plants in the genus *Desmodium* and applying a trap border crop to suppress pests (Hailu et al. 2018). Apart from being well studied for its effectiveness against stem-borers and fall armyworms, the technology provides multiple additional benefits in yield gains as well as ecological and social advantages unlike the simple intercropping techniques based on edible legumes (Pickett et al. 2014; Kassie et al. 2018; Midega et al. 2018; Niassy et al. 2022). In addition, the perennial nature of the companion crops in push-pull technology compliments well with other management practises like no- or low-till and crop residue management that are practised in conservation agriculture.

Challenges with invasive pests are predicted to get worse with changing climate patterns and increased movement of people and crop produce due to globalisation, necessitating devising urgent suppression measures that are effective and sustainable. The challenge is harder for polyphagous invasive pests such as *S. frugiperda* because of their ability to persist on diverse host plants between cropping seasons makes it harder to eradicate them. On the other hand, smallholder resource-poor farmers in low-income countries, who produce a larger share of the food consumed worldwide (between 30 - 70%) (Altieri 2009; Ricciardi et al. 2018) are the most vulnerable to such insect pest attacks. Therefore, efforts to increase productivity of food cropping systems should focus on these resource-constrained farmers by devising sustainable agricultural practices attuned to their contexts. Improvements on sustainable farming practices like the cereal push-pull technology are best positioned to help smallholder farmers increase yields while protecting the environment.

2.2 Intercropping in Sub-Saharan Africa

Intercropping is a crop diversification practice that aims at optimising agricultural production. Intercropping can be simply defined as co-existence of two or more crops in the field at the same time (Wezel et al. 2014). The goal of intercropping is to optimise the use of available spatial, temporal and physical resources to increase yield of agricultural produce (Midmore 1993; Brooker et al. 2015). Early on when plant domestication began, farmers must have realised some crops fared better together than others and adopted the practice of growing them in the same space and at the same time. The practice seeks to minimise negative interactions between plants in cropping systems and maximise positive ones.

Intercropping is especially attractive for subsistence smallholder farming in Africa where it is commonly practised alongside agroforestry (Abate et al. 2000; Brooker et al. 2015; Bjornlund et al. 2020). The practice offers diverse economic, food security and environmental benefits compared to the common intensive monoculture farming. For instance, farming in SSA depends on seasonal rainfalls that are characterised by short heavy wet periods during which crops are grown followed by long dry spells (Philippon et al. 2015; Bjornlund et al. 2020). Farmers typically have a few months to grow crops for food, feed and income generation and thus effective utilisation of available land and water resources is critical. In addition, the region's arable soil layer has been shown to be thin, prone to erosion and nutrient loss, unlike the rich soils in Europe and Asia (Bjornlund et al. 2020). Such fragile soils may not withstand heavy mechanised agriculture, typical of the green revolution agriculture practised in many developed countries. Thus, intercropping and similar practices are fitting and effective approaches for ensuring high crop yield per area with existing wetness patterns while maintaining soil stability and nutrients. The practice also reduces the need for artificial fertilisers that are not affordable to smallholder farmers and would cause more damage to soils over time.

Cereal-legume intercropping is one of the most common intercropping option among the diverse array commonly practised in SSA. Farmers in SSA usually mix cereals like maize and sorghum with legumes such as chickpeas, common beans, groundnuts and cowpeas (Karel 1993; Nampala et al. 2002; Ndzana et al. 2014; Ju et al. 2019). This allows them to diversify diets and thus nutrient sources as well as improving income prospects. Additionally, intercropping helps farmers mitigate the impact of adverse weather patterns such as drought and pest attack by having two or more crops with different resource utilisation patterns in a field (Himmelstein et al. 2017). Figure 2 depicts a mixed-cropping model integrated with animal husbandry that is common practice in SSA.

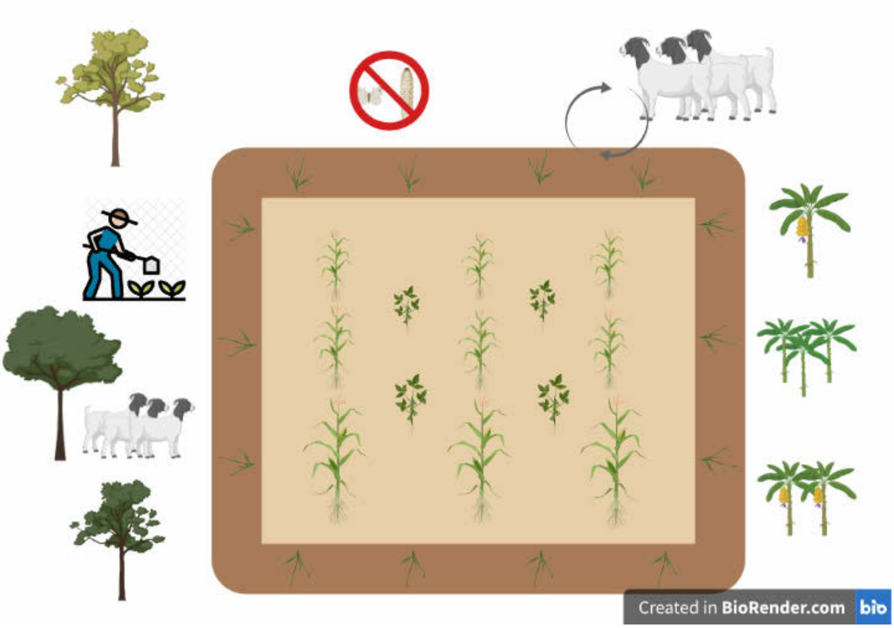

Figure 2. A farming model commonly practised by smallholder farmers in Sub-Saharan Africa. Farming is usually mixed cropping (intercropping, agroforestry) and integrated with livestock, creating a positive feedback loop where farming provides a source of food and income to farmers and feed to the animals. Manure from the animals goes back to improve soil fertility in farms. The systems are high in diversity of plants, which provides resilience against biotic and abiotic stresses such as insect pests), while helping farmers mitigate risk from adverse weather events that may impact harvest such as drought. Image by Aneth Bella David (2022)

Intercropping with perennial companion plants such as practised by the push-pull system for cereal pest management is less common. Push-pull technology was introduced to farmers in eastern and southern Africa as a method to manage stem-borers of cereals as well as the parasitic weed

Striga (Khan et al. 2000, 2002; Midega et al. 2017). The technology features the dynamics of mixed cropping in different spatial and temporal scales. It also combines several farm management approaches such as reduced tillage and off-season cover cropping geared into preserving soil structure and fertility. Uptake of the system was facilitated by the fact that agroforestry and intercropping were already common agricultural practices in the areas where push-pull was introduced, reaching more than 250,000 farmers since its establishment in the later 1990s to early 2000s (push-pull.net).

Maize push-pull technology however differs from the common intercropping practice not only in the perennial nature of the companion crops, but also their non-edible nature. While this may be among the factors limiting uptake of the technology and farmers do try to modify the practice to suit their needs (personal observations), push-pull technology does provide multiple additional ecological and economic benefits in addition to pest control. Both companion crops are good fodder for cattle, coming in especially handy during dry seasons where farmers either use them for their own animals or sell as fresh feed silage (Murage et al. 2015; Kassie et al. 2018; personal observations). In addition, the long term impacts of perennation in the field and environment become apparent with time, including preservation of the soil structure and moisture, increased organic matter as well as improvement of soil quality (Khan et al. 2016; Drinkwater et al. 2021), which are critical to the delicate soils of the region. The practice has been demonstrated to increase soil organic carbon stocks and enhance delivery of ecosystem services including increased food, fodder and fibre productivity. Further benefits in lowering mycotoxins play into economies of the cereal produce and food safety (Maxwell et al. 2018; Njeru et al. 2020). Looked at as a whole, the system delivers originally intended results, suppression of stem-borers, as well as diverse ecosystem services and keeps paying with time.

2.3 About push-pull farming for cereal pest management

The maize push-pull technology is an intercropping-based approach to management of herbivorous pests and weeds used by cereal smallholder farmers in Sub-Saharan Africa (SSA) (Murage et al. 2015; Kassie et al.

2018). The strategy combines different crop diversification practises like mixed cropping and management approaches such as reduced tillage to sustainably manage insect pests of cereals and increase yield.

In push-pull farming, an intercrop in the genus *Desmodium* spp. is grown between rows of the main cereal crop to suppress insect stem-borers (*Chilo partellus* and *Busseola fusca*) as well as the fall armyworms (*Spodoptera frugiperda*) (Midega et al. 2015; 2018), reportedly through constitutive release of repellent aerial volatiles (Khan et al. 2000; Kimani et al. 2000). At the same time, an attractive trap crop around the field attracts the pests away from the main crop and reduces larval survival through different mechanisms (Khan et al. 2010). The technology has been applauded for being affordable and effective way to increase maize and sorghum yield (up to 60% increase) by eliminating major pests of maize and sorghum from farmer fields (Khan et al. 2000).

This habitat management technology stands out from other intercropping practises common in SSA in that both the intercrop and trap crop are perennial, whereas other common intercrops are short-term food crops, for instance common beans and groundnuts. This in turn creates a system provides numerous benefits to both farmers and the environment over time. Apart from being pesticide free, reduced tillage practice and presence of cover by intercrop year round preserve moisture and soil structure (Khan et al. 2011; Campanhola & Pandey 2019). Under push-pull farming, yield increases come not only from reduced pests and parasitic weeds attack but also improved soil fertility from nitrogen fixation by the *Desmodium* spp. intercrops (Khan et al. 2014; Midega et al. 2018; Niassy et al. 2022). Smallholder farmers in the SSA region also commonly keep cattle in zero grazing fashion to supplement their income and diets. Practising push-pull farming has proved especially advantageous to farmers who also keep cattle since the technology increases their access to animal fodder especially in drier seasons of the year as both the inter- and trap crops are a good source (Kassie et al. 2018; Niassy et al. 2022).

Although individual components of the push-pull system show some benefits reducing pest populations in the farmer fields, maximum gains are demonstrably obtained from using the full package of the technology, with a repellent ‘push’ intercrop and attractant trap ‘pull’ crop. Figure 3 shows the maize push-pull technology in action.

Figure 3. Push-pull technology in action (left side pictures) compared to corresponding maize monoculture (pictures on the right) showing clear difference on maize health. The top pictures were taken at long-term trial sites at the international centre for insect physiology and ecology (ICIPE), Mbita, Kenya, while the middle and bottom pictures were obtained from farmer fields, also in Kenya. Pictures by Aneth Bella David (2017)

It’s not hard to see the appeal for the cereal push-pull farming technology; the combination of an effective and affordable restorative farming technology that contributes to ecological stability with multiple spinoff benefits has attracted a great deal of attention from researchers, farmers and even policy makers (Government of Rwanda 2011; Khan 2011; Nkurunziza 2021). While many ecological farming practices are criticised for the lack of demonstrable evidence of effectiveness in actual agro-settings, the evidence of functioning of push-pull farming in cereals has been meticulously documented for over two decades (http://www.push-pull.net/publications.shtml).

2.4 Companion crops in maize push-pull technology

The current push-pull practice for pest management in cereal farming uses *Desmodium* spp. as intercrops and *Brachiaria* cv. mulato II or Napier as trap crops. To reach this point, the evolution begins with early studies in the 1990’s that demonstrated wild forage crops *Sorghum vulgare* cv *sudanense* (Sudan grass) and *Pennisetum purpureum* (Napier grass) being more attractive to ovipositing stem-borers than maize while *Melinis minutiflora* (molasses grass) repelled the insect pests (Khan et al. 1997b; a). Although *S. vulgare* and *P. purpureum* were attractive to the stem-borers, they did not support the pest populations and either arrested their development (Napier grass) or attracted natural enemies leading to parasitisation of the larvae of herbivores, bringing down insect pests populations in the farmers’ plots (Khan et al. 2000). This was the basis and birth of a push-pull cereal pest management strategy employing stimulo-deterrent diversionary dynamics. The net impact was a significant reduction in stem-borer infestation on maize plants and thus increased yield.

Initial field observations were confirmed by laboratory studies that showed that volatile compounds from intact *M. minutiflora* plants were repellent to gravid stem-borers, but at the same time attractive to parasitoids. The volatile compounds from *M. minutiflora* were the same as those that are released by other plants after herbivory damage, termed herbivore induced plant volatiles (HIPV) and are known to be host cues for natural enemies (predator and parasites) including (3E)-4,8-dimethylnona-1,3,7-triene and β-caryophyllene (Khan et al. 1997a, 2000; Kimani et al. 2000). *M. minutiflora* was thus attractive to use for pest control because it released these compounds without herbivore damage i.e. constitutively.

Later, plants belonging to the genus *Desmodium* were identified while scouting for indigenous intercrops alternative to *M. minutiflora*. In particular, two *Desmodium* species were identified to be suitable to stem-borers suppression, *D. uncinatum* (silverleaf desmodium) and *D. intortum* (greenleaf desmodium), with the latter being more drought resistant. The mechanisms of pest suppression were described to be similar to those of *M. minutiflora*; through release of repellent semiochemicals with *D. uncinatum* reportedly emitting volatile compounds such as ocimene, nonatriene, a-cedrene and large amounts of other sesquiterpenes (Khan et al. 2000). Although not as effective at stem-borers suppression and attraction of parasitoids, *Desmodium* spp. had additional advantages over *M. minutiflora*; its ability to suppress the witchweed *Striga* spp., a devastating parasitic plant of cereals in Sub-Saharan Africa (Khan et al. 2000, 2002; Midega et al. 2017), resource complementarity with the main crop through nitrogen fixation (Khan et al. 2011; Campanhola & Pandey 2019) and ease of management between rows of maize. Later *Brachiaria* spp. was added to the list of companion crops as a trap crop for its tolerance to drought compared to Napier and Sudan grass (Khan et al. 2016). Figure 4 shows the intercrop greenleaf desmodium growing in the field.

However, the fact that *P. purpureum* is one of the host plants of *B. fusca* (Calatayud et al. 2014) and *Brachiaria spp.* for *S. frugiperda* (Nyamutukwa et al. 2022) brings into question the potential of the trap crops to carry over the stem-borer populations between seasons after maize harvest and thus removal of the main host. Calatayud et al. (2014) also questioned the evidence put forward describing mechanisms of push-pull farming, citing the rigorous host selection behaviour of gravid *B. fusca*, it seems unlikely that the stem-borers would lay eggs on a non-host plant that does not guarantee survival of their offspring. In addition, Finch and Collier (2012) questioned the validity and evidence of the purely chemical ecology based mechanisms of action in push-pull technology, arguing for a more biological perspective. Studies documenting impact of trap crops used in push-pull farming on stem-borers and FAW populations within and between seasons and subsequent cereal crop damage and yield loss are lacking.

Further, while there is sufficient evidence to support the benefits of push-pull farming on cereal yield increases and ecosystem benefits, the exact mechanisms of pest repellency via aerial volatiles of *Desmodium* spp. are not clear, necessitating the need for deeper investigation. In addition, the impact of the push-pull technology on belowground communities and their potential impact on volatile emissions from *Desmodium* spp. intercrops has not been investigated.

Figure 4. Greenleaf desmodium (*Desmodium intortum*) growing in a farmer's field in Tarime, Tanzania (left) and a close up view of a flowering desmodium plant trapping insects (right), also in farmer field in Tarime, Tanzania. Pictures by Aneth Bella David (2021)

2.5 The role of soil microbiome with special reference to cereal-legume intercropping; aboveground-belowground interactions and plant-soil feedback loops

Initial studies on push-pull technology focused on above ground components of the system, elucidating mechanisms and quantifying social and economic benefits. Recently, there has been some effort to include belowground activities and their contribution to not only crop productivity, but also long term ecological sustainability (Mutyambai et al. 2019; Drinkwater et al. 2021). This has evolved alongside a general holistic or systems approach to food production, where non-monetary benefits including social and environmental gains are considered when investigating and advocating farming practices.

Despite the shift, not much has been done with regard to the interactions between push-pull technology and belowground microbial communities. A few studies have looked at the impact of the technology on mycotoxins levels and mycotoxin producing fungi (Maxwell et al. 2017, 2018; Njeru et al. 2020) and plant-soil feedback impact on maize volatile emissions (Mutyambai et al. 2019), but in depth investigation is still lacking.

Belowground communities, especially soil microorganisms interact with plants via the root system to influence plant health in diverse ways, contributing to provision of ecosystem services that are critical to the stability of agro-ecological systems. Apart from the well-studied role of legume-associated rhizobia in nitrogen fixation and subsequent improvements in soil fertility (Andrews & Andrews 2017), soil microorganisms also help protect plants from disease causing pathogens, assist in acquisition of nutrients such as iron and phosphate from the soil and even modulate plant immune response through induced systemic responses (ISR) (Hayat et al. 2010; Mitter et al. 2013; Reva et al. 2020).

In recent years, studies have emerged that demonstrate the role of soil microorganisms and endophytes in modulating aboveground plant-insect relationships. Pangesti et al. (2013), Rashid & Chung (2017) and (White et al. 2019) review these tri-trophic interactions and their potential applications in cropping systems. General patterns of belowground-aboveground soil microbes-plants-insect interactions have started to become apparent. Plant growth-promoting rhizobacteria (PGPR) have been shown to negatively affect herbivore performance or abundance (Heinen et al. 2018), while mycorrhizal fungi seemingly to negatively affect generalist leaf chewers but having neutral or positive effect on specialist leaf chewers and phloem feeders. The impact of endophytes on phloem feeders seems to be drought dependent (Pineda et al. 2013; Heinen et al. 2018). These findings are promising, but they are still at an early stage of exploration, far from application in agricultural fields.

With regard to plant-soil microbes interactions, a general trend has been to understand interactions between plants and individual microorganisms, such as plant growth promoters such as *Bacillus* spp. and *Trichoderma* spp. and not whole soil microbial communities interactions. But this is changing as it has become apparent that microbes exist and function in complex networks and communities, making it important to understand their interdependence, network effect and functional redundancy.

The interaction between soil microbes and plants has been investigated in several other cereal-legume intercropping systems. Findings from both short term and long-term experiments consistently show that apart from increased yield, legume intercropping increases abundance and diversity of soil microbial groups, often enriching some of the groups of soil microorganisms. The shifts in soil microbial communities are often associated with changes in soil fertility, nutrient accessibility as well as increased enzyme activity. For example, intercropping sugar cane (*Saccharum* spp.) with legumes was shown to increase diversity and activity of soil microbes (Lian et al. 2019; Solanki et al. 2020; Malviya et al. 2021), which influenced increases in organic and dissolved carbon, total and available nitrogen as well as soil enzyme activity (Lian et al. 2019; Malviya et al. 2021). Other studies show the impact of peanut (*Arachis hypogaea*) intercropping with different crops such as cassava (*Manihot esculenta*) (Tang et al. 2020) or maize (*Zea mays*) (Li et al. 2018) where similar observations were made in both studies; higher overall abundance and diversity of soil microorganisms in intercropping practices, enrichment of beneficial microbial taxa and associated improvements on soil nutrients quantity and availability.

These and similar studies highlight the positive impact of legumes intercropping on soil microbial communities, which in turn affect soil health properties and subsequently, plant health and productivity. In push-pull technology, research has been concentrated on functioning and interactions between above ground part, neglecting below ground shifts and their impact on both plant health and ecosystem services provision.

3. Aims and objectives

Plant-soil microbial interactions have been investigated in intercropping and other farming practices in order to harness them for crop productivity. The interactions have been demonstrated to create lasting impacts in the soil and often leaving a legacy that may last for several subsequent cropping cycles growing in the same space, a phenomenon known as plant-soil feedback mechanisms (Veen et al. 2019). This has made it increasingly important to understand the impact of and interactions between agronomic practices and soil microbiota, given the critical role they play in influencing plant health and provision of ecosystem services. For the maize push-pull technology, most research has concentrated on ascertaining the agronomical gains, social and economic benefits as well as mechanisms of functions of aboveground parts. Constitutive release of volatile compounds from the intercrops, *Desmodium* spp., is reported as the chief mechanism of insect pest repellency. The volatile compounds are typically released by plants that have been challenged (induced) by herbivore damage. Literature on push-pull technology is devoid of studies on the interactions between the system and soil microbial communities, missing an opportunity to discern the impact of a relatively stable soil environment created by perenniation on soil microbial populations, and how they in turn shape plant health.

The aim of this study was therefore to first investigate the impact of long term cereal push-pull farming on soil microbial communities, and second in turn to determine whether the soil microbiome associated with *Desmodium* spp. played a role in the constitutive release of reported repellent volatile compounds and thus contributing to functioning of the technology. We also aimed at comparing the impact of different *Desmodium* species on soil microorganisms to inform future choices of intercrops for push-pull technology. Observations made from both the greenhouse and field studies on impact of soil microbiome on *Desmodium* spp. volatile emissions prompted further investigations into the attractiveness of *Desmodium* spp. as an oviposition host to adult moths (*Spodoptera frugiperda*) as well as a food source for resulting larvae compared to maize. We also assessed the physical properties of trichomes covering *Desmodium* spp. plants and their potential for reducing pest populations in the field.

4. Summary of findings and discussion

4.1 Long-term maize-*Desmodium* intercropping shifts structure and composition of soil microbiome with stronger impact on fungal communities

The initial goal of this research project was to investigate the impact of maize push-pull technology on soil microbial communities and how the microbes were linked to functioning of the technology and other ecosystem services that are rendered from using the technology. We began by evaluating the impact of long-term *Desmodium* intercropping and push-pull farming practices (14 - 18 years old plots) on soil microbial communities.

We show that long-term continued push-pull practice in maize farming causes a divergence in the composition and abundance of soil microbial taxa compared to monoculture. A general pattern that emerged is that of push-pull intercropping having a strong impact on abundance, richness and diversity measures of fungal taxa than bacteria. This signals a lower contribution of bacterial communities to the overall differences in abundance and diversity of soil microbes between push-pull and monoculture practices. Overall, fewer bacteria taxa were significantly enriched across all treatments (Figure 5). In this study we also uncovered taxa that were enriched in both maize monoculture and intercropping treatments. A large proportion of the enriched taxa were of fungal origin. We then postulate potential functional diversity of the enriched taxa in provision of ecosystem services focusing on the high abundance of saprophytes, endophytes as well as bio-protective fungi observed (Paper I).

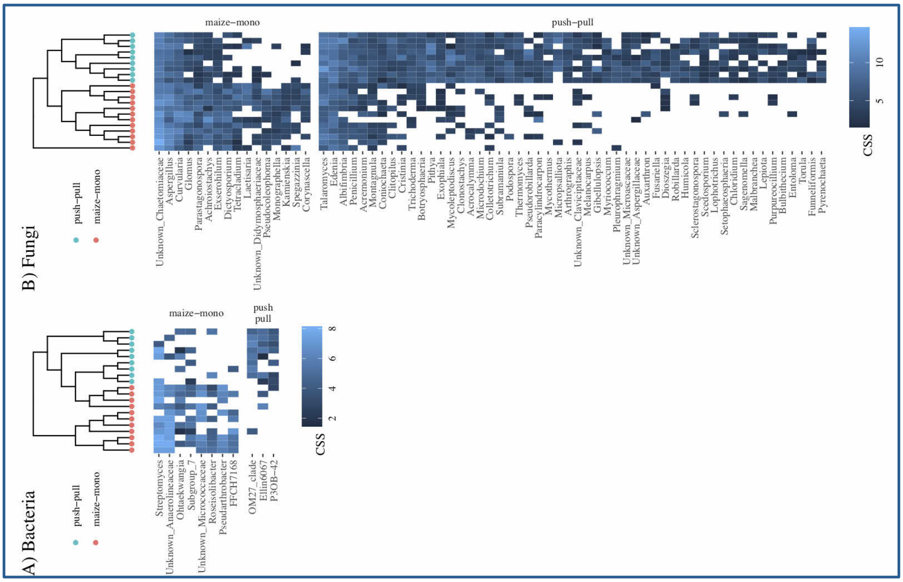

Figure 5. Differential abundance of soil bacterial (A) and fungal (B) taxa in long-term monoculture and push-pull/*Desmodium* intercropping plots. Push-pull farming/*Desmodium* intercropping enriched abundance and increased the diversity of fungal taxa than bacteria (Adapted from Mwakilili et al. 2021)

This is the first study reporting the impact of long-term push-pull and *Desmodium* intercropping on soil microbial communities, showing the benefits of perennial/long-term intercropping on formation of soil microbial populations. Legume plants are the most common type of intercrop in smallholder farming in SSA for their numerous benefits. They are not only nutritious (higher protein content to complement the starch cereal based diet) but also provide numerous ecosystem benefits beyond the obvious ones including increasing abundance and diversity of soil microorganisms, improving soil structure and fertility, protecting soil from environmental elements like wind and rain water, and more importantly, helping restore soil fertility through nitrogen fixation.

Other studies investigating the impact of intercropping on soil microorganisms uncovered similar patterns. Debenport et al. (2015) showed that long-term intercropping of millet (*Pennisetum glaucum*) and woody shrubs not only lead to higher millet yields but also increased the diversity of both bacterial and fungal soil communities, enriching some taxa at the same time. Experiments with leguminous intercrops has also been shown to cause shifts in composition and structure of soil microbial communities, which often go hand in hand with increases in soil nutrient stocks and availability (especially nitrogen, carbon and phosphorous) as well as higher enzymes activities in the soil, such as in the case of millet (*Panicum miliaceum*) and mung bean (*Vigna radiata*) intercropping (Dang et al. 2020) or melon (*Cucumis melo*) and cowpeas (*Vigna unguiculata*) intercropping (Cuartero et al. 2022).

Plants have evolved the ability to recruit microorganisms that are beneficial to their health, with unintended but often advantageous impacts to the ecosystem such as nutrient cycling and carbon sequestration. While considering choice of companion plants in intercropping systems it is important to also consider resultant belowground interactions to ensure compatibility and harness additional benefits that may emerge such as nutrient cycling and disease suppression. Perennial intercropping systems such as the *Desmodium*-based push-pull technology are especially attractive in conservation agriculture practices because they provide a relatively undisturbed soil environment for microbial communities and networks to form and stabilise, increasing rewards to the ecosystem. Future studies may investigate the role of soil microbiome on soil health and disease suppression in perennial intercropping settings.

4.2 A comparative study of the impact of five *Desmodium* species on soil microbiome reveals enrichment of select bacterial and fungal taxa

In the second objective, we investigated the impact of short-term cultivation of five different *Desmodium* species with different agronomic properties, on soil microbial communities. The *Desmodium* species were under consideration as additional intercrops in maize push-pull technology to fit wider agro-ecological zones. This study sought to complement other criteria such as agronomic performances during the selection process by informing on their impact on soil microbial communities.

Here we show that there were large overlaps in the comprising taxa associated with the *Desmodium* species, with a few unique taxa linked to each *Desmodium* species or shared between species (Figure 6). While the different *Desmodium* species seemingly exerted limited influence in diversity, richness and evenness indicators of microbial communities, differential abundance showed a slightly bigger impact on fungal communities than bacteria as reflected on the number of enriched taxa. This observation is consistent with findings made in our previous study (Appendix 1) on impact of maize push-pull farming and *Desmodium* intercropping on soil microbial communities. Contrary to expectations however, there was a high abundance of potentially pathogenic fungi like *Fusarium* and *Aspergillus* (Paper II).

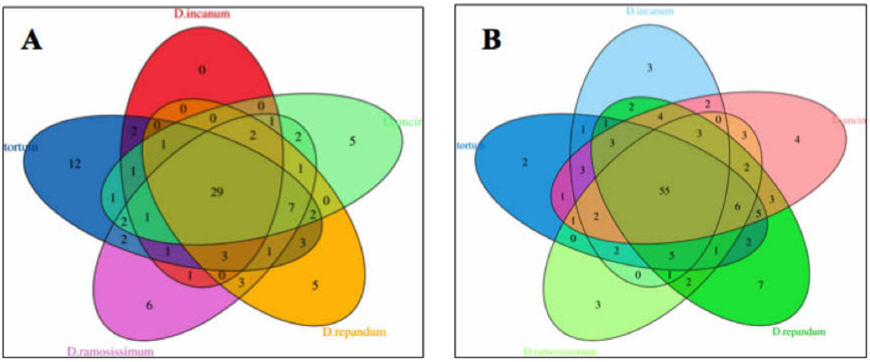

Figure 6. The composition of both bacterial (A) and fungal (B) taxa overlapped among the *Desmodium* spp. Plots, forming a large core microbiome group. Only a few taxa were uniquely associated with specific *Desmodium* species. This highlights a moderate influence of the species on soil microbial taxa for the cultivation period (Adapted from manuscript David et al. 2022)

This is the first study to compare the impact of different *Desmodium* species on soil microbial communities. Several *Desmodium* species, in particular *D. intortum* (greenleaf desmodium) and *D. uncinatum* (silverleaf desmodium) are important intercrops in the maize push-pull technology for insect pest management (Khan et al. 2010; Midega et al. 2018). The technology is effective, affordable and environmentally friendly, currently utilised by more than 250,000 farmers in eastern and southern Africa (http://push-pull.net/), the number is growing. Other studies on interactions between *Desmodium* spp. and soil microorganisms have focused on endophytes and nodule symbionts, with *Rhizobium*, *Bradyrhizobium* and *Mesorhizobium* being dominant taxa (Parker 2002; Xu et al. 2016; Toniutti et al. 2017).

The soil is a rich and diverse ecosystem harbouring a large biota. The soil biota, in particular soil microorganisms such as bacteria and fungi, play a critical role in the maintenance of soil health and thus supporting plant health and productivity. They are responsible for decomposition of organic matter, nutrient cycling and carbon sequestration (Jacoby et al. 2017; Saccá et al. 2017), with some groups of microorganisms interacting with plants directly and indirectly thus impacting plant health (Hayat et al. 2010; Mitter et al. 2013; Reva et al. 2020). Plants have in turn learned and evolved with soil microorganisms, recruiting those which are beneficial to them through the action of root exudates, modulating the composition depending on plant stage and needs. Thus when selecting companion crops to be planted together, such as in intercropping systems, it is critical to also evaluate compatibility for belowground interactions with soil microorganisms to ensure plant health.

In our previous study (Paper I) we discovered that long-term push-pull farming and *Desmodium* intercropping caused a clear divergence in composition and abundance on soil microorganisms compared to monoculture. The lack of significant differences in composition, structure and diversity of soil microbiome between *Desmodium* spp. plots and the bulk soil observed in this study may indicate that the shifts in soil microbial communities as a result of *Desmodium* spp. cultivation and thus associated ecosystem services take time to solidify. This is an important consideration in the context of soil conservation and restoration and conservation agricultural practices, and for farmers to have realistic expectations of gains in yield and improved soil health as well as harness benefits from the environment. Future studies may focus on quantifying microbial populations by using techniques that differentiate between active and non-active microbial taxa to further inform on the microbial shift process.

4.3 The push-pull intercrop *Desmodium* does not repel, but intercepts and kills pests

After the observations made in the previous above-mentioned studies, the next goal was to determine the impact of the soil microbiome and rhizobial inoculations on volatile emissions patterns by *Desmodium* spp. Surprisingly however, we observed no constitutive release of repellent volatiles from *D. intortum* and *D. uncinatu*m regardless of presence or absence of microbiome and inoculation by rhizobia. Upon herbivory of *Desmodium* spp. by *Spodoptera frugiperda*, slight increases in volatile emissions were observed (Figure 7a and 7b), but still at low levels compared to maize (*Zea mays*) and *M. minutiflora*, the later having been proven to constitutively release a wide array of insect-repellent and parasitoid-attractive volatiles in the past (Tolosa et al. 2019). Further, the amount of volatiles was negligible after seven days (Figure 7c).

These observations were made on greenhouse plants, we therefore collected and analysed *Desmodium* spp. volatile data from farmers' fields in Tanzania and Uganda. Similar observations were made; volatile emissions from *Desmodium* spp. were very low, even though some of the *Desmodium* plants sampled had clear signs of insect damage and thus potentially induced (Paper III). These low quantities of terpenoid volatiles emitted by *Desmodium* plants in the greenhouse and field conditions are not sufficient to support the chemical ecology-based pest repellent mechanisms in maize push-pull systems put forward thus far.

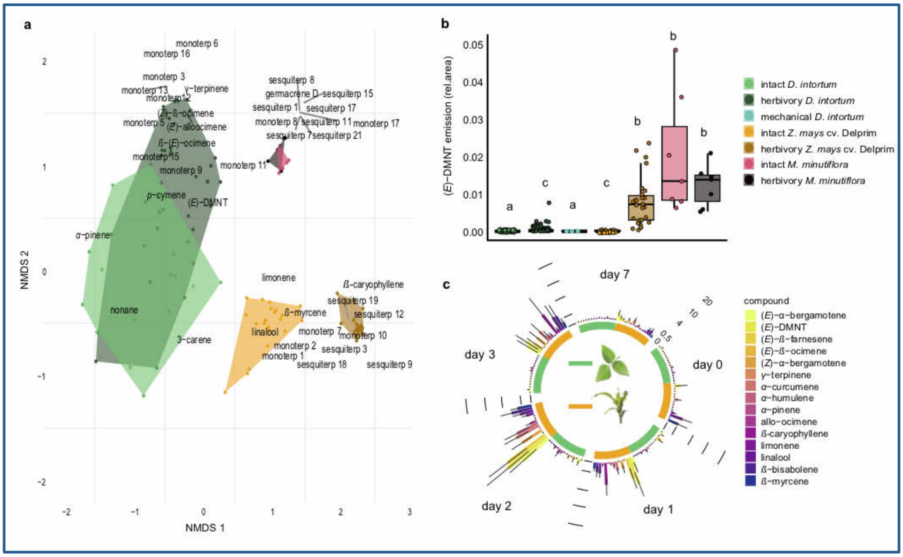

Figure 7. a) NMDS plot showing volatile emissions from *Desmodium intortum*, maize (*Zea mays*) and Melinis minutiflora both before and after herbivory, as well as after mechanical damage of D. intortum b) Relative amounts of E-DMNT released from *D. intortum*, maize and M. minutiflora both before and after herbivory, as well as after mechanical damage of *D. intortum*. E-DMNT is a key terpenoid volatile compound implicated in stem-borer repellency and attraction of parasitoid/natural enemies in the field c) Emission patterns of terpenoid compounds from *D. intortum* and maize over a course of 7 days post herbivory by *S. frugiperda*

The observed low constitutive volatile emissions from *Desmodium* spp. was surprising because literature on the maize push-pull technology has repeatedly reported the chemical volatile compounds as the chief mechanism through which the intercrop repels adult moths from laying eggs on host cereal plants (Khan et al. 2000, 2006). We therefore went further and assessed oviposition preference of adult *S. frugiperda* between maize and *Desmodium*. The experiments revealed equally interesting and unexpected findings; although *S. frugiperda* had a slightly higher tendency to lay eggs on maize (*Zea mays*) plants, they did not completely avoid *Desmodium* spp. and laid eggs on them as well (Paper III).

In addition, in feeding choice assays, neonate larvae of *S. frugiperda* preferred *Desmodium* spp. diet to maize, although their development was severely diminished that none of them went to pupa stage (Figure 8).

However, later stage larvae had a difficult time feeding and moving on *Desmodium* spp. plants, frequently being found stuck on stems and some completely immobilised to death (Figure 9c and 9d).

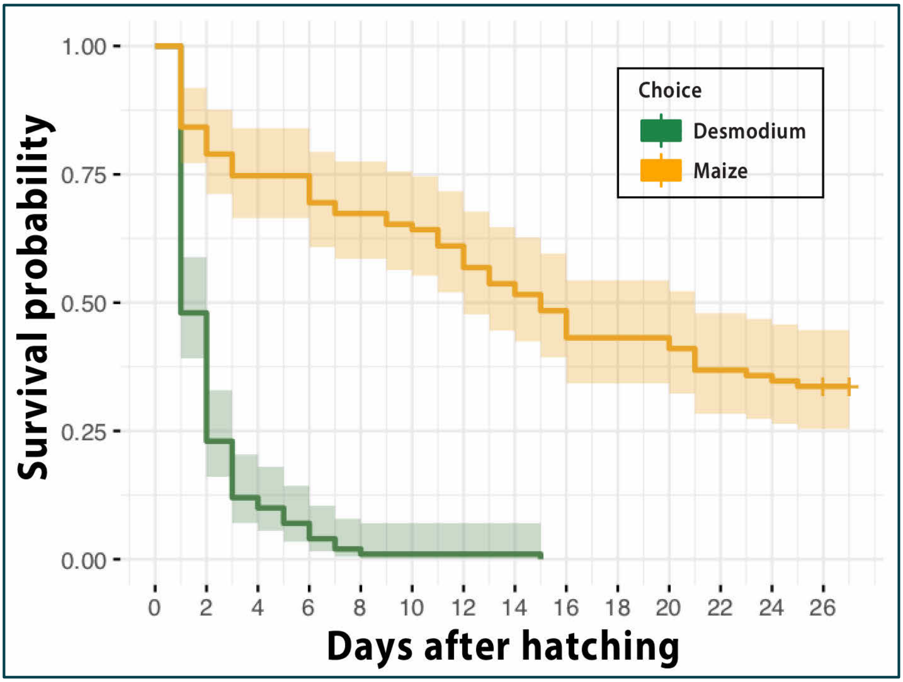

Figure 8. A chart of survival of neonate larvae of *Spodoptera frugiperda* on *Desmodium spp*. and maize (*Zea mays*). When the larvae fed on *Desmodium spp*. there was a sharp drop in the number of insects early on caused by high mortality rates. None of the larvae on *Desmodium spp*. diet reached pupa stage.

Further analysis and microscopic observations showed a mesh of straight and hooked trichomes of varying length covering *Desmodium* spp. stems and leaves (Figure 9a and 9b). Scanning electron microscope (SEM) showed that the sharp, glass like trichomes were composed of high amounts of Silicon (Si) (Figure 9e and 9f).

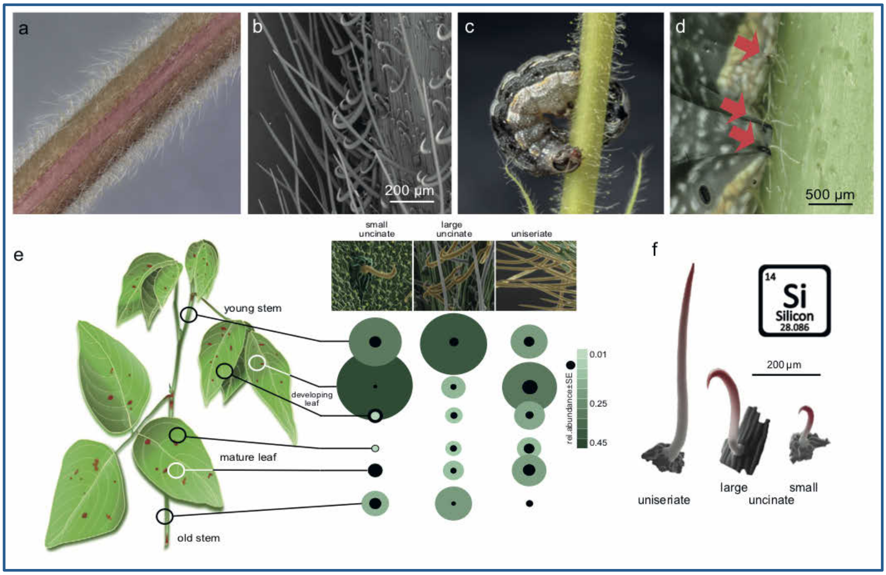

Figure 9. a) The dense networks of straight and hooked trichomes covering the stem of *Desmodium intortum* b) Trichomes of *D. intortum* under scanning electron microscopy (SEM) c) *Spodoptera spp.* larvae trapped by trichomes on *D. intortum* stem d) A close look of *D. intortum* trichomes piercing through the body of A *Spodoptera spp.* larvae e) & f) Quantification of the different types of trichomes on different parts of *D. intortum*

Based on these findings and observations, we propose a new set of mechanisms of action describing how pest populations and thus subsequent damage on the main cereal crop are reduced in push-pull technology using *Desmodium* as an intercrop.

First, *Desmodium* spp. seem to reduce insect pest populations in the field through trapping and immobilisation of the insects through a network of straight and hooked trichomes on its stems and leaves surface. This may impede the movement of spreading larvae from reaching more maize plants, especially in cases where larvae spread over a wide area like that of *S. frugiperda* (Kammo et al. 2019) or under high population pressure and larvae seek more plants to avoid competition (Calatayud et al. 2014).

Second, once trapped, the high amount of Silicon (Si) compounds making up the trichomes makes *Desmodium* spp. a poor diet when the larvae try to consume it. This slows or stops development of larvae entirely, thus further reducing insect pest populations below the economic threshold. Si content is one of the most important factors determining larval choice of host plant, with high Si accumulation and polymerisation on tissues increasing plant resistance to insects (Reynolds et al. 2009; Calatayud et al. 2016). In Juma (2012), silica content was demonstrated to be a significant barrier to dietary adaptation by *B. fusca* larvae potentially due to its impact on the insect mandibles that are worn off from the leaf abrasion upon continued consumption of plant parts with high Si content (Calatayud et al. 2016) as well tearing the gut lining.

Last, in general, the practice of mixed-cropping has been shown to reduce pest and general insect population densities in fields. The reduction in pest populations is achieved by either dilution or barrier effects where both the number of and access to suitable hosts is reduced. Presence of intercrops act to reduce pest populations by disrupting their ability to find oviposition hosts through physical, visual and masking effects especially when plants are young (Bybee-Finley & Ryan 2018). These theories are supported by observations of insect behaviour made in other intercropping settings including pheromone tracking behaviour of moths (Pearce et al. 2004; Vickers 2006), nectar searching by bumble bees (Nakamura & Kudo 2016) and host searching behaviour by parasitic wasps, where residence time of ‘foraging’ insects in a patch depends on the encounter rate of stimuli (such as filaments of pheromone, nectar-rich flowers, or prey). In the same vein, bark beetle attacks of spruce are significantly lower in mixed stands with deciduous trees than in spruce monoculture (Berthelot et al. 2021).

In the field, the additional benefit of improved soil fertility particularly in perennial legume intercropping such as in push-pull systems may further contribute to plant health and vigour thus reducing the impact of pest attack on cereal crops (Calatayud et al. 2014). Besides, plant volatiles appear to be effective pest repellents for a short distance only hence not able to prevent the insects from entering the field (Finch & Collier 2012).

The new proposed mechanisms, especially those related to insect entrapment and poor diet source warrant further investigations in the field to assess the mechanical barrier effect of not only *Desmodium* spp., but also other intercrops, with major focus on perennial, leguminous intercrops to maximise ecological benefits. In addition, the behaviour and ecology of the major insect pests of maize (stem-borers and FAW) is not uniform, with differing host ranges and competitiveness. Evidence from the field will be critical to ascertain effectiveness of the proposed mechanism in suppressing the differing insect pests as well as its impact on cereal yield, which is what farmers look for. Eventually, clearly described mechanisms may allow incorporation of food crops as intercrops that also help to significantly reduce pest attack, thus promoting food security and improved livelihoods. This may also allow expansion of the strategy into other cropping systems removing the limitation of the current push-pull technology applicability on large-scale farming.

5. Conclusions and future perspectives

In this study, we initially aimed at investigating the impact of long term maize push-pull intercropping practice on soil microbial communities and the potential role of the microbes in repellent volatile emissions by *Desmodium* spp. intercrops. We also aimed at comparing the impact of different *Desmodium* species with potential for integration into the push-pull system on soil microbial communities.

We show that long term maize push-pull intercropping (14 - 18 years) increases abundance of fungal taxa and diversifies soil microbial communities with a stronger influence on fungal than bacterial communities. We also uncover taxa that were enriched in both push-pull and maize monoculture practices, with the majority of those enriched in intercropping plots being fungi. The higher diversity in soil microbial communities may contribute to stable soil biota with diverse ecosystem benefits such as nutrient cycling and suppression of diseases causing taxa.

On the investigation of the impact of short-term cultivation (2 years) of five *Desmodium* species from different agro-ecological zones on soil microbial communities, we observed large overlaps in the composition of soil microorganisms associated with the *Desmodium* species, and no significant differences in the diversity and richness of the soil microbiome between the species. However, several taxa were enriched in different *Desmodium* spp. plots, and consistent with previous observations, the impact was stronger on fungal than bacterial communities. Contrary to expectations and previous findings, we observed a high abundance of *Fusarium* spp. and *Aspergillus* spp. Future studies on selecting intercrops should consider impact on and complementarity with soil microbial communities due to their role in provision of ecological services such as provision of nutrients to plants and suppression of pests and diseases.

We further conducted studies to determine the impact of soil microbiome and rhizobia inoculations on volatile emissions of *Desmodium* spp. To our surprise, we discovered that in greenhouse settings both *D. intortum* and *D. uncinatum* do not constitutively release large amounts of volatile terpenoid compounds previously widely reported. These findings were corroborated by data collected from the field. Further, we found out that adult moths (*Spodoptera frugiperda*) are not deterred from ovipositing on *Desmodium* spp. and their neonate larvae preferred *Desmodium* spp. to maize as a source of food although it does not support their growth and curbs their development. Older larvae found it difficult to move on *Desmodium* spp. often getting stuck on the plants for days before eventually dying. A deeper look on the leaves and stems of *Desmodium* spp. revealed a network of sharp, silicon-rich trichomes that was responsible for impeding larval movement. The trichomes also impair ingestion of the leaves and their digestion thus making *Desmodium* spp. a poor diet source.

In the light of these observations, we thus conclude that in maize push-pull technology, *Desmodium* spp. do not repel ovipositing adult moths through volatile emissions, but rather act as a mechanical barrier to dispersing larvae in addition to being a poor diet source. In addition, we hypothesise that intercropping maize and sorghum with *Desmodium* spp. reduces the chances of ovipositing adult moths from finding the main host plant potentially through barrier and masking effects as well as false landings on non-host that lower host searching success as observed in other crop diversification approaches.

The cereal push-pull technology is an effective eco-technology for pest and weed management in smallholder cereal farming in Sub-Saharan Africa. The technology offers multiple ecological benefits apart from increased grain yield including increasing diversity of soil microbial communities as shown in this study.

This thesis has brought forth a new paradigm on the functioning of the lauded push-pull technology for cereal pest management. Further studies should focus on testing the proposed mechanisms of action in field settings as well as investigating alternative intercrops (such as *Canavalia* spp. and others (Figure 10)). *Desmodium* is a non-edible intercrop, a factor that has impacted adoption of the technology since smallholder farming in SSA involves intercropping with edible legumes to diversify diets and reduce risk of crop loss. Incorporating edible leguminous intercrops with pest suppression properties based on the new proposed mechanisms into the practice will be more beneficial to farmers, providing both benefits of combating pests and improved food security. Due to the benefits of perennation observed in this study and elsewhere, emphasis should be placed on perennial intercrops adapted to specific contexts. This also opens up avenues for investigating high value legume crops that are edible but not commonly incorporated in diets in SSA or non-edible legume crops with commercial value.

Figure 10. Maize (*Zea mays*) intercropped with *Canavalia* spp. to investigate pest and weed suppression potential at Vi Agroforestry centre in Mara, Tanzania. Pictures by Aneth Bella David (2021)

6. Significance of the study

Smallholder farmers produce more than 50% of the food consumed worldwide, using less inputs and mechanisation (http://faostat.fao.org/) leaving a significantly lower damage to the environment. Most of these smallholder farmers are in rural, poor communities in the developing regions of the world, such as the Sub-Saharan Africa (SSA).

There is vast room to increase yield potential of smallholder farming through incremental improvements in the functioning of conservational diversified agricultural practices such as the push-pull technology for cereal pest management. These are likely to have more impact on food security and rural livelihoods with less environmental damage compared to intensification approaches, characteristic of cropping systems in many developed countries.

Findings of this study take a step further in understanding the impact of perennial intercropping systems on soil microbial communities, which have been shown to impact plant health and play critical roles in provision of ecosystem services. This is a starting point on harnessing aboveground-belowground interactions for restorative and sustainable crop production by optimising food production and provision of ecosystem services and stability.

Importantly, the study brings forth alternative mechanisms of pest management via intercropping with *Desmodium* spp. in push-pull technology. After years of research into the popular and effective cereal pest management technology, the technology has expanded and adopted by more farmers in east and south of Africa while inspiring more research to potentially translate the reported mechanisms in other agro-ecological settings. Lack of clear mechanisms of action of push-pull technology may have thrown off-track efforts to replicate the technology in other food production systems as well as causing loss of resources by trying to replicate pest repellency through volatile emissions.

Understanding the actual mechanisms of action and providing empirical evidence will help expand the benefits of the technology to wider contexts and maximise output with minimal resources. A deeper understanding of food cropping diversification strategies will also help to create transferable technologies that render diverse ecosystem services such as in the case of the cereal push-pull system.

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Popular science summary

Maize is a major staple crop in Sub-Saharan Africa (SSA), providing food and livelihood for about 300 million people in the continent. Among the major production constraints facing smallholder farmers of maize are the lepidopteran pests i.e. stem-borers and fall armyworms. Effective management of the pests by using chemical pesticides is hampered by the low income of the farmers as well as environmental and human health concerns associated with chemical inputs usage. Farmers in SSA therefore rely on a range of management approaches to control the pests, among them, polyculture practices such as intercropping and agroforestry.

Intercropping involves growing two or more different crops in the same piece of land at the same time. Smallholder farmers in SSA commonly practice intercropping where cereal crops are grown with legumes such as common beans, cowpeas and groundnuts. The practice helps farmers increase food diversity as well as mitigate crop loss risk in the event of adverse weather patterns such severe drought and flooding. The practice has also been shown to reduce insect pest populations compared to monoculture and thus increase yield.

Among the intercropping practices practiced by smallholder farmers in SSA is the cereal push-pull technology. The technology involves intercropping a leguminous plant, *Desmodium*, between rows of the cereal crop, and planting a grass border crop such as Napier *and Brachiaria*. The intercrop is said to repel insect pests from farmers' plots via aerial chemical compounds, while the trap crop, which is more attractive to the insects than the main crop, attracts them although it does not support their development.

Farmers in eastern and southern Africa employ the technology to control major pests of maize and sorghum, stem borers (*Busseola fusca* and *Chilo partellus*) and the invasive fall armyworms (*Spodoptera frugiperda*). If left uncontrolled, these pests can lead up to 100% cereal crop losses, devastating farmers and impacting food security. In addition, the technology effectively suppresses the parasitic witchweed, *Striga*, from the root exudates of the intercrop *Desmodium*. The main outcome of using the technology is increased cereal yield and with it improved food security and livelihood of the farmers. So far more than 250,000 farmers in Kenya, Tanzania, Uganda, Rwanda and Zambia have adopted the technology, with plans to disseminate the technology in Ethiopia and other southern African countries. The technology is an effective conservation agricultural approach to the management of major pests of cereal fitting for smallholder farming in SSA.

The appeal of the technology also comes from synergizing farmer needs with environmental sustainability. To farmers, besides crop protection, push-pull technology provides fodder year-round and additional income from selling seeds of the intercrops *Desmodium* and cuttings of Napier/*Brachiaria*. Environment-wise, push-pull technology has been shown to provide diverse ecosystem services including increasing soil organic matter, improving soil fertility through nitrogen fixation, conserving soil moisture and preserving soil structure.

Diverse aspects of functioning of the technology have been studied, however, little attention has been paid to the interaction with the soil microorganisms. The goal of this study was to investigate how the soil microbiome changed due to intercropping with *Desmodium*, and whether the soil microorganisms played a role in the ecosystem services that have been reported for the system, with an emphasis on the release of the reported aerial chemical compounds from the intercrop *Desmodium* that are responsible for repelling insect pests from farmers' fields.

We first investigated the impact of long term (14 - 18 years) maize push-pull farming on soil microorganisms compared to monoculture. We found out that long-term application of the push-pull technology increases the diversity of soil microorganisms, favouring fungi over bacteria microbes. The increased diversity of soil microbes has potential benefits in stabilising the belowground ecosystem and providing benefits associated with diverse microbial communities such as carbon sequestration, nutrient cycling and suppression of soil-borne diseases and pathogenic microbes. Also, we show that these changes in soil microbial communities take time to form thus strengthening the benefits of perennial intercropping practice.

When studying the role of soil microbes on the release of repellent chemical compounds previously reported to be released by the *Desmodium* intercrop, we came across surprising results. We found that *Desmodium* does actually not release the chemical compounds without a trigger, and when insect larvae induce them to do so, only small increases were observed, too low to effectively "push" insects from the field. Building on this observation, we then investigated the ability of the intercrop to ward off adult insect moths from laying eggs. Interestingly, we also found that insects laid eggs on *Desmodium* plants, which is unexpected if the plant was truly repellent.

We then went further and looked at the suitability of *Desmodium* as a source of food for the moth larvae. Here we found that the early stage larvae preferred the intercrop to maize, but they could not survive on it as they died before reaching pupal stage. The impact was worse on older larvae, which in addition to finding *Desmodium* as an unsuitable diet source, also struggled to move along the plants, getting trapped and immobilised. Further investigation revealed this was because of a dense network of plant hairs covering *Desmodium* leaves and stems. The plant hairs, also known as trichomes, were found to be made up of high silicon (Si) content, which contributes to making *Desmodium* a bad diet source by making its leaves and stem tough for the insects to chew and digest.

Based on these findings we propose new mechanisms of pest repellency in maize push-pull farming practice. We propose that *Desmodium* suppress insect pests by 1) restricting movement of larvae by trapping them as they move between maize plants, and 2) by being a poor diet source. In addition, the general practice of intercropping has been shown to reduce insect pest attack on the main crop through a number of mechanisms, including masking and barrier effects that make it harder for a pest to find its host plants.

These novel findings require further testing in the field to be verified. They also open an opportunity to evaluate edible intercrops with similar properties to replace *Desmodium*, which being non-edible reduces adoption rate by farmers and poses management challenges. Focus should be on perennial intercrops due to their additional benefits to farmers and the ecosystem. The push-pull technology described here is an effective and ecological approach for pest management in smallholder farming in SSA. Clearly understanding mechanisms underpinning its functioning would allow the technology to be adopted in other agro-ecological settings to effectively manage insect pests with minimal chemical inputs as well as inspire development of new approaches.

Populärvetenskaplig sammanfattning

Majs är en viktig stapelgröda i Subsahariska Afrika (SSA), vilken ger mat och uppehälle för ungefär 300 miljoner människor på kontinenten. Bland de större begränsningarna i produktionen för småskaliga odlare av majs är skadegörande fjärilsarter såsom stamborrare och majsfly (*Spodoptera frugiperda*). Ett effektivt växtskydd av dessa skadegörare med hjälp av pesticider begränsas av odlarnas låga inkomster men även av miljöhänsyn och risken för skador på den mänskliga hälsan. Odlare i SSA är därför beroende på en radda av skötselåtgärder för att kontrollera skadeinsekter, bland dessa finns polykulturella tekniker såsom samodling och agroskogsbruk.

Samodling innebär att odla två eller mer olika grödor på samma yta samtidigt. Småskaliga odlare i SSA brukar nyttja samodling där sädesslag växer tillsammans med ärtväxter såsom olika typer av bönor och jordnötter. Dessa system hjälper odlare att öka mängden odlad mat samtidigt som det minskar risken för att förlora all skörd vid extrema väderförhållanden såsom allvarlig torka och översvämning.
Samodlingstekniker har även visat sig att minska mängden skadeinsekter jämfört med monokulturella system och således vidare öka skörden.

Bland de samodlingssystem som nyttjas av småskaliga odlare i SSA finns finns push-pull tekniken. Detta system innebär att en ärtväxt, Desmodium, samodlas mellan radar av ett sädeslag, och att det plantas en gräsbarriär på kanterna av Napier eller *Brachiaria*. Det är sagt att samodlingsgrödan repellerar insektskadegörare från odlarnas gårdar via luftburna kemiska ämnen, medans fångstgrödor, som är mer attraktiv för insekterna än huvudgrödorna inte stödjer deras utveckling.

Bönder i östra och södra Afrika nyttjar detta system för att kontrollera besvärliga skadegörare på majs och sorghum, såsom stamborrare (*Busseola fusca* and *Chilo partellus*) och det invasiva majsflyt (*Spodoptera frugiperda*), som när de lämnas okontrollerade kan orsaka upp till 100% skördeförlust av sädesslaget, vilken är allvarligt för odlare och påverkar matsäkerheten. Som tillägg, denna teknik kan effektivt kontrollera det parasitiska gräset, *Striga*, med hjälp av rotexudat från samodlingsgrödan *Desmodium*. Den huvudsakliga effekten av att använda denna sorts system är ökade skördar och således förbättrad matsäkerhet och ekonomi hos de som odlar. Hittills har mer än 250 000 odlare i Kenya, Tanzania, Uganda, Rwanda och Zambia tillämpat denna teknik, med planer att sprida denna kunskap vidare till Etiopien och andra länder i södra Afrika. Denna teknik är ett effektivt sätt att bedriva regenerativt jordbruk för småskaliga jordbruk i SSA samtidigt som man hanterar problematiska skadeinsekter på sädesslag.

Lockelsen av att använda detta system kommer också från en synergieffekt mellan bondens behov och hållbarhet. För odlare, förutom växtskydd, så erbjuder push-pull tekniken foder över hela året och extra inkomster från försäljningen av frön från samodlingsgrödan *Desmodium* och sticklingar av Napier/*Brachiaria*. Miljömässigt så har push-pull tekniken visat sig erbjuda flera ekosystemtjänster, vilket inkluderar en ökning av jordens kolinlagring, en ökad bördighet genom kvävefixering, ökad vattenretention i jorden och bibehållen jordstruktur.

Flera aspekter av push-pull tekniken har studeras, men inte mycket fokus har lagts på interaktioner med det mikrobiella livet i jorden. Målet av denna studie har varit att undersöka hur jordens mikrobiom förändras vid samodling med *Desmodium*, och huruvida jordlevande mikroorganismer spelar roll i de ekosystemtjänster som har rapporterats för systemet, detta med fokus på emissionen av de luftburna kemiska ämnena från samodlingsgrödan *Desmodium* som repellerar de skadegörande insekterna från böndernas land.

Först undersökte vi hur en längre tids (14-18 år) av push-pull samodling med majs påverkade jordens mikroorganismer jämfört med monokultur. Vi upptäckte att en längre tids nyttjande av push-pull tekniken ökade diversiteten av mikroorganismer i jorden, med en större effekt på svampar än bakterier. Den ökade diversiteten av mikroorganismer i jorden har möjliga fördelar genom att stabilisera det underjordiska ekosystemet och erbjuder fördelar som är associerade med mer diversa mikrobiellt liv såsom kolinlagring, näringscirkulation och undertryckande av jordburna sjukdomar. Vi visar också att dessa förändringar i jordens mikrobiom tar tid att skapa, således förstärker det tillämpandet av perenna samodlingssystem.

När vi undersökte rollen som mikroorganismer i jorden spelar på emissionen av repellerande kemiska ämnen som tidigare har rapporterats komma från *Desmodium*, så förvånades vi över vad vi upptäckte. Vi märkte att *Desmodium* inte emitterar dessa kemiska ämnen utan anledning, och först när insektslarver åt på plantan, såg vi små ökningar, allt för låga för att effektivt kunna trycka bort insekter från fältet. Vidare undersökningar baserade på denna observation, visade att insekter la ägg på *Desmodium*, vilket hade varit förvånande om plantan verkligen var repellerande.

Vi gick då längre och tittate på hur lämplig *Desmodium* var som matkälla för majsflylarver. Här upptäckte vi att tidiga stadier av larverna föredrog samodlingsgrödan framför majs, men de överlevde inte och dog innan de hann bli puppor. Effekten var än värre på äldre larver, vilka fann att *Desmodium* var en olämplig födokälla, men också kämpade med att röra sig på plantorna, där de fångades och kunde inte röra sig. Vidare undersökningar påvisade att detta berodde på ett tjock nätverk av växthår som täckte blad och stammar på *Desmodium*. Dessa växthår, som också kallar trikomer, innehöll stora mängder kisel, vilket bidrog till att göra *Desmodium* en dålig födokälla genom att göra att växten alltför seg och hård för insekterna att tugga och smälta.

Baserat på dessa fynd så menar vi att det inte är repellerande egenskaper hos *Desmodium* som gör att push-pull tekniken fungerar utan att det är andra mekanismer. Vi föreslår istället att *Desmodium* undertrycker skadegörande insekter genom att 1) begränsa möjligheten för larver att röra sig, de fastnar mellan majsväxter, och 2) genom att vara en dålig födokälla. Som tillägg, så har generellt sett samodlingssystem visat sig minska attacker av skadeinsekter på huvudgrödor genom flera mekanismer, vilket inkluderar maskering och barriäreffekter vilket gör det svårare för skadeinsekten att hitta sina värdväxter.

Dessa nya fynd kräver vidare försök i fält för att bekräftas. De öppnar också upp för en möjlighet att utvärdera ätliga samodlingsgrödor med liknande egenskaper som *Desmodium*, som vilken genom att vara oätlig minskar graden av införande hos odlare och skapar utmaningar när det gäller skötsel. Fokus bör ligga på perenna samodlingsgrödor på grund av det mervärde det skapare både för bönder och ekosystem. Push-pull tekniken som beskrivs här är ett effektivt och ekologiskt sätt att hantera skadeinsekter på i småskalig odling i SSA. En klar förståelse av de underliggande mekanismerna skulle göra att liknande system skulle kunna utvecklas för andra agroekologiska system för att där effektivt kontrollera skadeinsekter med minimala kemikalieinsatser samtidigt som det skulle inspirera utvecklandet av nya tillvägagångssätt.

Abstract in Kiswahili

Kuitazama upya teknolojia ya sukuma-vuta: mbinu za faida za kiikolojia

Ikisiri

Sukuma-vuta ni teknolojia ya kiikolojia ya kilimo cha mseto inayotumika kudhibiti wadudu waharibuo nafaka hususani vipekechamashina au funza wa mabua (*Busseola fusca, Chilo partellus*), viwavijeshi vamizi (*Spodoptera frugiperda*) pamoja na magugu tegemezi yaitwayo viduha (*Striga*). Teknolojia hii hutumia mahusiano ya wadudu na mimea ili kudhibiti wadudu na, hivyo, kuongeza tija na wakati huohuo kudumisha ikolojia. Katika utafiti huu, tunaripoti kwa mara ya kwanza taathira za matumizi ya teknolojia sukuma-vuta kwa muda mrefu (miaka 14 - 18) kwenye jamii za vidubini vya udongoni. Pia, tunaonyesha taathira za kilimo cha muda mfupi cha spishi mbalimbali za *Desmodium* kwenye vidubini vya udongoni. Aidha, tulifanya majaribio katika vitalunyumba ili kuelewa jinsi vidubini vya udongoni vinavyochangia katika mimea ya *Desmodium* kutoa harufu kama njia kuu ya kufukuza wadudu waharibifu, kama ambavyo imekuwa ikiripotiwa kupitia teknolojia ya sukuma-vuta. Zaidi ya hayo, tulichunguza mambo mengine kadhaa: 1) kama kweli*Desmodium* inafukuza wadudu aina ya *Spodoptera frugiperda* wasitagie mayai katika mimea hiyo 2) uchaguzi wa chakula kinachopendelewa na mabuu ya *S. frugiperda* kati ya *Desmodium* na mahindi (*Zea mays*).

Matokeo ya utafiti huu yanaonyesha kwamba kutumia teknolojia ya sukuma-vuta au kilimo mseto cha nafaka na *Desmodium* kwa muda mrefu husababisha kubadilika kwa jamii za vidubini vya udongoni kwa kuongeza

uanuwai wa jamii za kuvu kuliko bakteria. Kwa upande mwingine, tunaonyesha kwamba mabadiliko ya muundo wa jamii za vidubini udongoni kutokana na kulima spishi mbalimbali za *Desmodium* kwa muda mfupi wa miaka miwili ni madogo. Hata hivyo, japokua mabadiliko ya ujumla ya jamii nzima za vidubini hayakuwa tofauti kati ya spishi za *Desmodium*, tuliweza kuainisha makundi ya vidubini ambayo wingi wake uliongezeka.

Kuhusu taathira za vidubini vya udongoni katika utoaji wa harufu inayofukuza wadudu kutoka kwenye *Desmodium*, tulibaini kuwa *Desmodium* haitoi harufu yenye misombo muhimu inayohusika katika kufukuza wadudu waharibifu, kinyume kabisa na maarifa yaliyozoeleka, bila kujali kama mimea ilioteshwa kwenye udongo hai wenye vidubini au ambao umeondolewa vijidudu vyote kwa joto kali la mashine ya otokleva. Hata baada ya kuliwa na wadudu, *Desmodium*ilitoa harufu kidogo sana ikilinganishwa na mahindi, ambapo tulibaini kuwa vidubini vya udongoni vinachangia kwa kiasi kidigo sana. Sambamba na hilo na kinyume na matarajio, ilibainika kwamba *Desmodium* haifukuzi nondo *S. frugiperda*, ambao mabuu yake (viwavijeshi vamizi) ni wadudu waharibifu sana wa mazao ya nafaka, kutagia mayai katika mimea hiyo ikilinganishwa na mahindi. Kwenye uchaguzi wa ulaji, ilionekana kwamba viwavijeshi vamizi wachanga walipendelea sana kula *Desmodium* kuliko mahindi lakini hawakukua vizuri katika mimea hiyo. Kwa viwavijeshi wakubwa, wao walipata shida zaidi kula na kujongea kwenye mimea ya *Desmodium.* Uchunguzi wa kina ulionesha kuwa mashina na majani ya *Desmodium* yalikuwa yamefunikwa na mtandao ulioshikamana wa nywelenywele zenye urefu tofautitofauti, baadhi zikiwa zimenyooka na nyingine zikiwa zimekunjika mithili ya ndoano. Nywelenywele hizi, ambazo zilionekana kuwa na kiasi kikubwa cha Silikoni, zilizuia mijongeo ya mabuu yakiwa kwenye *Desmodium* na hata kutoboa miili yao, mara nyingi yakiishia kufa baada ya siku chache. Kwa kuzingatia matokeo haya, kwenye kilimo cha sukuma-vuta, *Desmodium* haizuii nondo waharibifu wasitage mayai kwenye mazao kwa kutegemea harufu, ila inawezekana kwamba inafanya kazi kama mtego unaozuia wadudu wasifikie mashina mengi ya mazao. Pia, nywelenywele ngumu zenye kiasi kikubwa cha Silikoni zinafanya *Desmodium* kuwa chakula kibaya kinachoharibu midomo na matumbo ya mabuu wakijaribu kuila, hivyo kuunguza kiasi kikubwa cha wadudu kwa

kuwaua kabla hawajafikia kuwa nondo. Mafanikio makubwa ya teknolojia ya sukuma-vuta yamedhihirisha kwamba njia za kilimo cha kiikolojia kwa lengo la kudhibiti wadudu waharibifu na kuongeza tija zinafanya kazi kwa ufanisi. Hata hivyo, uelewa wa sayansi ya mbinu hizi, hasa za namna teknolojia za kiikolojia zinavyofanya kazi, ni muhimu ili kuziboresha zaidi na pia kuweza kuzitanua ili ziweze kutumika kwenye aina nyingine za kilimo-ikolojia.

Dhana muhimu: sukuma-vuta, *Desmodium*, vidubini vya udongoni, kilimo mseto, vipekechamashina, viwavijeshi vamizi, nywelenywele za mimea, *Spodoptera frugiperda*, funza wa mabua, kilimo cha ikolojia

Anwani ya mwandishi: Aneth Bella David, Chuo Kikuu cha Sayansi za Kilimo cha Uswidi, Idara ya Biolojia ya Ulinzi wa Mimea, Alnarp, Uswidi.

Acknowledgements

This work is the result of effort and contribution from so many people and entities. I am deeply grateful to all of them.

First to my **supervisors** who at times felt like academic parents, thank you for your guidance and support during this journey. You have helped me grow in so many ways than I can count. I still remember the beginning of my PhD journey interviewing with [Profs.] **Sylvester** and **Teun**, not imagining how far you would help me reach, thank you for your faith in me. May you be blessed abundantly.

To my **family, friends and friends who became family**, what can I say?
I have the most patient and understanding family in you; although you were always surprised that a PhD takes so long to finish but you always encouraged me to push through. You always reminded me that I know what I want and it is worthwhile enough to endure. You gave me a stable base from which to launch and come back to as needed. I am eternally grateful.

A special honor goes to my mother, **Fortunata Kilawe** a.k.a. mama Professa. I wouldn't have gotten where I am without your dedication and your instilling in me a commitment to education since childhood. *Elimu* was never up for discussion and I am grateful I picked that early on from you. I have no words to express my gratitude to and for you.

Asateni sana mama zangu **Mama Kelvin**, **Mama Asela** and **Mama Evance**, just to name a few. Your encouragement, wise words and most importantly, prayers, have helped so much. To my grandparents **Bibi Mangare** and **Babu Justine Kilawe**, I am so glad that you get to see this moment. My grandparents in heaven **Aneth (my namesake)** and **Anyambilile**, I wish that you were here to witness this feat**.** To my uncle **Francis** and aunt **Grace,** all I can say to you is a prayer for God to bless you abundantly and fill your cups with overflowing grace. To my uncles and aunts especially **Anna** and **Edwin**, cousins, sisters (**Sheila**, **Nare**, **Mariam** and **Sarah**) and brothers (**Godbless** and **Kelvin**), your faith in me kept me going when things took a tough turn, which was quite often. Thank you for believing in me when doubts and impostor syndrome visited (also quite often than I could manage, especially towards the end). Thank you for the prayers, encouragement, tips, suggestions and uplifting music you kept coming my way.

To fellow students at SLU, especially my East African family at SLU **Charles** and **Matthew**, to **Diya**, **Anna**, **Vaida** and **Advaith**. Life at our little Alnarp village was always interesting and warmer because of you presence. Special thanks to **Advaith** for making sure I was always fed with delicious (Indian) food during thesis writing. To my fellow PhD students in Tanzania, Sweden and elsewhere (**Nare**, **Jimmy**, **Koku Bernedether**, **Herieth**, **Deo pacha** and **Lwidiko**), thank you for the company.

I made new family in Sweden, especially from **Barbro** and **Anders**, and **Mattias**. Thank you for making my stay easier and memorable; full of Swedish food, experiencing Swedish traditions and for making the culture feel less foreign. I will always remember the taste of Surströmming and my skiing experience ☺

I am grateful to Staff at SLU Plant Protection Biology Department and at UDSM Department of Molecular Biology and Biotechnology for all the support with lab work and experiments. Special thanks to **dada Winnie** and **Charles** at UDSM and to **Elin** and **Minggang** at SLU. Many thanks to **Sebastian** for so much help; always being ready to help wit data analysis or GC-MS troubleshooting and with Swedish translations in this thesis. Thanks to research scientists in Tanzania (TARI) and Kenya (ICIPE); I appreciate all the help and guidance with both field and lab work, especially **Dickens Nyagol** at ICIPE, Mbita campus, and **Maureen** and **Levi** at ICIPE, Duduville campus. Thank you **Annagrace** for helping process images in this thesis. To **farmers** in Tanzania and Kenya, thank you. I learned so much from you all, complementing my “paper-based” knowledge.

I can’t end this note without thanking the **Academic twitter** who made me realise that I wasn't alone in what I was going through and I wasn’t a bad student. Academic twitter made the journey felt less lonely and provided delightfully distracting company during procrastination and doom scrolling episodes. As the Kiswahili saying goes, “*Msiba wa wengi sherehe*” ☺

To all family, friends, neighbours, co-workers and all well-wishers who contributed in one way or another, or made my life simpler in any way during this journey, I thank you all. To all those I may have forgotten to mention here out of pure human error and those who I cannot name here, I value your contribution and *asanteni sana sana*. It indeed does take a village.

Plant Soil (2021) 467:437–450
https://doi.org/10.1007/s11104-021-05082-w

ORIGINAL ARTICLE

Long-term maize-*Desmodium* intercropping shifts structure and composition of soil microbiome with stronger impact on fungal communities

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Received: 10 January 2021 / Accepted: 8 July 2021 / Published online: 14 August 2021
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Abstract
Purpose Push–pull is an intercropping technology that is rapidly spreading among smallholder farmers in Sub-Saharan Africa. The technology intercrops cereals with *Desmodium* to fight off stem borers, eliminate parasitic weeds, and improve soil fertility and yields of cereals. The above-ground components of push–pull cropping have been well investigated. However, the impact of the technology on the soil microbiome and the subsequent role of the microbiome on diverse ecosystem benefits are unknown. Here we describe the soil microbiome associated with maize—*Desmodium* intercropping in push–pull farming in comparison to long-term maize monoculture.

Methods Soil samples were collected from long-term maize—*Desmodium* intercropping and maize monoculture plots at the international centre for insect physiology and ecology (ICIPE), Mbita, Kenya. Total DNA was extracted before16S rDNA and ITS sequencing and subsequent analysis on QIIME2 and R.

Results Maize—*Desmodium* intercropping caused a strong divergence in the fungal microbiome, which was more diverse and species rich than monoculture plots. Fungal groups enriched in intercropping plots are linked to important ecosystem services, belonging

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Responsible Editor: Birgit Mitter.

Teun Dekker and Sylvester Leonard Lyantagaye shared last authorship.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-021-05082-w.

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to functional groups such as mycorrhiza, endophytes, saprophytes, decomposers and bioprotective fungi. Fewer fungal genera were enriched in monoculture plots, some of which were associated with plant pathogenesis and opportunistic infection in humans. In contrast, the impact of intercropping on soil bacterial communities was weak with few differences between intercropping and monoculture.

Conclusion Maize—*Desmodium* intercropping diversifies fungal microbiomes and favors taxa associated with important ecosystem services including plant health, productivity and food safety.

Keywords Soil microbiome · Push–pull farming · Intercropping microbiome · 16S · ITS · *Desmodium* spp

Introduction

Push–pull technology is an ecological habitat management strategy for the control of major pests of cereals, particularly maize and sorghum. Since its inception in the 90's, the technology has spread to smallholder farmers across southern and eastern Africa who use it to manage stem-borers (*Busseola fusca* and *Chilo partellus*) and fall armyworm (*Spodoptera frugiperda*) attacks on cereal crops thus increasing yield (Midega et al. 2018). The technology exploits the chemical ecology of a leguminous intercrop belonging to the genus *Desmodium*, which 'pushes' stem-boring insects from the main crop reportedly through its volatile compounds that signal an unfavourable egg-laying environment. At the same time, a grass trap crop such as *Brachiaria* spp. or *Cenchrus purpureus*, commonly known as napier grass, is planted as border vegetation to 'pull' the insects towards itself without supporting their development (Khan et al. 2003, 2010).

Over the years several additional benefits of the *Desmodium*-based intercropping system have been uncovered and indicate diverse soil-based mechanisms that warrant further study. *Desmodium* employs allelopathic mechanisms that dramatically reduce infestation of the parasitic weed *Striga hermonthica* to cereal crops, further adding to yield increases (Khan et al. 2002). Moreover, the technology provides other ecological benefits contributing to improved cereal crops yield, including nitrogen fixation by *Desmodium*, soil structure improvement and a mulching effect in the fields. Finally, the technology reportedly reduced the incidence of human pathogenic fungal toxins in maize kernels (Njeru et al. 2020; Owuor et al. 2018). In addition, both *Desmodium* and the trap crops are a reliable source of animal fodder, particularly in drought periods, as farmers do not uproot them between farming seasons (Khan et al. 2010). The overall impact is increased cereal yield with minimum chemical inputs. The technology is effective and, importantly, affordable for smallholder farmers in Sub-Saharan Africa.

Whereas the components of the cereal—*Desmodium* push–pull farming system and their underlying mechanisms have been well investigated, one of the areas that has received no attention is its interaction with the soil microbiome. Intercropping is increasingly adopted as a sustainable alternative to monoculture production systems. The cropping practice provides diverse ecosystem services, some of which are immediate and pronounced (such as productivity, pollinator support, pest and disease reduction, nitrogen fixation (Bybee-Finley and Ryan 2018; Nourbakhsh et al. 2019), whereas others are acquired over a longer time. In push–pull cropping systems the effects on and impact of the soil microbiome fall in the latter category and have been, in part for that reason, little studied.

Soil microorganisms promote plant health and productivity through direct and indirect mechanisms mediated through root systems (van der Heijden et al. 2008). Plants use their roots exudates to actively influence the microbial assemblages in the rhizosphere often favouring those that offer survival benefits (Liu et al. 2021). Therefore, it is of interest to explore the impact of maize—*Desmodium* on soil microbial profiles as the first step to understand their contribution on the effectiveness of the farming system. Microbiome studies are increasingly used to discern potential impacts of farming practices such as intercropping on abundance, structure and diversity of soil microbiota, which in turn provide plants with other benefits such as higher mineral nutrients availability (Johansen & Jensen 1996; Tang et al. 2014). Studies in cereal—legume intercropping systems have shown changes in soil microbial structures as well as benefits on plants mediated by soil microbes. For instance, in a study by Li et al. (2018), an increase in yield as well as overall diversity of soil bacteria was observed in maize—peanut intercropping systems. The study observed a higher abundance of beneficial soil bacteria in intercropping systems, where belowground interactions were either complete or partial when compared to monoculture. Increases in soil microbial biomass as well as nutrient availability, especially N, P and C have been observed in multiple cereal—legume intercropping systems, such as that of wheat (*Triticum aestivum*), maize (*Zea mays*), and faba bean (*Vicia faba*) intercropping (Song et al. 2007), and durum wheat (*Triticum turgidum durum*) intercropped with either chickpea (*Cicer arietinum*) or lentil (*Lens culinaris*) (Tang et al. 2014).

In that light, the current study compared the diversity of soil microorganisms between long-term maize—*Desmodium* and maize monoculture plots. Specifically, amplicon sequencing (16S rDNA and ITS) was used to investigate the differences in soil bacterial and fungal population structures between long-term maize—*Desmodium* intercropping and maize monoculture practices in a context of potential ecological benefits.

The mapping of the soil microbiomes demonstrated that the fungal microbiome was particularly diversified in maize—*Desmodium* intercropping plots compared to maize monoculture plots. The results are discussed in the context of reported benefits around maize—*Desmodium* intercropping in push–pull farming by inferring known ecological functions of taxa contributing to the observed difference. This is the first step towards understanding soil microbial diversity in push–pull technology for optimal exploitation of their potential ecosystem benefits in plant health and productivity. Further studies are recommended to discern key determinants of the observed differences and their importance in ecosystem (dis) services. Knowledge and translation of this knowledge into other cropping systems could advance sustainable food production through fostering belowground microbial communities that support plant health and productivity.

Methodology

Sampling site

To compare soil microbial profiles between maize monoculture and maize—*Desmodium* intercropping maize farming, we obtained soil samples from long-term (14–19 years old) experimental plots at the International Centre for Insect Physiology and Ecology (ICIPE), Mbita campus, Kenya (0°25.877 S 34°12.425 E). The campus has clay-loam soil type, receives approximately 900 mm of rainfall per annum, has a mean annual temperature of 27 °C, and is located at an altitude of approximately 1200 m above sea level.

The samples were collected from three sets of plots established between 1998 and 2003. The first set of plots consisting of a maize monoculture and push–pull plots was established in 1998 (30 m by 30 m). The plots had *D. uncinatum* (silver-leaf desmodium) as the intercrop while Sudan grass (*Sorghum sudanense*) was the trap crop. The second set of plots was established in 1999 (6 × 6 m) to study the ability of *Desmodium* intercropping to suppress *Striga*. These plots were not surrounded by border/trap crops but were separated from other plots by 2 m buffer spaces. The third set of plots was established in 2003 (5 × 6 m) to compare efficiency of food legumes and *Desmodium intortum* (greenleaf desmodium) intercrops in *Striga* suppression. Phosphorus, in the form of di-ammonium phosphate (DAP), was applied in each plot at planting at the rate of 60 kg/ha. Nitrogen was applied after thinning of maize, in the form of calcium ammonium nitrate (CAN), at the rate of 60 kg/ha (Midega et al. 2014). The plots were also not surrounded by a border/trap crop but they were separated from other plots by 2 m buffer spaces. In the plots established in 1999 and 2003, only plots of maize monoculture and maize—*Desmodium* intercropping were selected for sampling.

In all plots, maize (medium maturing commercial hybrid 513 variety) was planted at a spacing of 0.75 m between rows and 0.3 m within rows while *Desmodium* was planted through drilling method within a row. Plant population (maize) was therefore the same in any set of plots.

Soil sample collection

Soil samples were collected during the cool dry season in July 2017, when the maize was mature and just before harvesting. We collected seven samples from each site; four samples from maize monoculture and three from intercropped/push–pull plots. Each individual sample was made up of three 15–18 cm deep soil cores that were collected from random spots in each selected plot away from the edges. For monoculture, sampling was done between rows of maize plots while in intercropped plots, it was done close to *Desmodium* spp. roots system (growing in rows between maize rows). Afterwards, each soil sample was homogenized and sieved through a 4 mm wire mesh. About 200 g of soil sub-sample was collected and stored at − 20 °C until further processing.

DNA extraction and sequencing

DNeasy Powersoil kit (Qiagen, Manchester, UK) was used for total DNA extraction from the soil samples following the manufacturer's protocol. Briefly, 0.25 g soil was added to PowerBead Tubes containing a lysis buffer and vortexed for a few seconds. The resulting mixture was centrifuged at 10,000*g* for 30 s before discarding the pellet and centrifugation of the supernatant in spin columns. Tris–HCl solution was used to wash off DNA from the spin column. A Nanodrop spectrophotometer and gel electrophoresis were used to assess the quality of the extracted DNA. The DNA samples were stored at − 20 °C until further processing.

DNA sequencing was done at Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa). Primers targeting the V1-V3 region of 16S rDNA gene of the bacteria (27F and 518R primer pairs) were used to amplify DNA under the following PCR conditions: initial denaturation at 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 30 s, primer annealing at 60 °C for 30 s, and extension at 72 °C for 30 s, with a final elongation at 72 °C for 5 min. For fungi, ITS1F and ITS2 primer pairs targeting ITS1 were used for PCR amplification under the following conditions: 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 30 s, primer annealing at 50 °C for 30 s, and extension at 72 °C for 1 min. Final elongation was held at 72 °C for 5 min.

Resulting amplicons were gel purified, end repaired and Illumina specific adapter sequence were ligated to each amplicon (NEBNext Ultra II DNA library prep kit). Following quantification, the samples were individually indexed (NEBNext Multiplex Oligos for Illumina Dual Index Primers Set 1), and another AMPure XP bead-based purification step was performed. Amplicons were then sequenced on Illumina's MiSeq platform, using a MiSeq v3 kit with 600 cycles (300 cycles for each paired read and 12 cycles for the barcode sequence) according to the manufacturer's instructions. Demultiplexed 300 bp paired-end reads were obtained.

Bioinformatics and statistical analysis

FASTQC (Wingett and Andrews 2018) was used to assess the quality of raw sequence reads after which QIIME2 v2020.8 was used for quality control, construction of a feature table, taxonomic classification and diversity analyses (Bolyen et al. 2019). Briefly, the dada2 plugin (Callahan et al. 2016) was used to trim and truncate poor regions of both the 16S and ITS raw reads. The truncation and trimming were set to –p-trim-left-f 8, –p-trim-left-r 8; and –p-trunc-len -f 290, –p-trunc-len-r 260, for the 16S; while for the ITS, parameters used were p-trim-left 22, –p-trunc-len 299. Bacterial taxonomic assignment was done using feature-classifier classify-sklearn (Bokulich et al. 2018; Pedregosa et al. 2011), only including reference genes that were classified to at least genus level, by using SILVA v.138 97% database (Quast et al. 2013) pre-trained to V1-V3 region of 16S. For ITS, we used UNITE v8.2 reference database (Nilsson et al. 2019) pre-trained to ITS1.

The resulting feature table was converted into biom format (using QIIME2's export tool), and then imported into R (R Core Team 2020) using "qiime2R" (Bisanz 2018). For visualising the number of amplicon sequence variants (ASVs), genera, families and orders present in the dataset we filtered out everything that was present only once at each level and then Venn diagrams were produced using function vennCounts from package "limma" (Ritchie et al. 2015). Then, everything that was unassigned at family level was filtered out.

For constructing dendrograms, primary component analysis (PCA) and heatmap data was transformed using CSS (cumulative sum scaling) by using a package "metagenomeseq" (Paulson et al. 2013). To perform a principal component analysis (PCA), we used package "recipes" (Kuhn and Wickham 2020), and annotated ellipses using a Khachiyan algorithm from package "ggforce" (Pedersen 2020). Dendrograms were constructed using a jaccard index from package "vegan" (Oksanen et al. 2020), with a presence absence standardization, and plotted using "ggtree" (Guangchuang et al. 2017).

Species diversity (Shannon) and richness (chao1) were calculated on untransformed and unfiltered data using "vegan" through the package "phyloseq" (McMurdie and Holmes 2013), while evenness was calculated as the Shannon index divided by the natural logarithm of the total number of species. All indices were tested for significance using a two tailed Student's t-test.

Differential expression analysis was done on untransformed but filtered data based on a negative binomial distribution through "DESeq2" (Love et al. 2014). The resulting log2fold changes were shrunken using the adaptive shrinkage estimator from package "ashr" (Stephens et al. 2020). Genera were deemed to significantly impact treatments if they had an adjusted p-value smaller than 0.05 (Wald test), and an absolute log2fold change of over one, which was then visualised on a volcano plot modified from package "EnhancedVolcano" (Blighe et al. 2020). The result from the differential expression analysis also was used to group data in the heatmap and label significant genera in the PCA. All data was manipulated using "tidyverse" (Wickham et al. 2019) and visualised using "ggplot2" (Wickham 2016).

Results

Composition and abundance of soil microbiome in maize monoculture and maize—*Desmodium* intercropping plots

When considering the total number of ASVs (taxonomic units), a moderate divergence was observed between maize monoculture and intercropping plots. The difference becomes less pronounced at the order, family and genus levels with a high degree of overlap observed (Fig. 1). The number of fungal ASVs was higher than that of bacteria, indicating a higher richness of soil fungal communities. More bacteria ASVs (1934) were identified from monoculture plots than maize—*Desmodium* intercropping plots (1333 ASVs). For fungal communities however, the number of ASVs was higher in intercropping (1262 ASVs) than monoculture plots (1085 ASVs). At the genus level, monoculture plots were composed of more bacteria than fungal taxa (195 vs 162 genera), whereas the fungal genera made the larger proportion in intercropping plots than bacteria (284 vs 225 genera).

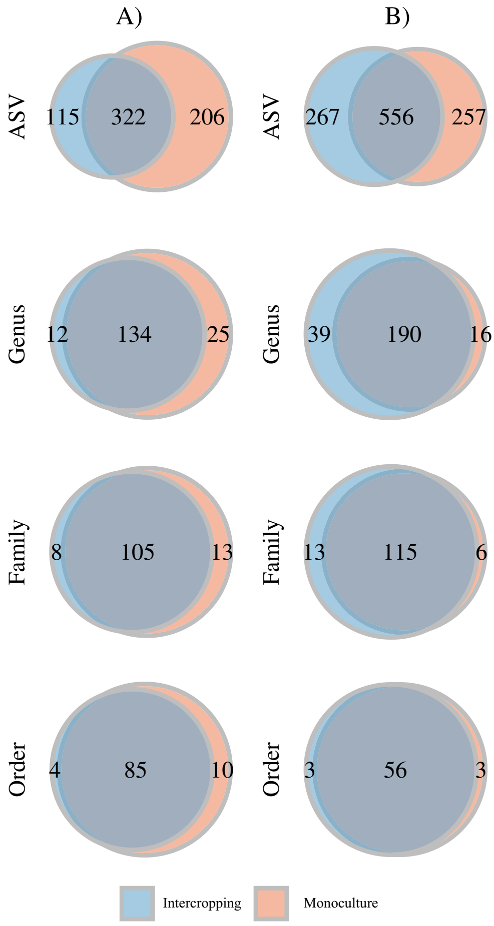
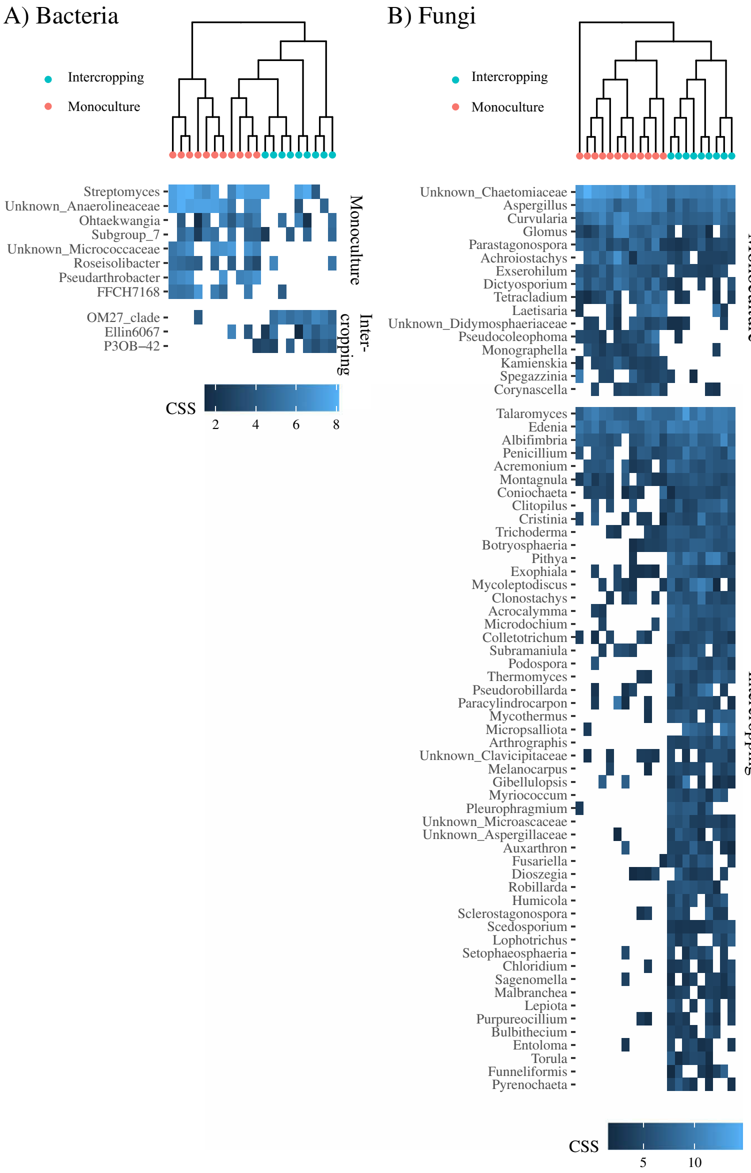


Fig. 1 Venn diagrams showing common and unique taxonomic units as well as the degree of overlap at the genus, family and order levels for **a** bacteria and **b** fungi communities in monoculture and maize—*Desmodium* intercropping plots

In spite of considerable/strong overlap, the two cropping systems separated clearly based on Jaccard dissimilarity index (dendrograms on Fig. 2a, b). Furthermore, differential abundance analysis revealed several genera that were enriched in either monoculture or intercropping plots. Bacterial taxa showed few differences in abundance between the cropping systems, whereas the abundance of fungal taxa showed stark contrasts. Fungal taxa were more enriched in

Fig. 2 Differential abundance of bacteria genera (**A**) and fungal genera (**B**) in monoculture or maize—*Desmodium* intercropping plots. The abundances were normalized by cumulative sum scaling (CSS). The dendrogram on the left was produced by using a Jaccard dissimilarity index, with a presence-absence standardization; each node corresponds to one sample



intercropping than monoculture plots, whereas an opposite trend was observed for bacterial taxa (Fig. 2, for a full heatmap of all bacterial and fungal taxa, see supplementary Figs. 1 and 2).

Statistical analysis confirmed that bacteria contributed little to the microbial divergence between the two treatments: only four bacterial genera were significantly abundant in monoculture while only one genus was significantly more abundant in maize—*Desmodium* intercropping plots (Fig. 3a). Among fungal genera, the trend was reversed, with more genera being enriched in maize—*Desmodium* intercropping (52 genera) than monoculture plots (16 genera) (Fig. 3b).

Fewer bacterial taxa were classified at the genus level due to limited information in classification databases, limiting further analysis and dissection of the findings. In contrast, a large proportion of fungal genera were identified. In maize monoculture plots, several fungal genera were enriched including plant pathogens *Curvularia*, *Parastagonospora* and *Tetracladium* as well as human opportunistic pathogens such as *Aspergillus* and *Exserohilum*. Only a few of the fungal genera enriched in monoculture plots are known for beneficial ecosystem services, notably the mycorrhizal genus *Glomus* and endophytic *Laetisaria* (Fig. 3b). In maize—*Desmodium* intercropping plots, noteworthy abundant fungal genera include saprophytic fungi like *Pithya*, *Albifimbria*, *Acremonium*, *Pseudorobillarda* and *Cristinia*, mycorrhizal and endophytic fungi like *Edenia*, *Acrocalyma* and *Colletotrichum*, as well as fungal groups known for plant bio-protection such as *Talaromyces*, *Penicillin*, *Clonostachys* and *Trichoderma*. A few pathogenic genera were also enriched in intercropping plots, for example, *Mycoleptodiscus*, a genus of fungi reported to cause disease in legumes (Fig. 3b).

The impact of maize—*Desmodium* intercropping on diversity of soil microbial populations

Comparing overall diversity of soil microbial populations, no statistically significant difference was found among bacteria genera between monoculture and maize—*Desmodium* intercropping plots (Shannon index $p = 0.246$, Fig. 4). In contrast, fungal genera in maize—*Desmodium* intercropping were significantly more diverse compared to monoculture plots (Shannon index $p = 0.047$). Likewise, the richness of bacterial genera did not significantly differ between the two farming systems (Chao1 estimator $p = 0.238$), whereas that of fungal genera was significantly higher intercropping plots (Chao1 estimator $p = 0.012$). Evenness of both fungal and bacterial communities was not significantly different in both treatments (Fig. 4).

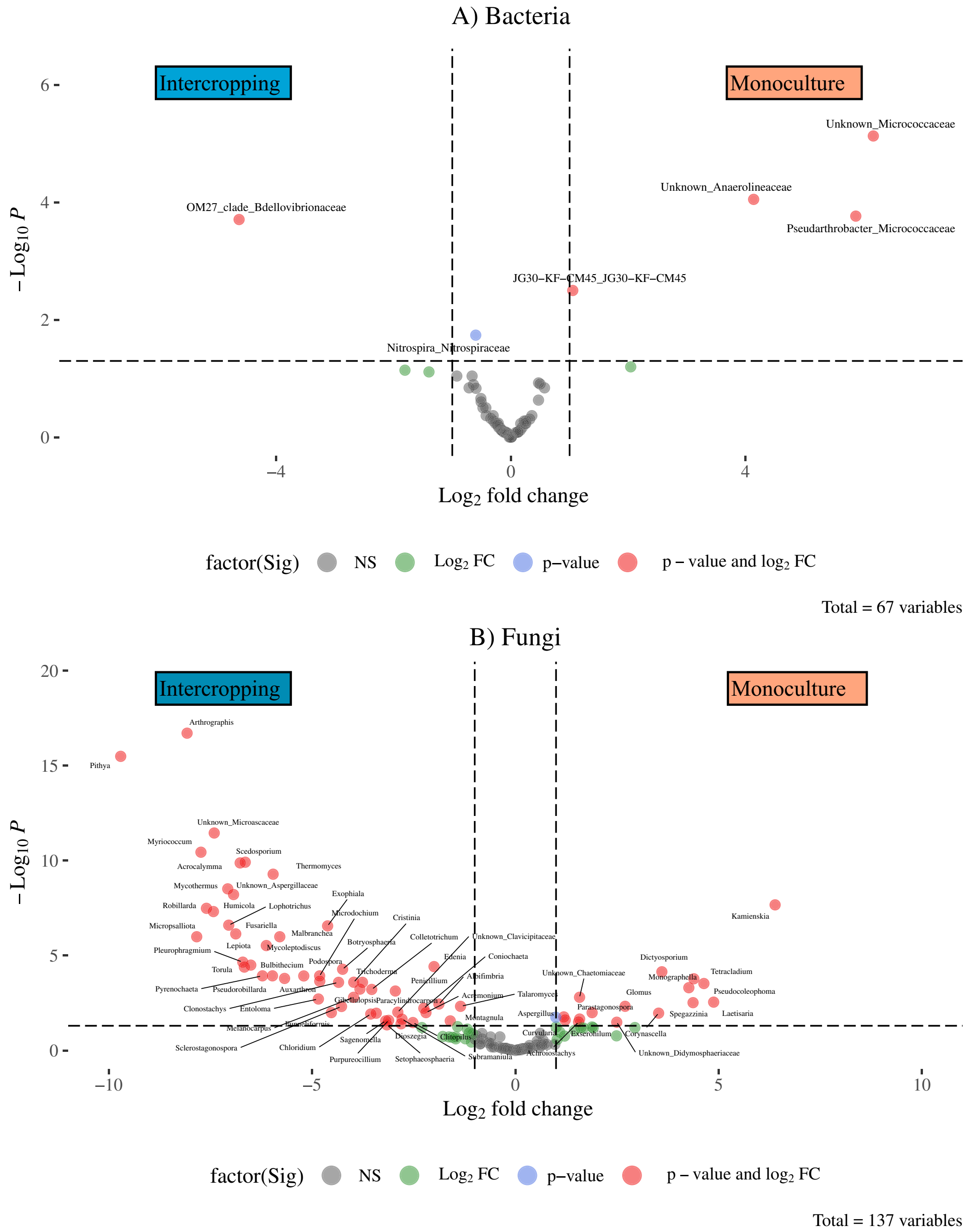
The impact of maize—*Desmodium* intercropping on the soil microbiome is also reflected in beta diversity measures. As noted above, the impact on the two farming practices on bacterial populations communities is weaker compared to that on fungi. Although the PCA plots for both bacterial and fungal communities show clear separation based on cropping practice, the separation was much stronger in fungal taxa (Fig. 5b). Cropping practises contributed to a major extent to the variation observed, with fungal taxa showing a clear non-overlapping clustering pattern between monoculture and intercropping plots along PC1, which contributed to a total of 30% of the variation (Fig. 5b). In bacterial taxa the separation was clearest again on PC1, but the total contribution of PC1 to the variation was only 19% and did not fully separate the cropping practises (Fig. 5a).

Discussion

Abundance and differential abundance of taxa and their potential functional significance

A large proportion of the fungal taxa that were abundant in maize—*Desmodium* intercropping plots appear to fulfil a saprophytic role, including *Talaromyces*, *Trichoderma, Penicillium* and *Colletotrichum* (see supplementary Table II). Presence of these taxa may indicate higher carbon sequestration in intercropping plots that is enhanced by the perennial intercropping system (Cong et al. 2015). Other enriched fungi genera likely confer more distinct ecosystem services, such as forming mycorrhizal (Ravnskov et al. 2006;) and endophytic associations with plant roots that promote plant growth activities through increased nutrient supply (Díaz-González et al. 2020; Macías-Rubalcava et al. 2008; Munasinghe et al. 2017). The fungi may also directly or indirectly stimulate production of beneficial secondary metabolites and protection against pathogens and insect pests (Hiruma et al. 2016; Zin and Badaluddin 2020). Indeed, effects can be indirect and intricate, for example,*T. atroviride* was shown to promote growth and herbivory resistance of maize against S*podoptera frugiperda*, possibly linked to induction of the jasmonic acid pathway leading to heightened induced defence (Contreras-Cornejo et al. 2018). In general, a positive correlation between soil microbe composition and productivity of plants above ground has been reported in most systems (Schnitzer et al. 2011), with positive effect on above ground biodiversity and biological control.

How direct and indirect microbial and plant interactions in the rhizosphere contribute to the diverse ecosystem services observed in push–pull intercropping needs further study. For instance, a recent study


A) Bacteria
Intercropping
Monoculture
Unknown_Micrococcaceae
Unknown_Anaerolineaceae
Pseudarthrobacter_Micrococcaceae
OM27_clade_Bdellovibrionaceae
JG30–KF–CM45_JG30–KF–CM45
Nitrospira_Nitrospiraceae
$-\mathrm{Log}_{10}\,P$
6
4
2
0
−4
0
4
Log_2 fold change
factor(Sig)
NS
Log_2 FC
p–value
p – value and $\log_2$ FC
Total = 67 variables
B) Fungi
Intercropping
Monoculture
Arthrographis
Pithya
Unknown_Microascaceae
Myriococcum
Scedosporium
Acrocalymma
Thermomyces
Mycothermus
Unknown_Aspergillaceae
Exophiala
Robillarda
Humicola
Lophotrichus
Microdochium
Cristinia
Micropsalliota
Fusariella
Malbranchea
Botryosphaeria
Colletotrichum
Unknown_Clavicipitaceae
Lepiota
Mycoleptodiscus
Pleurophragmium
Edenia
Coniochaeta
Podospora
Torula
Bulbithecium
Trichoderma
Albifimbria
Penicillium
Pyrenochaeta
Pseudorobillarda
Auxarthron
Acremonium
Talaromyces
Gibellulopsis
Paracylindrocarpon
Clonostachys
Entoloma
Montagnula
Aspergillus
Funneliformis
Melanocarpus
Sagenomella
Dioszegia
Clitopilus
Curvularia
Sclerostagonospora
Chloridium
Purpureocillium
Setophaeosphaeria
Subramaniula
Achroiostachys
Kamienskia
Dictyosporium
Unknown_Chaetomiaceae
Monographella
Tetracladium
Glomus
Pseudocoleophoma
Parastagonospora
Spegazzinia
Laetisaria
Exserohilum
Corynascella
Unknown_Didymosphaeriaceae
$-\mathrm{Log}_{10}\,P$
20
15
10
5
0
−10
−5
0
5
10
Log_2 fold change
factor(Sig)
NS
Log_2 FC
p–value
p – value and $\log_2$ FC
Total = 137 variables

◂**Fig. 3** Volcano plots showing bacterial (**A**) and fungal (**B**) genus level features that are differentially and significantly abundant in monoculture and maize—*Desmodium* intercropping plots. Red dots represent genus entities that are significantly abundant in each group with log2 fold change greater than 1. The grey and green dots represent the genus features whose abundance is similar between the two farming systems and the blue dots represents values where the p—value is significant between the treatments, but where the log2 fold change is smaller than one

showed that maize grown in soil from push–pull plots displayed a higher induced-defence response, including higher release of induced volatiles and lower herbivore damage compared to that growing on soil from monoculture (Mutyambai et al. 2019). Soil microbiota may be a missing link explaining the observed differences in maize direct and indirect defence pathways. The increased abundance of several soil fungal groups noted in intercropping plots in this study, such as *Edenia* and *Clonostachys* species, is particularly noteworthy in this context. Species belonging to these genera are associated with increased plant health, biocontrol of plant diseases and increased resistance against herbivore damage on plants (Iqbal et al. 2018; Macías-Rubalcava et al. 2008; Poveda et al. 2020).

Recent papers reported lower incidences of maize ear rot and associated mycotoxins (aflatoxins and fumonisins) (Owuor et al. 2018) as well as lower rate of infection of maize kernels with *Fusarium verticillioides* and *Aspergillus flavus* (Njeru et al. 2020) in smallholder farmers' push–pull plots compared to monoculture. Push–pull thus appears to promote food safety by reducing the risk of mycotoxins entering the human food chain, although the mechanisms remained unclear. Interestingly, in the current study, a lower relative abundance of *A. flavus* was indeed associated with maize—*Desmodium* intercropping cropping. However, no association was found for *F. verticillioides*, a mycotoxin producing fungus in maize. The earlier reported lower incidence of ear rot infections may thus be partially explained by the shift in relative abundance of key species in intercropping/push–pull plots, causing competition between taxa and lowering mycotoxin incidence levels. Suppression of some taxa through fungal competition or biocontrol is a common phenomenon. Sarrocco et al. (2019) found that *Fusarium graminearum*, a plant pathogen and mycotoxin producer, was controlled by competition from other fungi, including *Clonostachys*, and *Trichoderma,* both of which were found in higher abundance in maize—*Desmodium* intercropping plots than monoculture in this study. Further research on how mycotoxin incidence in maize kernels can be reduced by interactions between mycotoxin producing fungi and other soil microbes in maize—*Desmodium* intercropping would help in devising strategies to increase food safety through more healthy plant production systems.

Diversity of cropping systems links to diversity in soil microbiome

In this study, long-term maize—*Desmodium* was associated with a higher diversity of soil microbial communities, with a stronger shift observed in fungal populations. Other studies have reported a similar trend where cereal—legume intercropping increases overall diversity of soil microorganisms. Such observations have been made in wheat—soybean intercropping (Bargaz et al. 2017), maize/wheat—faba bean intercropping (Wang et al. 2020) and millet—mung bean intercropping (Dang et al. 2020). While intercropping with annual legumes may cause a temporary shift in the soil microbial profiles, the impact of perennial crops and intercrops, such as *Desmodium* spp., on soil microbial diversity is likely to be stronger and more resilient.

Diversifying cropping systems, often by using legumes as an intercrop, were originally for purposes other than increasing biodiversity, such as food security, pest control (push–pull), green manure, or to avoid negative plant-soil feedback and soil legacy (Stagnari et al. 2017). However, ripple effects on biodiversity and ecosystem services have become apparent and maize—*Desmodium* intercropping and/or push–pull farming is a good example of this. The system was initially designed to combat stem-borers of maize and sorghum, but additional ecosystem services gradually emerged to include combating parasitic weeds of cereals (such as *Striga* spp.), increase soil nitrogen and carbon, and even reducing incidence of mycotoxins in maize (Balaso et al. 2019; Cook et al. 2007; Xu et al. 2018; Owuor et al. 2018; Njeru et al. 2020). This study adds to these benefits by describing a diversification of the soil microbial communities, with a particularly strong shift in the composition of fungal taxa. By itself diversity in ecosystems is generally regarded as increasing stability,

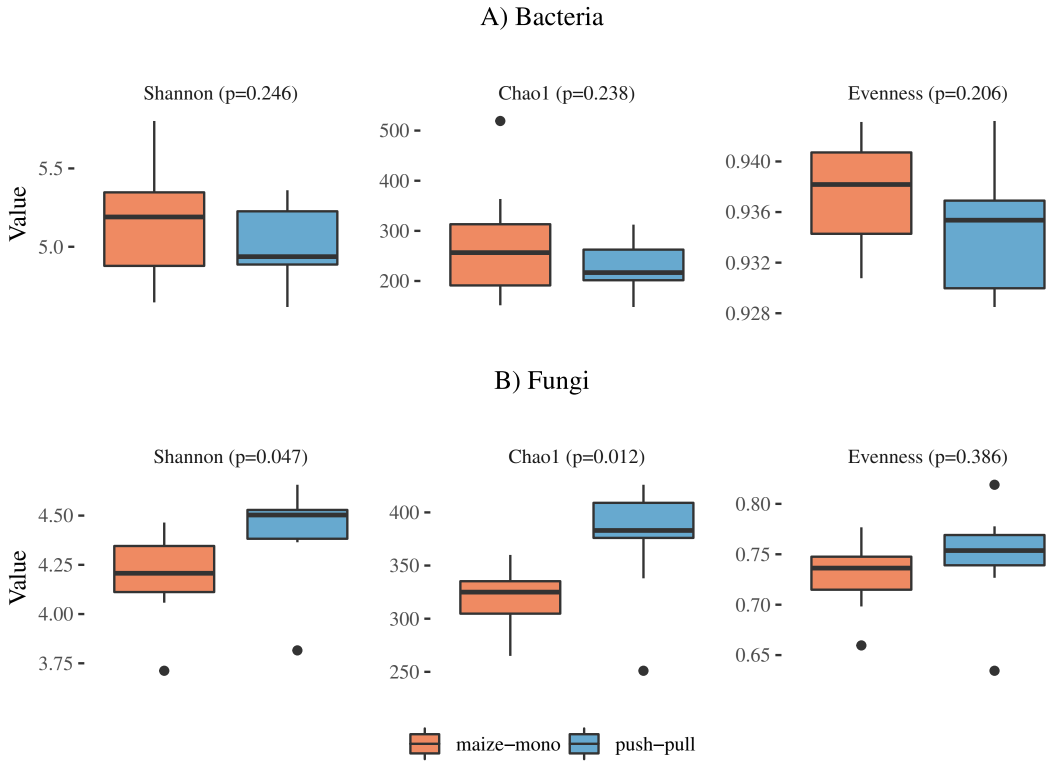


Fig. 4 Diversity index (shannon), richness (chao1) and evenness (shannon/ln[number of species]) of bacterial (**a**) and fungal (**b**) genera across monoculture and maize—*Desmodium* intercropping (push–pull) plots. The box indicates the interquartile range (25–75%), whereas points are deemed to be outliers to the whiskers when they exceed 1.5 times the interquartile range in either direction of the hinges of the box

resilience and productivity (Prieto et al. 2015), mostly as a consequence of resource complementarity and functional redundancy (Cleland 2011; Rosenfeld 2002).

Looking at bacterial populations, the discussion is limited by two factors; fewer taxa that are significantly enriched in either of the farming systems and limited classification (identification) at the genus level. Nevertheless, the genus *Nitrospira* is one of the identified genera that was enriched in maize—*Desmodium* intercropping plots. Species of this genus are known for their ability to perform the complete nitrification process (oxidation of ammonia) during nitrogen fixation, unlike other nitrifying bacteria in which the process occurs in two different organisms (Koch et al. 2015). An enriched presence of *Nitrospira* spp. in intercropping plots suggests involvement in nitrogen fixation, potentially contributing to increased nitrogen supply in the soil and in turn leading to a higher maize yield as previously reported (Khan and Pickett 2008).

Concluding remarks

This study has shown that long-term maize—*Desmodium* intercropping causes a complex shift in composition of the soil microbiome compared to maize monoculture. Many functions of soil microbial communities arise through complex interactions and ecosystem services may therefore not be readily attributed to a single taxon, but arise as an emergent property of system, although exceptions

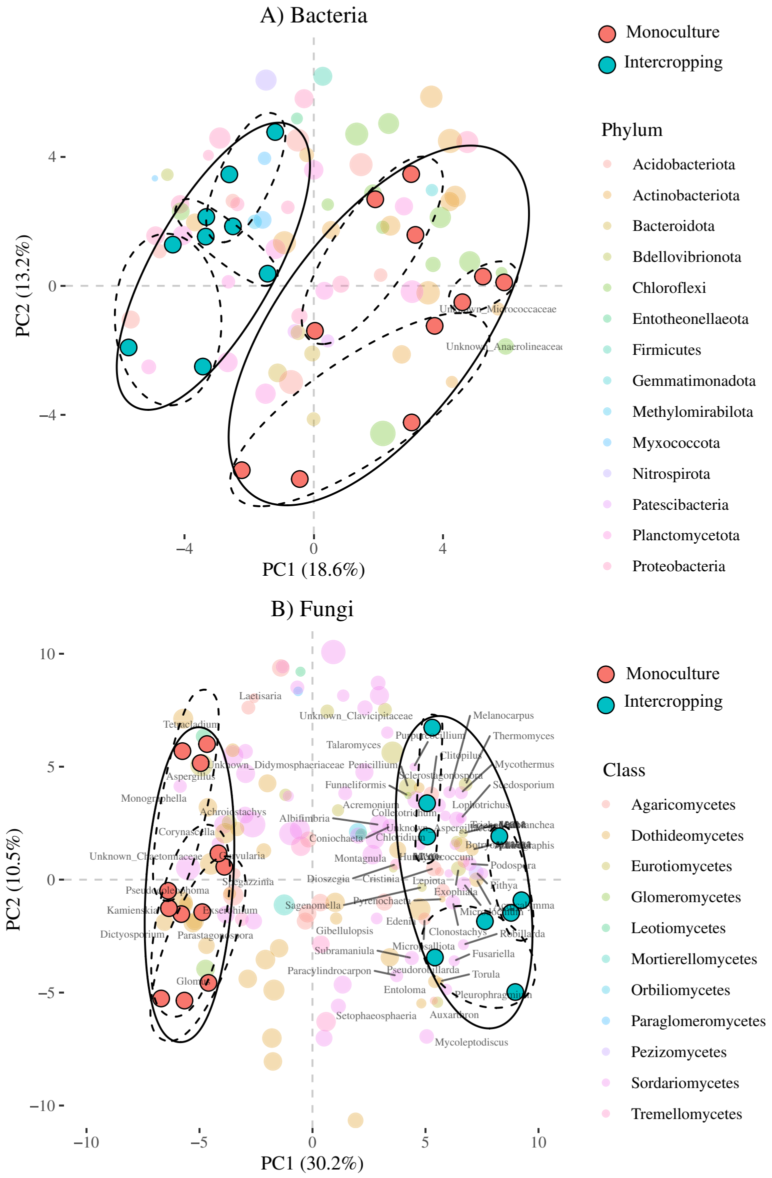


Fig. 5 Principal component analysis of genus level communities of soil bacteria (**A**) and fungi (**B**) in long term maize monoculture and maize—*Desmodium* intercropping/push–pull farming plots. Solid ellipses around each treatment were drawn using the Khachiyan algorithm. Dotted ellipses represent samples from the same experiment in each treatment. In the background are scaled up eigenvectors sized according to average abundance and color-coded according to bacterial and fungal families

exist (Reva et al. 2019). Given the increasing accessibility of sequencing technologies, metagenomics and other DNA-based analyses should be included as an integral part of intercropping studies for improvement of crop health and productivity. Metagenomics data can facilitate interpretation of complex community structure and composition in the light of plant productivity, plant health, and more broadly, ecosystem health.

Acknowledgements This research was made possible with funding from SIDA of Sweden through the University of Dar es Salaam, Tanzania, within the sub-programme 'Sustainable Agricultural Productivity—Processing and Value Chain to Enhance Food Security in Tanzania (ADM, TD, SLL), and The Swedish Research Council (621-2014-4816, TD). We thank the laboratory and field staff at ICIPE Thomas Odhiambo and Duduville campuses for their generous support in sample collection and processing and DNA extraction, especially Dickens Nyagol, Levi and Maureen.

Authors' contributions Conceptualization and advice: TD, SLL, ADM; Methodology and advice: TD, SLL, SLH, ADM, BA, CM, FM; Formal analysis and investigation: ADM, SLH, KSM, SLL, TD; Writing—original draft preparation: ADM, KSM, TD, SLL Writing, review and editing: all authors. Funding acquisition: TD, SLL, FM Supervision: TD, SLL, FM, CM, BA. All authors approved the submitted version for publication.

Funding Funding for this research was provided by a Research Capacity Building Program from Swedish International Development Cooperation Agency (SIDA) to University of Dar es Salaam (UDSM)—(ADM, TD, SLL) and the Swedish Research Council (621-2014-4816, TD).

Availability of data and material The datasets generated during and analysed during the current study are available in the Genbank Sequence Read Archive (SRA) repository under project number PRJNA667690 linked here https://www.ncbi.nlm.nih.gov/bioproject/PRJNA667690/.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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II

A comparative study on the impact of five *Desmodium* species on soil microbiome reveals enrichment of selected bacterial and fungal taxa

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Abstract

Introduction: Several *Desmodium* spp. are used as intercrops in push-pull pest management system to repel insect herbivores. In addition, *Desmodium* suppresses the parasitic weed *Striga*, and diversifies the soil microbiome with negative impacts on fungal. We investigated the impact of a 2-year cropping of five *Desmodium* species on soil microbiome populations.

Methodology: Total DNA was obtained from root zone soil samples collected from a two-years-old common garden experiment with replicated plots of five *Desmodium* spp. at the international center for insect physiology and ecology (ICIPE), Mbita, Kenya. Subsequently, 16S and ITS DNA sequencing were performed and the data was analyzed by using QIIME2 and Calypso.

Results: Our findings show significant differences in composition and abundance of specific microbial taxa among the *Desmodium* plots and the bulk soil, with a stronger shift observed for fungal community profiles than bacteria. There was, however, no significant difference in overall diversity, richness and evenness of microbial communities among the *Desmodium* plots and the bulk soil. Similarly, beta diversity analysis did not reveal a significant association of variation to specific *Desmodium* spp. plots.

Discussion and conclusion: This is the first study to compare impact and association of whole soil microbiomes to different *Desmodium* species. Whereas long-term *Desmodium* cropping clearly shifts whole microbiome communities, no significant difference in overall diversity and richness of microbial populations was observed among the studied plots. However, there was a divergence of individual taxa reflected on their increased abundance in association to specific *Desmodium* spp., pointing towards potential impact on ecosystem services. These findings indicate that significant shifts in whole microbial populations due to *Desmodium* spp. and thus potentially provision of associated ecosystem services require longer cultivation periods to solidify. Future studies should focus on techniques that monitor real-time changes in microbial populations such as RNA-seq to ascertain live and dead microbes, and thus infer ecological services.

Keywords: Desmodium spp., soil microbiome, 16S, ITS, push-pull technology

INTRODUCTION

Push-pull technology for management of Lepidopteran insect pests of cereals employs a stimulo-deterrent mechanism, where *Desmodium* spp. intercrops play a critical role. Smallholder farmers in Eastern and Southern Africa grow *Desmodium* spp. between rows of cereal crops such as maize and sorghum to lower populations of deleterious insect pests away from the main crop. At the same time, *Brachiaria* cv. Mulato or Napier grass (*Pennisetum purpureum*) is planted on the border of the fields to attract and trap insect pests (Pickett et al., 2014). The technology effectively and sustainably controls stem-borers (*Chilo partellus* and *Busseola fusca*) and recently fall armyworms (*Spodoptera frugiperda*). In addition, the root exudates od Desmodium spp. intercrops suppress the parasitic weed *Striga*, endemic in Eastern Africa, thus leading to increases in cereal yield from reduced attack of both insect pest the weed (Midega et al., 2015, 2018).

Desmodium spp. is a genus of flowering plants in the Fabaceae family of about 350 species that grow mainly in tropical and subtropical zones worldwide. Species of this genus find numerous uses including in traditional medicine (Ma et al., 2011; Farid et al., 2018) and a commonly used as animal fodder (Heuzé et al., 2015, 2017). More importantly, species within the *Desmodium* genus were selected for use in a push-pull farming system through careful studies by scientists in Kenya, delivering multiple benefits in smallholder cereal cropping systems. Being leguminous plants, cultivation of *Desmodium* spp. also improves soil fertility through a range of mechanisms including nitrogen fixation, which is enhanced by the perennial nature of *Desmodium* spp. In addition, the intercrop builds soil organic matter reserves and promotes aggregate formation both of which can improve soil moisture conservation (Drinkwater et al., 2021). These soil health benefits likely further contribute to increases in yield of cereal crops, making push-pull an attractive system for smallholder farmers.

The diverse aspects of plant-plant and insect plant interactions in push-pull technology have been well investigated and documented. However, belowground interactions, especially with focus to soil microorganisms have not been investigated, despite the numerous ecological services provided by push-pull technology, in addition to pest control, some with clear indications of the role of soil microorganisms. Taking into account belowground interactions between plants and soil communities is of paramount importance when selecting intercrops considering the contribution of both individual species as well as whole soil microbiomes on plant health and ecosystem functioning (Compant et al., 2019). Yet this area is poorly researched especially regarding soil microbial shifts caused by closely related species or cultivars.

The composition and diversity of soil microbes at a particular location is determined by both biotic and abiotic factors, with aboveground vegetation having the largest influence (Philippot et al., 2013). Plant-soil microbes interactions are mediated through root exudates that provide an important source of carbon for microorganisms as well as signaling compounds (Haichar et al., 2014). In turn, soil microorganisms and nematodes play key roles in maintenance of soil structure and function through provision of critical ecological services. For instance, soil microbes play key roles in decomposition of organic matter and cycling of nutrients, carbon sequestration, promotion of plant health through bio-protection (Jacoby et al., 2017; Saccá et al., 2017) with recent studies suggesting that plant-associated microbes including those in the soil are involved in regulating plant-insect herbivore interactions (Friman et al., 2021; Grunseich et al., 2020; Pangesti et al., 2015). These findings suggest direct implications for farming practices where insect pests continue to devastate productivity. Understanding how specific crop plants modulate overall soil microbiota, and not just individual species, may enhance existing benefits as well as unlock new avenues for sustainable plant health and productivity improvement.

In a previous study we characterized the difference in soil microbial composition, structure and diversity in long term push-pull plots compared to maize monoculture (Mwakilili et al., 2021). Several other studies reporting on benefits of push-pull technology that are clearly linked to soil microbial communities suggest a deeper role than currently known. For example, a study by Njeru et al. (2020) revealed that maize coming from push-pull plots had lower levels of mycotoxins and mycotoxin-producing fungi compared to that from monoculture. In a separate study, the frequency of occurrence of a mycotoxin-producing fungus *Aspergillus flavus* was lower in push-pull than maize monoculture plots (Maxwell et al., 2017). These findings are in line with our previous study where we show that push-pull farming and *Desmodium* intercropping in cereal farming impact diversity of fungal communities more than bacteria (Mwakilili et al., 2021), manipulation of fungal communities in the soil to promote competitive beneficial filamentous fungi has been established as a method to manage mycotoxins in cereals (Sarrocco et al., 2019). In a similar vein, another study demonstrated that maize growing on soil from long-term push-pull plots produced higher amounts of secondary metabolites and experienced lesser herbivory than maize growing on soil from corresponding maize monoculture plots (Mutyambai et al., 2019). Although not investigated, these observations point towards the role of *Desmodium* intercrops in shaping soil microbial communities in push-pull farms and the subsequent microbial activities in plant-soil feedback mechanisms. Although we have already shown that long-term push-pull farming (*Desmodium* spp. intercropping) cause significant shifts in composition and structure of soil microorganisms, however, the time-scale of such changes, and whether the impact on soil microbial communities may be different between *Desmodium* species due to potential differences in composition of root exudates, is unknown. Understanding the differences in soil microbial associations to *Desmodium* spp. may show inter-linkage to the health of *Desmodium* spp. and their abilities to survive under diverse environmental stresses.

In the current study, we investigated the impact of five *Desmodium* species on soil microbial profiles. Currently, two *Desmodium* spp. are commonly used in push-full farming as intercrops, *D. intortum* (greenleaf desmodium) and *D. uncinatum* (silverleaf desmodium). These species have demonstrated several challenges including sensitivity to drought (*D. intortum*) and difficulty of producing seeds (personal communication). In search of more resilient *Desmodium* varieties suited for the varying African climates, several *Desmodium* species accessions were compared for their ability to withstand abiotic stresses including drought tolerance, these are *D. incanum, D. repandum, D. uncinatum, D. intortum* and *D. ramosissimum,* where *D. incanum* and *D. ramosissimum* were shown to exhibit stronger drought tolerance than the other *Desmodium* species, as well as stronger capability to suppress *Striga* weeds (Midega et al., 2017). We thus aimed at complementing the selection of the *Desmodium* spp. as intercrops in cereal push-pull farming by providing insights on their impact on soil microbial populations.

In the present study we show the composition of soil microbial communities in plots cultivated with five different *Desmodium* spp. in comparison to the bulk soil. We also highlight diversity measures as well as enriched taxa associated with each *Desmodium* spp. and the bulk soil.

METHODOLOGY

Sampling site

Soil samples were collected from ongoing common garden experimental plots at the International Centre for Insect Physiology and Ecology (ICIPE), Mbita campus, Kenya (0°25′S, 34°12′E). Mbita is located on the eastern shores of Lake Victoria, 1125 m above sea level. The area receives about 1001 mm rainfall per year and has an average annual temperature of 22.6 °C. Sampling was done during the cool dry season in July 2017. The soil type of the area is sandy loam/black cotton soils.

Soil samples

Soil samples were collected from the common garden plots in which five different species of *Desmodium* had been growing for two years grown in 7.8 m^2 plots in a completely randomized design. The five species have been under evaluation for use in push-pull systems in different agro-ecological regions of Kenya and included *Desmodium* spp*.: D. ramosissimum, D. repandum, D. uncinatum, D. intortum* and *D. incanum*. All plots were treated equally with no additives throughout the cultivation period. The plots relied on seasonal rainfalls and irrigation during dry season. A 2 m buffer strip of bare soil from which control bulk soil samples were collected, separated the plots from the surrounding uncultivated grass-covered land.

For each treatment, three samples were collected. Three plots were selected from each *Desmodium* spp. treatment, with each plot representing one sample. Each sample was made up of a composite of three 15 – 18 cm deep cores taken randomly across *Desmodium* plots close to the roots (root zone). A total of three bulk soil control samples were also collected from the buffer zone where plants were constantly removed so that bare soil was left. Here also each sample was made up of a composite of three 15 – 18 cm deep cores. Afterwards, the composite soil samples from each plot and the buffer zone were homogenized and sieved through a 4 mm wire mesh. About 200 g soil subsample was then collected and stored at -20 ºC for further analysis.

DNA extraction and sequencing

DNeasy Powersoil kit (Qiagen, Manchester, UK) was used for total DNA extraction from the soil samples following the manufacturer’s protocol. Nanodrop spectrophotometer and gel electrophoresis were used to assess the quality, size and quantity of the extracted DNA. DNA samples were stored at -20 ºC.

For bacterial communities, the V1-V3 region of the 16S rDNA gene was targeted with primer pairs 27F and 518R while ITS1F and ITS2 primer pairs were used for fungi targeting the ITS1 region.

Resulting amplicons were gel purified, end repaired and illumina specific adapter sequence were ligated to each amplicon (NEBNext Ultra II DNA library prep kit). Following quantification, the samples were individually indexed (NEBNext Multiplex Oligos for illumina Dual Index Primers Set 1), and another AMPure XP bead based purification step was performed. Amplicons DNA sequencing was done at Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa) on Illumina MiSeq platform using a MiSeq v3 kit with 600 cycles (300 cycles for each paired read and 12 cycles for the barcode sequence) according to the manufacturer’s instructions. Demultiplexed 300bp paired-end reads were obtained.

Bioinformatics and statistical analysis

FASTQC (Wingett & Andrews, 2018) was used to assess the quality of raw sequence reads. The reads were then imported into QIIME2 v2020.11 (Bolyen et al., 2019) where quality control, construction of a feature table and taxonomic classification were performed. In summary, quality control was done by using the dada2 plugin (Callahan et al., 2016) by trimming and truncating both the 16S and ITS reads to remove low quality parts. Taxonomic assignment was done by using feature-classifier classify-sklearn (Bokulich et al., 2018) by using pre-trained classifiers. Bacterial taxonomic assignment was based on Greengenes reference database (DeSantis et al., 2006) pre-trained on V1-V3 region of the 16S, while for the fungi, the UNITE v8.2 reference database (Nilsson et al., 2018) pre-trained to ITS1 was used. Important commands and parameters used are highlighted in Table 1.

Table 1*: Commands and parameters used during data analysis in Qiime2*

Function	Command and parameters	Platform
Trimming 16S sequences	--p-trim-left -f 8 --p-trim-left -r 8	qiime2
truncation 16S sequences	--p-trunc-len -f 290 --p-trunc-len -r 260	qiime2
Trimming ITS sequences	-- p-trim-left 10	qiime2
Truncation ITS sequences	--p-trunc-len 299	qiime2

Further, the feature table was converted into biom format (using qiime 2 export tool), and then imported into calypso V8.84 (http://cgenome.net:8080/calypso-8.84) (Zakrzewski et al., 2017) where further statistical and diversity analyses were performed. Before the analyses in calypso, samples with less than 1000 sequence reads, taxa with less than 0.01% relative abundance and taxa with over 50% zeroes were filtered out. Feature reads counts were normalized by total sum of squares (TSS) and transformed by both cumulative sum-scaling (CSS) and log2 to account for the non-normal distribution of taxonomic counts.

In calypso, different quantitative measures were analysed and plotted including taxa abundance and differential abundances in the treatments. Bray-Curtis distance metric was used to perform multivariate statistical testing and generate relevant plots for beta diversity estimation among the *Desmodium* spp. and control plots. Alpha diversity measures Shannon index, richness and evenness were also calculated as well as differential abundance and group association analyses.

RESULTS AND DISCUSSION

In this study, we hypothesized that continuous cultivation of *Desmodium* species for 2 years caused shifts in soil root zone microbial community structure, composition and diversity relative to the bulk soil. With aboveground vegetation having been shown to exert the biggest influence on composition and structure of soil microbial populations (Hooper et al., 2010, 2015) we further hypothesized that the impact on soil microbial communities would diverge between *Desmodium* species due to potential variation in composition of root exudates. It was expected that the different species *Desmodium* would attract different assemblages of soil microorganisms in the root zone, with potential implications on health and functioning of *Desmodium* and the ecosystems of which they are part, such as in push-pull farming.

The findings show different aspects of soil microbial communities associated with the studied *Desmodium* species in contrast to the bulk soil; 1) differences in composition and abundance of soil microbes between *Desmodium* plots and bulk soil 2) highlight dominant soil microbial taxa associated with *Desmodium* spp., 3) unique and common microbial groups associated with *Desmodium* spp. as well as 4) diversity measures of soil microbial communities.

Composition and abundance of soil microorganisms

A total of 15 bacterial and 8 fungal phyla were identified in all soil samples. The most abundant bacterial phyla were Chloriflexi (23%), Actinobacteria (21%) Cyanobacteria (15%), Acidobacteria (14%), Proteobacteria (8%) and Planctomycetes (8%). Other phyla included Bactroidetes, Gammatimonadetes, Nitrospirae, Elusimicrobia, Firmicutes and Armatimonadetes while two phyla were unclassified. Relative abundances of the identified bacterial phyla are shown in figure 1A.

The majority of the fungal microbes belonged to the phylum Ascomycota (84%), followed by Basidiomycota (7.6%). Other phyla included Chytridiomycota (0.16 %), Glomeromycota (0.9 %) and Mortierellomycota (0.9 %). One phylum was unclassified and another unidentified. The relative abundances of fungal phyla in all plots are shown in Figure 1B.

At the genus level, most observed bacterial genera were unclassified due to the limitations of the classification databases in addition to potentially novel soil bacteria genera that may have not been classified in the past. However, among the few that were identified *i.e.*, *Rhodoplanes*, *Gemmata*, *Nitrospira*, *Bradyrhizobium*, *Balneimonas*, *Streptomyces* and *Steroidobacter* occurred in varying abundances in all *Desmodium* spp. plots and bulk soil. The abundances of the 30 most abundant bacterial genera including those mentioned above are shown in figure 2.

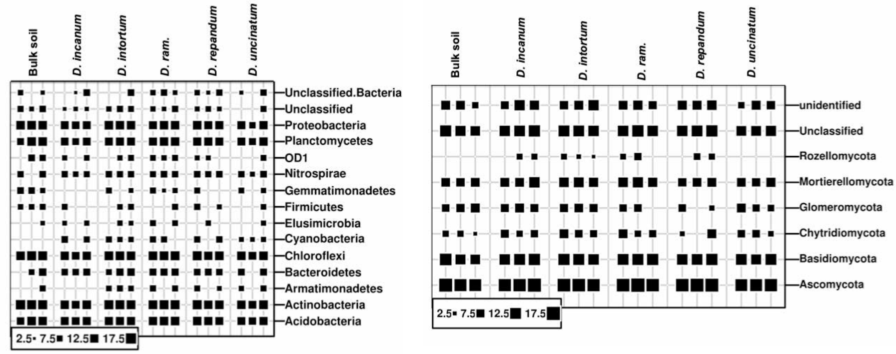

Figure 1: Bubble plots showing relative abundances of bacterial (left) and fungal (right) phyla in *Desmodium* spp. root zone soil and bulk soil. The relative abundances are shown as bubbles, with the size of the bubble being directly proportional to the relative abundance. Relative abundance was calculated from read counts (ASVs) normalized by total sum of squares (TSS) and transformed by cumulative sum scaling (CSS).

Compared to bacterial taxa, the majority of the abundant fungal genera were classified, as shown in figure 3, allowing for theorization of function based on literature. Both the *Desmodium* spp. plots and bulk soil harbored a diverse number of genera in varying abundances, with the genus *Fusarium* being the most abundant taxa in both *Desmodium* species plots and the bulk soil. Other abundant genera identified are *Didymella, Chaetomium, Cladorrhinum, Stachybotrys* and *Curvularia*.

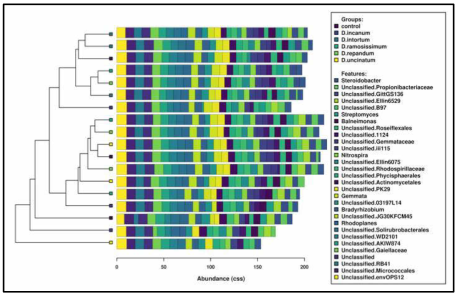

Figure 2: A clustered bar chart showing relative abundance of 30 most abundant soil bacterial genera in Desmodium spp. root zone soil and bulk soil. Most of the bacteria genera were unclassified and thus unidentified. The relative abundance was calculated from read counts (ASVs) normalized by total sum of squares (TSS) and transformed by cumulative sum scaling (CSS).

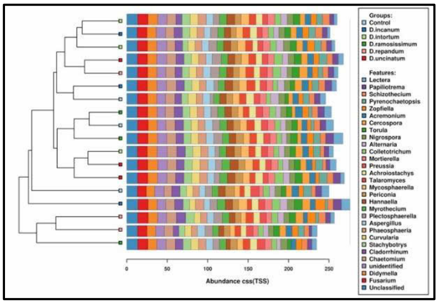

Figure 3: Clustered bar chart showing relative abundance of 30 most abundant soil fungal genera in Desmodium spp. root zone soil and bulk soil. Most of the fungal genera were classified and identified. The relative abundance was calculated from read counts (ASVs) normalized by total sum of squares (TSS) and transformed by cumulative sum scaling (CSS).

Studies on shifts of soil microbial populations as a result of *Desmodium* spp. cultivation are scant. Literature on soil microbes and *Desmodium* spp. is populated by research on endophytes and nodule symbionts, in particular bacterial endosymbionts. Endosymbionts and nodule bacteria of other leguminous plants have been widely characterized and studied for their role in nitrogen fixing, an important ecological function. For *Desmodium* spp. (Parker, 2002) isolated several *Bradyrhizobia* species from *D. grahamii* nodules while (Toniutti et al., 2017) did the same from *D. incanum*. Most of the endosymbionts isolated from different *Desmodium* species in these studies tend to fall under three rhizobia genera; *Rhizobium*, *Bradyrhizobium* and

Mesorhizobium (Xu et al., 2016). While investigating plant-endosymbionts relationships is important for plant health and productivity due to their intimate relationship with plant physiology, the importance of free living and the rhizosphere microbiome microorganisms cannot be overlooked, not the least because they are the source of the endophytes recruited by plants (Xiao et al., 2017). Free-living soil microbes also interact with plants through direct and indirect mechanisms that impact their health and productivity. In this study for example, some of the abundant bacterial groups identified are linked to varying activities in the soil that contribute to provision of ecosystem services. For example, *Nitrospira* spp. are known for their ability to fix nitrogen and potentially increasing supplies in the soil (Lu et al., 2020) while species of both *Streptomyces* and *Bradyrhizobium* are commonly known as biofertilizers (Htwe et al., 2019).

Contrary to expectations, fungal genera known to harbor plant pathogenic species were found in high abundance in both *Desmodium* plots and the bulk soil. These included *Fusarium*, *Gibberella* and *Didymella* genera (Figure 2). Although *Fusarium* is a ubiquitous genus with many harmless species, other species of this genus cause serious crop losses due to their pathogenicity and mycotoxin production that affect animals and human beings alike (Summerell, 2019). However, *Fusarium* species may form endophytic relationships with legumes such as *F. solani* and *Medicago truncatula* (Skiada et al., 2020) and become opportunistic when a favorable environment in the soil/plant is present.

In addition to *Fusarium*, we observed the presence of *Aspergillus* among the most abundant taxa in both *Desmodium* spp. plots and the bulk soil. Several species of the genus *Aspergillus* including *A. flavus*, *A. parasiticus* and *A. fumigatus* also produce potent mycotoxins that spoil cereal crop harvests and are harmful to human beings (Barkai-Golan, 2008). In our previous study, *Aspergillus* spp. were found in high abundance in soils of maize monoculture compared to long-term push-pull farms that employed *Desmodium* intercrops (Mwakilili et al., 2021). Similarly, (Maxwell et al., 2017) reported lower frequency of *Aspergillus flavus* in maize cobs from monoculture plots than in *Desmodium* intercropping push-pull systems, but an opposite trend for *A. parasiticus*. It is possible that the soils of the area are rich in these fungal taxa and the time under *Desmodium* spp. cultivation was too short to induce a significant change in populations abundance like in the discussed studies. In addition, without further analysis using higher resolution techniques such as whole genome sequencing (WGS) metagenomics, it is not possible to discern the specific *Fusarium* and *Aspergillus* species observed in the present study but the findings of this study point to a possibility of reduction of these taxa with continued cultivation of *Desmodium* spp.

The rest of the abundant genera were those ubiquitous in nature, containing beneficial, neutral and pathogenic fungi of plants and human beings, such as *Chaetomium, Cercophora*, *Colletotrichum* and *Plectosphaerella*.

Common and unique soil microbial taxa among the *Desmodium* spp. plots

Comparison of the composition of soil microorganisms between the *Desmodium* spp. plots revealed the core microbiome of 29 bacteria and 55 fungi genera (Figure 4). Further, the microbiome and taxa that were uniquely associated with each *Desmodium* plot were identified. From the findings, the composition of taxa overlapped among the *Desmodium* plots, with *D. intortum* being associated with the largest number of unique bacterial genera (12) while *D. repandum* plots harbored the largest number of unique fungal genera (7). These two *Desmodium* spp. may be the most effective in recruiting and maintaining diverse microbial groups compared to others. Converesly, no unique bacterial genera were associated with the *D. incanum* plots (Figure 4A).

Although the core fungal microbiome was larger than bacterial, most of the taxa were shared among the *Desmodium* plots causing the proportion of unique fungal taxa associated with individual *Desmodium* spp. plots to be lower. In general, most of the *Desmodium* spp. plots shared at least one taxon with each other, with *D. rammossisimum*, *D. repandum* and *D. intortum* sharing the largest number of both bacteria (7) and fungal genera (6) amongst themselves (Figure 4). This may indicate that their microbial recruitment strategies and root exudates composition are similar, possibly from a genetic makeup that is not very far from each other. The complete list of core, unique and pan genera is in supplementary tables 1 - 6.

While an association with more unique taxa in itself may not be an indication of direct and indirect activities of soil microbes that impact plant health, the ability of plants to recruit and support diverse microorganisms contributes to a more stable and resilient rhizospheric ecosystem (Wu et al., 2018). *D. intortum* and *D. uncinatum* are the commonly used intercrops in push-pull farming while the other *Desmodium* species have not been widely adopted despite some of them showing moderate to high drought tolerance, and *Striga* suppression. Of particular importance is *D. rammossisimum*, which along with *D. incanum,* showed the highest level of drought resistance and biomass retention in a previous field study (Midega et al., 2017). Although the study did not investigate the role of soil microbiome in the drought tolerance, other studies have demonstrated the ability of whole soil microbiomes to confer plants with the ability to tolerate abiotic stresses including tolerance to drought

(Zolla et al., 2013; Vurukonda et al., 2016; Huang et al., 2017). In addition to finding the link between belowground diversity and abiotic stress tolerance, it may be useful to investigate the potential of mixed-intercropping in push-pull systems by combining more than one *Desmodium* spp. to leverage both below- and above-ground benefits offered by different species.

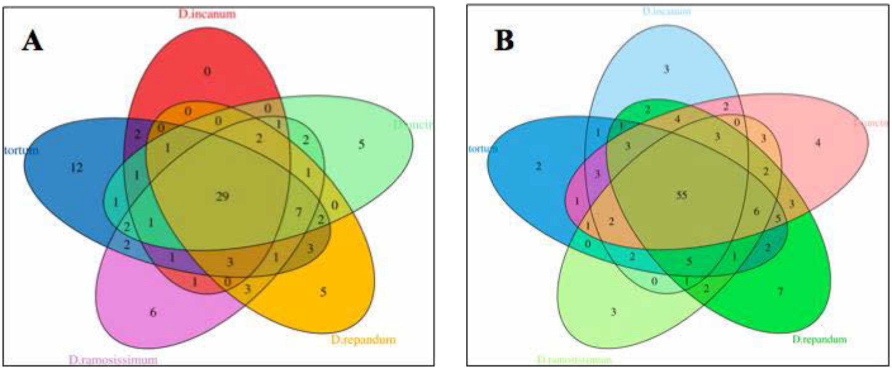

Figure 4: Venn diagram showing core, pan and unique soil bacterial (A) and fungal (B) genera from the 5 studied Desmodium species plots. The plots shared a large core genome of bacteria (29) and fungi (55) with few unique genomes associated with each *Desmodium*

Diversity measures

We also analysed different measures of diversity and richness of the soil microbiome among the *Desmodium* spp. plots and the bulk soil. Comparing the diversity of soil microbes within each treatment (alpha diversity), we found no significant difference/variation of both bacterial (Supplementary figure 1) and fungal (Supplementary figure 2) communities through diversity measures of richness and evenness.

Similarly, analysis of diversity between the treatments (beta diversity) did not reveal any significant association of the soil microbial populations to the different treatments i.e. *Desmodium* spp. plots or the bulk soil. This indicates the overall variation of the soil microbial communities composition between plots was random and not significantly altered by the cultivation of *Desmodium* spp. compared to the bulk soil, as observed by absence of distinct clustering patterns in PCoA plots (Figure 5).

We expected to see a more pronounced impact of the *Desmodium* spp. cultivation on the diversity and divergence of soil microbial communities compared to bulk soil. Our observations suggest that two years is not a sufficient time window for a noticeable influence of *Desmodium* spp. on whole shifts in belowground microbial communities. In a previous study, we reported the impact of long term (14 - 18 years) *Desmodium* intercropping on the composition and diversity of soil microbial profiles (Mwakilili et al., 2021) where a strong shift of fungal communities was observed in push-pull plots compared to maize monoculture plots.

Other studies have indeed suggested that microbial based plant-soil feedback is a slow process in that although aboveground vegetation has the largest influence on assemblages and alterations of soil microbial communities, the process may take several years to form stable structures (Eisenhauer et al., 2011; Vukicevich et al., 2016). Given a longer period, the patterns of the impact of the *Desmodium* spp. on soil microbial communities may emerge, and with them, other emergent differential benefits conferred by soil microorganisms on *Desmodium* plant health and other ecological services.

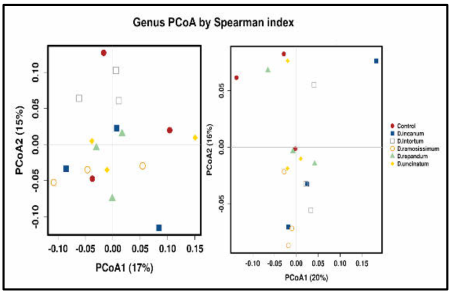

Figure 5: PCoA plots of soil bacterial (left) and fungal (right) genera (OTUs) across the Desmodium spp. root zone soil and bulk soil based on Spearman correlation. The clustering patterns reveal no association between the different treatments and microbial populations, indicating no clear impact of the Desmodium species.

Differential abundances of individual taxa

Despite the lack of significant difference in overall diversity and richness of the soil microbial populations, several bacterial and fungal taxa were enriched in specific *Desmodium* spp. plots as well as in the bulk soil (Figure 6 and 7). A larger proportion of the significantly abundant taxa are fungal (23) compared to only four bacterial taxa. Of the four significantly abundant bacteria taxa, one taxon code-named JG30KFCM45 was abundant in all treatments. By contrast, the genus *Agromyces* was significantly abundant in *D. intortum* plots only (Figure 7). *Novosphingobium* and *Craurococcus* were other significantly abundant bacterial genera, both having high abundance in *D. repandum* plots. *Novosphingobium* was in addition found in significantly higher abundance in *D. intortum* plots and *Craurococcus* in *D. uncinatum* plots and the bulk soil.

Among the fungal taxa that were significantly abundant, 12 of them were the most common being significantly abundant across most of the treatments. *Mycosphaerella*, *Hannaella* and *Cercospora* were the most ubiquitous significantly abundant fungal taxa irrespective of treatment. On the other hand, other fungal groups were significantly abundant in few treatments such as *Pseudaleuria* and *Fusidum*, which were enriched in only two treatments (*D. incanum* and *D. ramosissimum)*. In addition, three of the five *Desmodium* spp. plots harbored a substantial percentage of the significantly abundant taxa; these are *D. uncinatum*, *D. incanum* and *D. intortum* (Figure 7.)

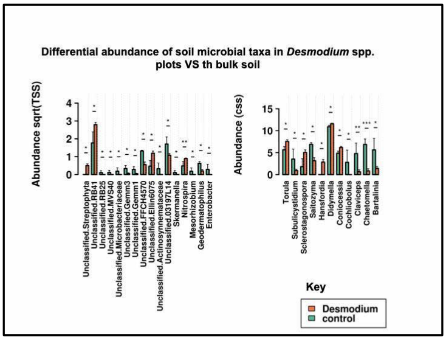

Figure 6: Comparison of differential abundance of bacterial (left) and fungal (right) taxa between *Desmodium* spp. root zone soil and bulk soil highlighting taxa significantly abundant in either treatment. (ANOVA, where * = p<0.05, ** = p<0.01, *** = p<0.001)

As in our previous long-term study on maize-*Desmodium* intercropping soils (Mwakilili et al., 2021) where we investigated impact of push-pull technology that employs perennial *Desmodium* spp. intercrops, soils diverged more in fungal than bacterial communities. The studied plots were between 14 and 18 years old and employed *D. intortum* and/or *D. uncinatum* as perennial intercrops. To our knowledge, there are no other studies that investigate the impact of *Desmodium* cropping on whole soil microbial populations. Understanding which shifts in soil microbiomes are associated with plant health and productivity is, however, important to be able to more efficiently reap the benefits of ecologically intensified cropping systems.

Without experimental verification, we can only speculate about the role of and potential ecosystem services rendered by differentially abundant taxa in *Desmodium* plots. The bacterial genera *Novosphingobium* and *Agromyces*, both significantly enriched in *Desmodium* spp. plots, harbor species with the ability to degrade compleorganic compounds, with the later also being able to resist heavy metals. Members of the taxa have been reported to show capability of degrading complex organic compounds such as xylan (Rivas et al., 2004) and aromatic compounds (Sohn et al., 2004; Liu et al., 2005). These properties may be essential in releasing nutrients from complex organic matter as well as degrading toxic compounds and thus potentially improve the ability of plants to survive in harsh environments.

Several fungal taxa were significantly abundant in *Desmodium* plots but not in the bulk soil, including *Pseudaleuria*, *Phialophora*, *Hansfordia* and *Fusidium*. These genera comprise common soil and wood saprotrophs most of which have known ecological functions. The genus *Pseudaleuria* for instance has been associated with healthy soils and disease suppression in pea fields (Xu et al., 2012), while some species of *Phialophora* cause soft rot of wood and other root diseases especially in wheat and other species show plant protection properties (Zriba et al., 1999; Karunasekera & Daniel, 2013). The remaining fungal genera that were significantly abundant in *Desmodium* spp. plots belong to endophytic groups, and often possess beneficial plant protection and growth promotion activity. These include *Hannaella*, a genus of endophytic fungi (Gonzaga et al., 2015), *Chrysosporium*, whose species have been shown to produce plant hormones (Hamayun et al., 2009), and *Lecanicillium*, a genus comprising species that display a wide range of growth promotion and protection activities against pathogens, insects and nematodes on plants (Goettel et al., 2008; Nicoletti & Becchimanzi, 2020). Other fungal groups highly abundant in *Desmodium* plots, but present in the bulk soil in small amounts were *Paracamarosporium*, *Microasus*, *Leptodiscella* and *Humicola*.

Conversely, species of some fungal genera that were significantly abundant in the bulk soil compared to the *Desmodium* plots (i.e. *Ascochyta*, *Chaetomella* and *Graphium*) have been reported to cause diseases in plants. For example, species of the genus *Ascochyta*, a teleomorph of *Didymella* spp., cause blights of cereals and legumes (Tivoli & Banniza, 2007)

Although not conclusive, these findings point in a slow divergence, whereby cultivation of *Desmodium* spp. favors growth and replication of specific groups of microbial taxa. Most of the microbial groups found in significantly higher abundance in *Desmodium* spp. plots are either ubiquitous harmless microbes or have previously been noted for conferring ecosystem services such as improved access to nutrients from the soil, suppressing harmful and disease-causing microbes. Indeed, reports of lower mycotoxin producing fungi in push-pull plots where *Desmodium* spp. are used as intercrops (Maxwell et al., 2017; Owuor et al., 2018; Njeru et al., 2020) as well as associational resistance in maize grown on soil from long term push-pull fields (Mutyambai et al., 2019) may be the first clue about the important role of *Desmodium* spp. in shaping soil microbial communities leading to diverse ecological benefits related to food production and safety. These observations warrant further dissection.

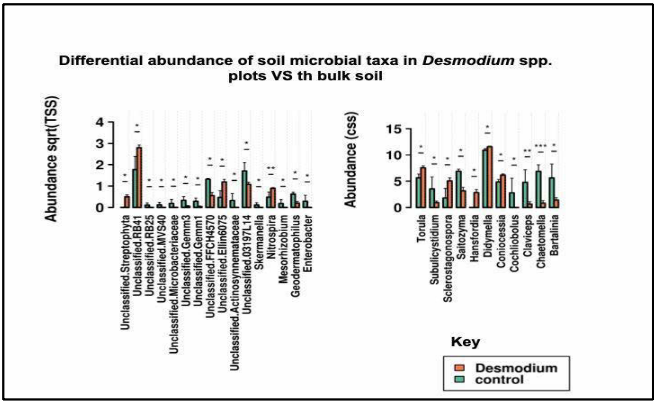

Figure 6: Comparison of differential abundance of bacterial (left) and fungal (right) taxa between *Desmodium* spp. root zone soil and bulk soil highlighting taxa significantly abundant in either treatment. (ANOVA, where * = p<0.05, ** = p<0.01, *** = p<0.001)

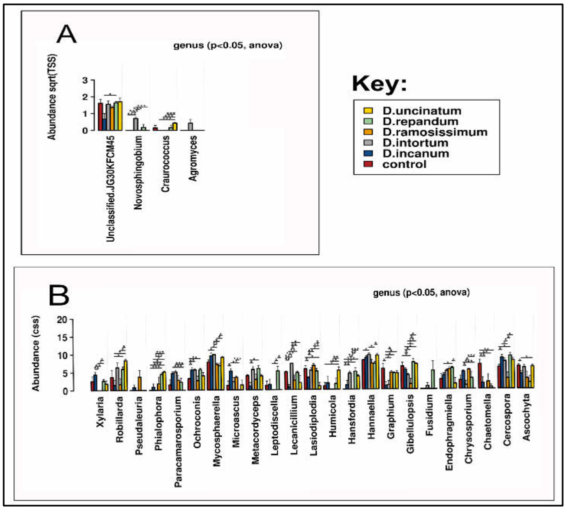

Figure 7: A bar chart showing significantly abundant bacterial (A) and fungal (B) taxa among *Desmodium* spp. root zone soil and bulk soil obtained by t-test pairwise comparisons (where *p<0.05, **p<0.01, ***p<0.001). Error bars depict standard error.

CONCLUSION

In this study, we hypothesized that continuous cultivation of *Desmodium* species shifts in soil microbial populations structure, composition and diversity relative to non-cultivated bulk soil. In addition, we hypothesized that the impact on soil microbial communities would be different among different *Desmodium* species. Although cultivation of *Desmodium* spp. leads to significant increases in abundance of selected bacterial and fungal taxa, no significant difference in overall diversity of soil microbial communities both within plots and between plots. Soil microbial communities interact with plants and play a key role in restoring resilience of soils for provision of ecosystem services in farming systems. However, as shown in this study, shifts in microbial populations are more intricate long-term processes than anticipated without short-term incentives. A longer period of cultivation is undoubtedly required for clearer patterns of changes in the composition and abundance of the soil microbial communities. Aboveground vegetation has been demonstrated to play the most significant role in shaping soil microbial communities in long-term studies. This fits well with the nature of push-pull farming, being a perennial *Desmodium* spp. based intercropping technology whose numerous benefits become apparent with time, adding to unseen belowground ecological services of the technology.

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ACKNOWLEDGEMENT

We are grateful for the support of ICIPE staff during soil sample collection and processing, including Dickens Nyagol, Levi and Moureen. Special thanks to Emma Huri, Will English and Paul Eagan for reviewing early drafts of the manuscript.

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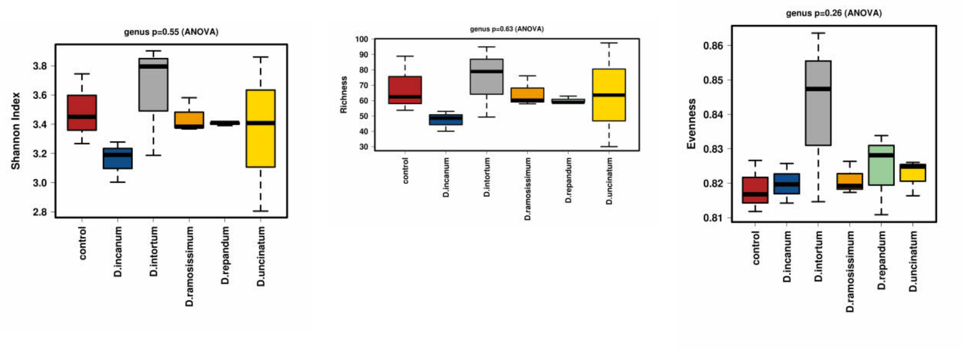
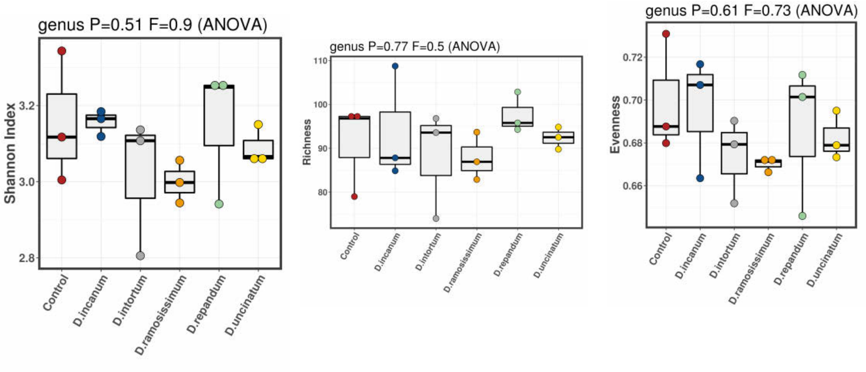
Supplementary materials

Supplementary figures



Supplementary figure 1: Box plot representation of alpha diversity measures (from left, Shannon index, richness and evenness measures) of soil bacterial communities in all treatments. Alpha diversity measures compare diversity of microbial populations within each treatment. The figures show variation in the diversity in composition of soil bacterial taxa (ANOVA, p = 0.55) in the treatments was not significant. In addition, there was no significant difference in richness (ANOVA, p = 0.63) and evenness of the taxa (ANOVA, p = 0.26).



Supplementary figure 2: Box plot representation of alpha diversity measures (from left, Shannon index, richness and evenness measures) of soil fungal taxa in all treatments. Alpha diversity measures compare diversity of microbial populations within each treatment. The figures show variation in the diversity in composition of soil fungal taxa (ANOVA, p = 0.51) in the treatments was not significant. The same observation was made for richness (ANOVA, p = 0.77) and evenness (ANOVA, p = 0.61) measures.

Supplementary tables

Supplementary table 1: Unique bacterial taxa that occur in only one of the *Desmodium* spp. plots

	Abundance					**OCC**				
Taxa	*D. incanum*	*D. intortum*	*D. ramosissimum*	*D. repadum*	*D. uncinatum*	*D. incanum*	*D. intortum*	*D. ramosissimum*	*D. repandum*	*D. uncinatum*
D. repandum										
X5B12	0	0	1.697	3.267	2.043	0	0	0.33	0.67	0.33
X03196A21	0	0	0.887	2.137	0.373	0	0	0.33	0.67	0.33
Unclassified CV90	1.62	0	0	2.483	2.02	0.33	0	0	0.67	0.33
OM27	1.79	1.69	0	2.347	1.227	0.33	0.33	0	0.67	0.33
Dolo_23	0	1.837	2.013	4.357	2.07	0	0.33	0.33	0.67	0.33
D. intortum										
Unclassified TM73	1.363	3.15	0	0	0	0.33	0.67	0	0	0
Unclassified TM71	0	3.087	0	1.533	1.49	0	0.67	0	0.33	0.33
Unclassified TM7	0	2.867	1.697	0	0.84	0	0.67	0.33	0	0.33
Unclassified B07_WMSP1	0	3.39	1.057	2.193	2.127	0	0.67	0.33	0.33	0.33
Unclassified Acidimicrobiales	0	4.227	0	0	0	0	0.67	0	0	0
Sphingomonadaceae	0	6.11	0	1.533	2.257	0	1	0	0.33	0.33
Rhizobiaceae	2.027	6.79	2.007	2.143	1.49	0.33	1	0.33	0.33	0.33
Pseudomonadaceae	0	2.993	0	0	1.147	0	0.67	0	0	0.33
Micrococcaceae	0	3.843	1.877	0	0	0	0.67	0.33	0	0
Microbacteriaceae	0	4.287	1.74	1.727	1.467	0	0.67	0.33	0.33	0.33
Chloroflexaceae	0	2.663	0	0	0	0	0.67	0	0	0
Entotheonellaceae	1.53	1.237	0.977	0.727	0	0.33	0.67	0.33	0.33	0
D. uncinatum										
Unclassified S085	2.313	0	1.597	1.927	3.147	0.33	0	0.33	0.33	0.67
Unclassified C0119	0	1.317	1.27	0.873	2.5	0	0.33	0.33	0.33	0.67
Acetobacteraceae	0	0	0	1.623	4.167	0	0	0	0.33	1
Haliangiaceae	1.217	0	0	0	2.893	0.33	0	0	0	0.67
Flavobacteriaceae	1.91	0	0	0.93	3	0.33	0	0	0.33	0.67
D. ramosissimum										
Unclassified Planctomycetes	0	1.767	3.38	1.227	2.03	0	0.33	0.67	0.33	0.33
Geodermatophilaceae	1.97	0	3.203	1.58	1.467	0.33	0	0.67	0.33	0.33
Unclassified CL50015	0	0	2.707	0	1.863	0	0	0.67	0	0.33
Unclassified CCU21	0	1.64	2.953	0	0	0	0.33	0.67	0	0
Ardenscatenaceae	0	0	2.397	0	0	0	0	0.67	0	0
Cystobacteraceae	0	1.84	3.403	1.667	1.993	0	0.33	0.67	0.33	0.33

Supplementary table 2: Core bacterial taxa found in all five *Desmodium* spp. plots

Taxa	**Abundance**					**OCC**				
	D. incanum	*D. intortum*	*D. ramosissimum*	*D. repandum*	*D. uncinatum*	*D. incanum*	*D. intortum*	*D. ramosissimum*	*D. repandum*	*D. uncinatum*
Unclassified WD2101	6.907	8.047	8.003	7.903	9.037	1	1	1	1	1
Unclassified TK10	3.433	6.087	5.48	6.197	3.84	0.67	1	1	1	0.67
Unclassified Solirubrobacterales	4.977	8.123	8.12	8.103	7.993	0.67	1	1	1	1
Unclassified Roseiflexales	3.82	5.517	4.273	5.79	6.367	0.67	1	0.67	1	1
Unclassified RB41	9.843	8.983	9.593	9.55	9.147	1	1	1	1	1
Unclassified PK29	5.047	7.01	7.073	6.903	7.213	0.67	1	1	1	1
Unclassified Phycisphaerales	6.607	5.78	6.207	6.507	7.523	1	1	1	1	1
Unclassified Myxococcales	3.367	5.403	5.347	4.84	4.157	0.67	1	1	0.67	0.67
Unclassified Micrococcales	9.463	9.267	9.503	9.17	9.493	1	1	1	1	1
Unclassified JG30KFCM45	4.28	7.8	7.397	8.03	7.997	0.67	1	1	1	1
Unclassified.envOPS12	10.257	9.947	10.337	10.513	10.44	1	1	1	1	1
Unclassified.B97	4.1	3.87	5.86	6.573	5.81	0.67	0.67	1	1	1
Unclassified Actinomycetales	7.403	6.567	6.5	6.847	4.71	1	1	1	1	0.67
Unclassified 1124	5.327	5.573	6.08	4.877	5.98	1	1	1	1	1
Unclassified 03197L14	5.397	6.95	6.647	6.85	6.8	1	1	1	1	1
Unclassified	7.76	8.997	8.553	8.907	8.367	1	1	1	1	1
Sinobacteraceae	5.673	4.927	5.863	6.597	5.897	1	1	1	1	1
Rhodospirillaceae	4.283	6.443	6.507	6.777	6.343	0.67	1	1	1	1
Propionibacteriaceae	7.827	7.4	7.213	7.32	7.303	1	1	1	1	1
Pirellulaceae	5.007	2.693	7.127	3.753	3.837	0.67	0.67	1	0.67	0.67
Nitrospiraceae	6.353	6.003	5.727	6.257	6.477	1	1	1	1	1
mb2424	4.46	4.063	4.283	3.973	3.92	0.67	0.67	0.67	0.67	0.67
Hyphomicrobiaceae	9	8.623	5.86	8.547	5.53	1	1	0.67	1	0.67
Gemmataceae	5.153	7.9	8.437	8.873	5.623	0.67	1	1	1	0.67
Gaiellaceae	7.563	9.32	8.613	9.243	9.26	1	1	1	1	1
Ellin6075	7.76	6.017	7.217	7.143	5.01	1	1	1	1	0.67
Chitinophagaceae	7.147	6.767	7	6.837	4.51	1	1	1	1	0.67
Bradyrhizobiaceae	7.75	8.513	7.817	7.77	7.787	1	1	1	1	1
AKIW874	8.443	8.107	8.443	8.217	8.647	1	1	1	1	1

Supplementary table 3: Pan bacterial taxa shared by several *Desmodium* spp. plots

Taxa	*Desmodium* spp. plots sharing taxa	**Abundance**					**OCC**				
		D. incanum	*D. intortum*	*D. ramosissimum*	*D. repandum*	*D. uncinatum*	*D. incanum*	*D. intortum*	*D. ramosissimum*	*D. repandum*	*D. uncinatum*
Unclassified Streptophyta	DIN, DIT, DRP, DUN	4.437	4.617	1.717	3.997	4.38	0.67	1	0.33	0.67	1
Unclassified Sphingobacteriales	DIN, DIT	3.05	1.727	1.17	0	0	0.67	0.67	0.33	0	0
Unclassified S0208	DIT, DRM, DUN	0	3.14	3.497	1.917	3.5	0	0.67	0.67	0.33	0.67
Unclassified Pla4	DRM, DRP	0	1.257	4.253	5.897	1.96	0	0.33	0.67	1	0.33
Unclassified MVP88	DIN, DIT, DRM	2.75	2.457	2.937	1.597	1.407	0.67	0.67	0.67	0.33	0.33
Unclassified MND1	DIN, DRM	4.293	1.687	4.137	2.47	1.863	0.67	0.33	0.67	0.33	0.33
Unclassified iii115	DIT, DRM, DRP, DUN	2.677	6.84	6.697	6.417	5.007	0.33	1	1	1	0.67
Unclassified H39	DRM, DRP	1.75	1.393	3.03	2.95	1.623	0.33	0.33	0.67	0.67	0.33
Unclassified GittGS136	DIT, DRM, DRP, DUN	2.55	6.823	6.343	6.283	4.24	0.33	1	1	1	0.67
Unclassified Gemmatimonadetes	DRM, DUN	0	1.213	1.82	0	3.057	0	0.33	0.67	0	0.67
Unclassified Ellin6529	DIN, DRM, DRP, DUN	7.59	1.66	5.15	5.513	5.263	1	0.33	0.67	0.67	0.67
Unclassified Ellin329	DRM, DUN	0	0	3.357	1.767	2.71	0	0	0.67	0.33	0.67
Unclassified DRC31	DIN, DIT, DRM, DUN	3.667	4.293	3.73	2.157	4.273	0.67	0.67	0.67	0.33	0.67
Unclassified Bacteria	DIN, DRM, DRP, DUN	3.303	2.193	4.293	4.367	3.067	0.67	0.33	1	1	0.67
Unclassified AKIW781	DIT, DRM	1.583	4.997	4.923	1.533	1.883	0.33	0.67	0.67	0.33	0.33
Unclassified agg27	DRM, DRP	1.583	0	4.717	2.453	1.85	0.33	0	1	0.67	0.33
Unclassified Acidobacteria5	DIN, DRM, DUN	3.303	1.513	3.147	1.73	3.843	0.67	0.33	0.67	0.33	0.67
Unclassified ABY1	DIN, DIT, DRM, DRP	3.167	3.03	4.493	2.507	1.787	0.67	0.67	1	0.67	0.33
Unclassified 03196E2	DIT, DRM, DRP, DUN	0	4.723	3.32	2.847	3.363	0	1	0.67	0.67	0.67
Streptomycetaceae	DIN, DIT, DRM, DRP	4.877	7.603	4.927	4.86	2.07	0.67	1	0.67	0.67	0.33
Sporichthyaceae	DIT, DUN	2.383	6	1.91	2.12	3.857	0.33	1	0.33	0.33	0.67
Solirubrobacteraceae	DIT, DRM, DRP	1.513	4.36	3.967	4.803	2.327	0.33	0.67	0.67	0.67	0.33
RB40	DRM, DRP, DUN	0	0	2.5	3.013	2.303	0	0	0.67	0.67	0.67
Pseudonocardiaceae	DIT, DRM, DRP, DUN	2.077	6.127	5.44	3.947	4.13	0.33	1	1	0.67	0.67
Planctomycetaceae	DIT, DRP, DUN	1.74	5.013	1.727	5.023	3.34	0.33	1	0.33	1	0.67
Oxalobacteraceae	DIT, DRP	0	5.167	1.407	3.857	0	0	1	0.33	1	0
Nocardioidaceae	DIT, DRM, DUN	1.237	6.503	3.963	1.417	3.803	0.33	1	0.67	0.33	0.67
mitochondria	DIT, DRP	2.067	3.203	1.277	3.69	1.267	0.33	0.67	0.33	0.67	0.33

Micromonosporaceae	DIN, DIT, DRM, DRP	4.697	5.693	5.28	5.023	2.49	0.67	0.67	0.67	0.67	0.33
Kouleothrixaceae	DIT, DRM, DRP, DUN	2.14	3.95	3.537	4.087	6.123	0.33	0.67	0.67	0.67	1
FCH4570	DIT, DRP, DUN	2.21	4.333	2.17	3.897	4.82	0.33	0.67	0.33	0.67	0.67
Euzebyaceae	DIN, DIT	3.69	2.583	1.347	0	1.467	0.67	0.67	0.33	0	0.33
Cytophagaceae	DIN, DIT, DUN	3.51	3.41	1.74	0	3.723	0.67	0.67	0.33	0	0.67
Comamonadaceae	DIT, DRM, DRP, DUN	0	3.76	3.087	3.107	3.11	0	0.67	0.67	0.67	0.67
Caldilineaceae	DIT, DRM	0	3.097	2.77	1.727	1.693	0	0.67	0.67	0.33	0.33
Bacillaceae	DIT, DRP	1.91	2.853	1.757	3.183	1.66	0.33	0.67	0.33	0.67	0.33
A4b	DIT, DRM, DRP, DUN	1.91	6.167	3.937	2.95	6.837	0.33	1	0.67	0.67	1

Legend: DRM = *D. ramosissimum*, DUN = *D. uncinatum*, DIN = *D. incanum*, DIT = *D. intortum*, DRP = *D. repandum*

Supplementary table 4: Unique soil fungal genera in *Desmodium* spp. plots

	Abundance					**OCC**				
Taxa	*D. incanum*	*D. intortum*	*D. ramosissimum*	*D. repandum*	*D. uncinatum*	*D. incanum*	*D. intortum*	*D. ramosissimum*	*D. repandum*	*D. uncinatum*
D. intortum										
Zygosporium	0	2.007	0	2.213	0	0	0.67	0	0.33	0
Knufia	2.563	2.907	1.35	0	0	0.33	0.67	0.33	0	0
D. ramosissimum										
Stephanonectria	1.677	0	3.767	1.25	0	0.33	0	0.67	0.33	0
Solheimia	1.377	0	2.31	0	0	0.33	0	0.67	0	0
Pseudaleuria	0.787	0	3.71	0	0	0.33	0	0.67	0	0
D. repandum										
Fusidium	0	0.823	0	5.15	0	0	0.33	0	0.67	0
Atractiella	1.887	0	0	3.71	0	0.33	0	0	0.67	0
Veronaea	0.68	0	0	3.207	0	0.33	0	0	0.67	0
Veronaea	0.68	0	0	3.207	0	0.33	0	0	0.67	0
Leptodiscella	1.43	0	0	5.193	0	0.33	0	0	1	0
Lophiostoma	0	0	0	3.447	0	0	0	0	0.67	0
Pseudocoleophoma	1.2	1.39	1.317	3.323	1.203	0.33	0.33	0.33	1	0.33
Stachylidium	1.283	0.777	1.28	2.66	0	0.33	0.33	0.33	0.67	0
D. uncinatum										
Alfaria	0	0	0	0.81	3.207	0	0	0	0.33	0.67
Bipolaris	0	1.407	0	0	2.157	0	0.33	0	0	0.67
Humicola	1.767	0	0	1.553	5.287	0.33	0	0	0.33	1
Scedosporium	1.667	0	0	0	3.437	0.33	0	0	0	0.67
D. intortum										
Basidioascus	3.403	0	1.237	1.133	1.317	1	0	0.33	0.33	0.33
Coprinopsis	2.473	0	1.267	1.25	1.397	0.67	0	0.33	0.33	0.33
Cintractia	4.23	1.163	0	1.02	1.317	0.67	0.33	0	0.33	0.33

Supplementary table 5: Core soil fungal genera in *Desmodium* spp. plots

	Abundance					**OCC**				
Taxa	*D. incanum*	*D. intortum*	*D. ramosissimum*	*D. repandum*	*D. uncinatum*	*D. incanum*	*D. intortum*	*D. ramosissimum*	*D. repandum*	*D. uncinatum*
Acrophialophora	5.49	4.757	6.757	5.287	6.283	0.67	0.67	1	0.67	1
Acrocalymma	6.81	7.013	6.59	5.067	4.137	1	1	1	1	0.67
Acremonium	7.217	6.587	7.41	6.187	7.233	1	1	1	1	1
Achroiostachys	7.677	7.29	8.593	7.46	8.333	1	1	1	1	1
Alternaria	8.01	7.943	5.62	7.26	7.477	1	1	1	1	1
Auxarthron	6.653	2.8	7.147	6.637	5.607	1	0.67	1	1	1
Hannaella	8.793	9.61	7.223	7.197	9.42	1	1	1	1	1
Chaetomium	11.24	11.043	11.267	11.47	11.1	1	1	1	1	1
Cercospora	8.61	7.663	2.937	9.15	7.94	1	1	0.67	1	1
Cercophora	5.093	4.193	3.497	4.157	2.927	1	0.67	0.67	1	0.67
Ceratobasidium	5.28	5.187	5.807	4.303	5.483	0.67	0.67	1	0.67	1
Coniocessia	6.797	5.253	6.187	6.42	6.08	1	1	1	1	1
Colletotrichum	7.377	7.077	6.867	7.967	8.893	1	1	1	1	1
Clonostachys	5.303	7.7	6.09	6.637	6.57	1	1	1	1	1
Clitopilus	6.677	3.537	4.977	6.007	4.38	1	1	1	1	0.67
Cladorrhinum	10.203	9.113	9.76	9.103	9.95	1	1	1	1	1
Fusarium	12.41	12.4	12.677	12.367	12.51	1	1	1	1	1
Fusariella	4.65	4.17	4.263	5.14	4.85	0.67	0.67	1	1	1
Curvularia	8.443	9.413	9.153	8.093	9.56	1	1	1	1	1
Zopfiella	8.053	7.237	7.53	8.203	5.23	1	1	1	1	1
Westerdykella	2.783	2.837	4.343	4.553	4.057	0.67	0.67	1	1	1
Aspergillus	8.397	8.65	8.927	9.093	8.543	1	1	1	1	1
unidentified	11.223	12.163	11.077	10.993	11.963	1	1	1	1	1
Unclassified	13.243	13.347	13.433	13.427	13.343	1	1	1	1	1
Trichoderma	6.453	3.653	5.28	7.35	5.293	1	0.67	1	1	1
Torula	7.47	7.507	6.41	7.98	8.38	1	1	1	1	1
Thanatephorus	5.4	4.383	5.237	2.65	5.427	1	0.67	1	0.67	1
Tetracladium	5.29	4.397	5.76	3.077	5.297	1	1	1	0.67	1
Talaromyces	8.123	8.203	8.127	8.773	7.823	1	1	1	1	1
Stachybotrys	8.873	8.663	9.29	9.047	9.483	1	1	1	1	1
Didymella	11.513	11.857	11.423	11.21	11.937	1	1	1	1	1
Idriella	6.173	7.343	4.953	6.847	4.867	1	1	1	1	1
Scytalidium	2.867	3.267	3.45	4.98	4.857	0.67	0.67	1	1	1
Sclerostagonospora	6.433	5.563	6.033	4.26	2.93	1	1	1	0.67	0.67
Schizothecium	7.75	6.747	7.007	7.133	6.203	1	1	1	1	1
Lectera	6.803	4.467	8.783	5.543	6.347	1	1	1	1	1
Papiliotrema	8.117	5.773	7.68	5.44	6.88	1	1	1	1	1
Ochroconis	5.643	5.597	2.613	5.67	3.973	1	1	0.67	1	1

Nigrospora	7.843	7.717	6.977	6.463	7.037	1	1	1	1	1
Neurospora	4.72	6.52	5.123	5.317	3.083	0.67	1	1	1	0.67
Myrothecium	8.727	8.487	8.377	8.303	9.12	1	1	1	1	1
Mycosphaerella	9.53	9.87	7.107	6.773	9.087	1	1	1	1	1
Mortierella	8.087	8.487	8.187	6.55	8.117	1	1	1	1	1
Microdochium	6.34	4.86	5.637	5.32	4.483	1	0.67	1	1	1
Metarhizium	2.487	2.973	2.717	3.187	2.967	0.67	0.67	0.67	1	1
Sarocladium	2.847	2.827	4.097	3.143	2.783	0.67	0.67	1	0.67	0.67
Roussoella	4.16	1.8	4.513	3.24	3.65	1	0.67	1	1	0.67
Preussia	7.49	6.87	8.66	8.417	6.853	1	1	1	1	1
Plectosphaerella	7.757	7.833	9.143	10.33	8.673	1	1	1	1	1
Phaeosphaeria	9.237	9.123	8.973	7.823	9.417	1	1	1	1	1
Periconia	7.45	8.23	8.787	7.567	8.81	1	1	1	1	1
Penicillium	6.42	5.02	3.773	6.08	4.653	1	1	0.67	1	0.67
Paracremonium	7.017	6.397	6.817	7.197	6.603	1	1	1	1	1
Pyrenochaetopsis	6.607	8.16	4.22	5.697	9.577	1	1	0.67	1	1
Purpureocillium	4.237	3.407	4.653	5.9	5.603	1	0.67	1	1	1

Supplementary table 6: Pan fungal taxa shared among *Desmodium* spp. plots

		Abundance					**OCC**				
Taxa	*Desmodium* spp. plots sharing taxa	*D. incanum*	*D. intortum*	*D. ramosissimum*	*D. repandum*	*D. uncinatum*	*D. incanum*	*D. intortum*	*D. ramosissimum*	*D. repandum*	*D. uncinatum*
Sporisorium	DRM, DUN	1.283	1.88	2.37	0	2.553	0.33	0.33	0.67	0	0.67
Stagonospora	DRM, DUN	1.21	1.877	3.24	0.637	4.307	0.33	0.33	0.67	0.33	0.67
Saitozyma	DIN, DIT, DRM, DRP	4.013	3.357	4.84	1.893	1.7	0.67	0.67	1	0.67	0.33
Setophaeosphaeria	DIT, DRM, DUN	2.06	4.107	2.447	5.357	3.783	0.33	0.67	0.33	1	0.67
Subulicystidium	DRM, DRP	0.877	0	1.247	1.747	0.717	0.33	0	0.67	0.67	0.33
Robillarda	DIT, DRM, DUN	1.43	6.347	1.677	5.923	8.303	0.33	1	0.33	1	1
Rhodosporidiobolus	DIN, DRM, DRP, DUN	3.857	1.51	4.563	1.283	2.307	1	0.33	1	0.67	0.67
Rhizophlyctis	DIN DIT DUN	2.92	2.21	0	2.15	3.86	0.67	0.67	0	0.33	1
Pseudorobillarda	DIN, DRM, DUN	4.777	2.077	0.797	2.623	1.977	1	0.33	0.33	0.67	0.67
Psathyrella	DIN, DRP	2.933	1.843	0.593	2.563	0	0.67	0.33	0.33	0.67	0
Podospora	DIN, DIT, DRP	5.607	2.99	1.14	3.45	0	1	0.67	0.33	0.67	0
Poaceascoma	DIN, DRM, DRP, DUN	4.207	1.607	5.013	2.65	3.617	1	0.33	1	0.67	0.67
Phialophora	DRP, DUN	0.877	0	1.84	4.433	5.077	0.33	0	0.33	1	1
Montagnula	DRP, DUN	0	0	0.94	2.83	3.1	0	0	0.33	0.67	0.67
Monosporascus	DIN, DIT, DRM, DUN	2.307	3.147	1.75	0	4.593	0.67	0.67	0.67	0	0.67

Monographella	DIN, DRP	2.88	2.187	1.217	2.287	1.583	0.67	0.33	0.33	0.67	0.33
Modicella	DRM, DUN	2.71	0	6.063	0	3.71	0.33	0	1	0	0.67
Macrophomina	DIN, DUN	2.133	1.1	1.857	0	3.797	0.67	0.33	0.33	0	0.67
Metacordyceps	DIT, DRM, DRP, DUN	1.047	5.647	2.877	5.913	3.847	0.33	1	0.67	1	1
Magnaporthe	DIN, DIT, DRM, DRP	1.923	2.877	2.303	2.187	0	0.67	0.67	0.67	0.67	0
Microascus	DIN, DIT, DRM	5.227	2.263	3.483	0	1.363	1	0.67	1	0	0.33
Leucosphaerina	DIT, DRP, DUN	0.787	2.07	1.157	1.88	2.147	0.33	0.67	0.33	0.67	0.67
Leptospora	DIT, DRP	1.5	2.847	0	2.32	0	0.33	0.67	0	0.67	0
Kamienskia	DIT, DUN	1.423	4.737	1.237	0	4.053	0.33	1	0.33	0	1
Hirsutella	DIT, DRP	1.317	4.01	0	2.527	1.237	0.33	1	0	0.67	0.33
Hansfordia	DIT, DRP, DUN	1.2	4.433	0	4.943	3.627	0.33	1	0	1	1
Myrmecridium	DIN, DIT, DRP, DUN	3.267	3.807	1.45	2.777	4.107	0.67	0.67	0.33	0.67	1
Paracamarosporium	DIN, DIT, DRM, DRP	4.653	5.08	2.793	2.19	0	1	1	0.67	0.67	0
Xylaria	DIN, DRP, DUN	4.39	0	0	2.6	1.797	1	0	0	1	0.67
Conocybe	DIT, DRM, DRP, DUN	1.467	5.103	5.533	5.383	7.487	0.33	1	1	1	1
Cryptococcus	DIN, DRM, DRP, DUN	5.06	3.767	0.94	3.047	3.873	1	1	0.33	0.67	1
Dendryphiella	DIT, DRM, DRP, DUN	1.977	2.673	4.493	6.237	4.98	0.33	0.67	1	1	1
Chrysosporium	DIN, DRM, DRP, DUN	4.817	1.1	5.337	3.023	0	1	0.33	1	0.67	0
Chalara	DIT, DRM, DUN	1.767	4.12	2.24	1.403	2.71	0.33	0.67	0.67	0.33	0.67
Botryosphaeria	DRM, DRP, DUN	2.14	1.783	4.58	3.887	2.583	0.33	0.33	1	1	0.67
Boerlagiomyces	DIN, DIT	3.367	2.36	1.467	1.67	0	0.67	0.67	0.33	0.33	0
Bartalinia	DRP, DUN	0.95	1.14	0	1.917	3.01	0.33	0.33	0	0.67	0.67
Ascochyta	DIN, DIT, DRM, DUN	4.113	5.887	2.917	1.73	6.107	1	1	0.67	0.33	1
Arxiella	DIT, DRP, DUN	1.38	3.663	1.423	2.89	4.987	0.33	0.67	0.33	0.67	1
Arthrographis	DRM, DRP	2.147	1.42	2.133	2.997	0	0.33	0.33	0.67	0.67	0
Arthrobotrys	DIN, DRP, DUN	2.25	1.327	1.207	3.863	4.48	0.67	0.33	0.33	1	1
Arachnomyces	DIN, DRP, DUN	2.977	0	1.267	2.1	2.637	0.67	0	0.33	0.67	0.67
Aplosporella	DIT, DRM, DRP, DUN	1.97	6.16	6.327	3.047	4.147	0.33	1	1	0.67	0.67
Antennariella	DIN, DRM, DRP, DUN	4.553	2.427	7.043	5.81	7.487	1	0.33	1	1	1
Wardomycopsis	DIT, DRM, DRP, DUN	1.747	2.523	5.187	3.877	3.383	0.33	0.67	1	1	0.67
Vishniacozyma	DIT, DRM, DRP, DUN	1.65	3.52	3.633	4.053	4.527	0.33	0.67	0.67	1	1
Funneliformis	DIN, DIT, DUN	3	2.743	0	0.743	3.857	0.67	0.67	0	0.33	0.67
Exserohilum	DIN, DIT, DRM	2.84	4.47	2.637	1.02	1.517	0.67	1	0.67	0.33	0.33
Entoloma	DIN, DUN	1.983	1.06	1.823	0	2.393	0.67	0.33	0.33	0	0.67

Endophragmiella	DIN, DIT, DR, DRP	3.85	4.993	5.493	5.787	1.483	1	1	1	1	0.33
Dioszegia	DIN, DIT, DUN	2.42	4.447	0	1.783	3.67	0.67	1	0	0.33	1
Graphium	DRM, DRP, DUN	0.98	1.163	4.62	4.45	4.457	0.33	0.33	1	1	1
Gibellulopsis	DIN, DIT, DRP, DUN	5.427	3.913	1.397	7.47	6.967	1	1	0.33	1	1
Lecanicillium	DIT, DRM, DRP	0.68	7.267	2.567	4.737	1.917	0.33	1	0.67	1	0.33
Lasiodiplodia	DIN, DIT, DRM, DRP	3.303	5.347	6.67	5.09	0.963	1	1	1	1	0.33

Legend: DRM = *D. ramosissimum,* DUN = *D. uncinatum,* DIN = *D. incanum,* DIT = *D. intortum,* DRP = *D. repandum*

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The push-pull intercrop *Desmodium* does not repel, but intercepts and kills pests

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Over two decades ago, scientists developed a push-pull intercropping strategy that received critical acclaim for synergizing food security with ecosystem resilience in smallholder farming. The strategy suppresses Lepidopteran pests in maize through a combination of a repellent intercrop (push), commonly *Desmodium* spp., and an attractive, dead-end border crop (pull). Key is the intercrop's constitutive release of volatiles that repel herbivores. Surprisingly, however, we found that *Desmodium* does not constitutively release volatiles, and only minimally upon herbivory. Further, in oviposition choice settings, *Spodoptera frugiperda*, a devastating invasive pest, was not repelled by *Desmodium* volatiles. In search of an alternative mechanism, we found that neonate larvae strongly preferred *Desmodium* over maize. However, their development stagnated and none survived. In addition, larvae were frequently seen impaled and immobilized by the dense network of silica-fortified, non-glandular trichomes. Thus, entirely different from repelling adult moths, *Desmodium* intercepts and decimates dispersing offspring. As a hallmark of sustainable pest control, maize-*Desmodium* intercropping has inspired countless efforts trying to emulate a stimulo-deterrent diversion in other cropping systems. However, detailed knowledge of the actual mechanisms is required to rationally improve the strategy, and translate the concept into other cropping systems.

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Main text

Since the dawn of agriculture, humanity has been in an arms race with insect pests. Traditionally, a set of integrated cultivation strategies tailored to local settings helped keeping pests at bay, including associational resistance through varietal mixtures and intercropping[1–3]. With the advent of agrochemicals, monocultures superseded traditional strategies. However, their profound externalities on ecosystem resilience and global climate[4,5] have resuscitated interest in more sustainable alternatives, frequently grafted on traditional strategies. Trending terms such as agroecology, and climate smart, regenerative or organic agriculture evidence the search for solutions that harmonize food production and pest control with ecological sustainability. Some innovative practices have been important sources of inspiration. Among these, the push-pull strategy in which maize is intercropped with the legume, *Desmodium*, is arguably the most well known[6].

Push-pull aims to reduce the abundance of insect pests in crops through repelling the pest in the crop, while simultaneously providing attractive sources to trap the pest out (formalized by Miller and Cowles[7]). Using this 'stimulo-deterrent diversion' principle, a push-pull strategy was devised to combat Lepidopteran pests in sub-Saharan smallholder maize farming[8,9]. Embroidering on the common practice of smallholder farmers to intercrop maize with e.g. edible pulses, the strategy uses the perennial fodder legume *Desmodium* as intercrop in maize plots. *Desmodium* reportedly constitutively releases large amounts of terpenes (such as (*E*)-4,8-dimethyl-1,3,7-nonatriene ((*E*)-DMNT), (*E*)-*ß*-ocimene and cedrene) that repel ('push') lepidopteran pests and attract natural enemies ('pull')[10-12]. A 'dead-end' host sown as border crop (another 'pull' component), typically napier grass, complements the strategy as it induces oviposition in Lepidoptera, but reduces larval survival compared to maize[11–13]. This cropping strategy reduces infestations of various Lepidoptera pests, including *Chilo partellus* and *Busseola fusca*, as well as *Spodoptera frugiperda*, a polyphagous invasive pest that is ravaging maize and vegetable production and threatens food security in sub-Saharan Africa[14,15]. Strongly propagated by institutions and governments[16–21], this intercropping strategy has found widespread adoption in East Africa. As a hallmark of sustainable pest control, it also serves as a tremendous source of inspiration for intervention strategies in other cropping systems.

The 'push' volatiles reported in previous studies[11,12] are typically released by plants after induction by herbivory. This begs the question of why *Desmodium* releases these volatiles constitutively. Push-pull maize-*Desmodium* intercropping causes substantial shifts in below-ground ecosystems, including increased soil microbe diversification, increased soil nitrogen and carbon, increased plant defense through plant-soil feedback, and suppression of parasitic weeds and pathogenic microbes[22,23]. We therefore verified if the 'constitutive' release of volatiles was, in fact, induced or enhanced by soil-borne interactions. The root-microbe interactions are of particular interest, given the intimate association of legumes with specific microbial groups e.g. rhizobia and mycorrhizae. Indeed, soil and root-microbe interactions can induce pathways that lead to release of volatiles[e.g., 22,24].

Surprisingly, however, *D. intortum*, which is by far the most commonly used intercrop in push-pull technology[10], did not release volatiles constitutively at all (Figure 1a, b, Extended Data, Figure 2 and 3). This was independent of the soil in which *D. intortum* was grown, whether live soil (organic potting soil, organic clay Swedish soil or African clay loam soil from *D. intortum* plots), autoclaved soil, or autoclaved soils inoculated with mycorrhiza or rhizobacteria (Extended

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Data, Figure 4, 5 and 6). None of the previously reported terpenes[12] were constitutively released, nor any terpene or other volatiles that are typically released upon herbivory. Similar results were obtained with *D. uncinatum* (Extended Data, Figure 7). In contrast, we did confirm that *Melinis minutiflora*, a Poaceae used previously as a push intercrop, constitutively releases a diverse blend of terpenes in large quantities (Extended Data, Figure 2, 3 and 8). Clearly, independent of soil interactions, *Desmodium* does not constitutively release volatiles.

Although the constitutive release of volatiles is an important precondition for push-pull, inadvertent herbivory of *Desmodium* could have induced volatile release reported in earlier studies. However, *D. intortum* only minimally released induced volatiles when either mechanically damaged or when fed upon by *S. frugiperda* larvae (Figure 1a-d, Extended Data, Figure 2 and 3). This contrasted with maize, which, in line with previous studies[25–27], released large amounts of herbivore-induced volatiles in response to herbivory, with emission peaking between 24 and 48 hrs following infestation, and declining over the course of 7 days (Figure 1c). Herbivory of *M. minutiflora* did not significantly boost release of volatiles above the already high constitutive release (Figure 1b, Extended Data, Figure 2 and 3).

Arguably, greenhouse conditions are not representative of field conditions and additional, unknown factors in the field may cause the release of volatiles by *Desmodium*. We therefore analyzed 50 headspace samples from *D. intortum* from seven locations in Tanzania and Uganda. Also under field conditions, terpene release by *D. intortum* was minimal (Figure 2, Extended Data, Figure 8), and possibly induced by herbivory that was visible on most sampled plants. Thus, regardless of whether constitutive or induced, *Desmodium* does not release terpene volatiles, or any other volatiles, in large quantities in the field. Although it cannot be excluded that other conditions or herbivores may induce higher release of reported volatiles, our data with numerous samples under different growth conditions, and from different geographic regions show that this must be very rare, and can therefore not be at the core of a generic strategy. In contrast, maize, all of which displayed some herbivore damage, did release typical herbivore induced volatiles[25,26] (Fig 2, Extended Data, Figure 8), with variations likely due to differing levels of and age since herbivore infestations, which could not be controlled in the field.

Ironically, if the mode of action in maize-*Desmodium* push pull was repellent terpene volatiles, induced maize itself would appear a much better push candidate than *Desmodium*. Although the lack of volatiles emitted made it highly unlikely that *Desmodium* repels lepidopteran pests, we double checked this in bioassays. In a wind tunnel, gravid *S. frugiperda* were given a choice between maize plants with either *D. intortum* or artificial plants in the background (Extended Data, Figure 1). Adult females landed and oviposited on either maize plant equally, underlining that *D. intortum* volatiles indeed did not repel gravid *S. frugiperda* (Figure 3c).

Evidently, to explain the suppression of lepidopteran pests using *Desmodium* as intercrop, one needs to invoke a different mechanism than ‘stimulo-deterrent diversion’ or ‘push-pull’. To investigate possible alternatives we scored female *S. frugiperda* oviposition preference, larval feeding preference, and larval survival on maize and *Desmodium*. First, in two-choice tests *S. frugiperda* preferred oviposition on maize over *Desmodium*. However, the preference was not strong, as females also oviposited on *Desmodium*. In the field, one could perhaps expect a further shift toward *Desmodium*, particularly when maize is small and *Desmodium*, a perennial, well developed. However, irrespective of female oviposition choice, many lepidopteran larvae are known to disperse from the plant on which they hatched. Neonate larvae typically ‘parachute’

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between plants using silk threads[28–30], whereas later larval stages actively disperse across the soil surface in search for new host plants[30–32]. Given the dense, continuous ground cover of *Desmodium* in the interrows, stochastically the large majority of dispersing larvae would end up in *Desmodium*, particularly when maize plants are small and *Desmodium*, a perennial, large. We therefore verified the preference and survival of *S. frugiperda* larvae on *Desmodium* compared to maize. Surprisingly, first instar larvae strongly preferred *D. intortum* over maize, both in choice and in leaf area consumed (Figure 3d,e). However, their development stagnated, with hardly any larva molting to the second instar, and none completing their development (Figure 3f, Extended Data, Figure 9).

In addition to stagnating development, we found that larvae, particularly later larval instars, moved slowly on *Desmodium* leaves and stems, while many were immobilized entirely. Closer scrutiny of *D. intortum* surfaces revealed a dense network of non-glandular, uniseriate and uncinate trichomes, with densities and a distribution depending on the surface type (Figure 4a - d, f, Extended Data, Figure 10a). The stems and main veins of the leaves were particularly densely populated with uncinate trichomes. First instar larvae were somewhat freely moving and grazing between trichomes (Extended Data, Figure 10b,c), but older larvae were seen impaled and immobilized by these trichomes (Figure 4c,d, Extended Data, Figure 10d-f). Occasionally, even ovipositing *S. frugiperda* were immobilized with their ovipositor on *D. intortum* (Extended Data, Figure 10g). Whereas trichomes were flexible at the base, they were fortified with silica toward the tip (Figure 4f), equipping the plant with an effective mechanism to obstruct, damage and immobilize herbivores. Also beneficial insects (Extended Data Figure 10i) and even vertebrates can be trapped by *Desmodium*[33]. Similar structures are also used by many other plant species[34–36], and may serve multiple purposes including seed dispersal[37,38].

We thus infer that in the field *Desmodium* affect fitness of lepidopteran larvae, both directly and indirectly. First, *Desmodium* entices larval feeding, but truncates larval development. Second, trichomes on *Desmodium* hinder movement, damage the cuticle and even entirely immobilize larvae on the plant, increasing developmental time, exposure to natural enemies and overall mortality[39,40]. Third, the ingestion of trichomes will damage the intestinal lining and affect digestion, development and survival[40,41]. Indeed, while first instar larvae easily fed around the trichomes, larger larvae did ingest trichomes as evidenced by trichomes found in larval frass. Effectively, rather than functioning as a repellent intercrop, *Desmodium* appears to be a developmental deathtrap for larvae.

Clearly ‘push’ does not describe the mode-of-action of *Desmodium*. Instead, the plant exhibits properties reminiscent of a ‘pull’ crop, a ‘dead-end host’. Although superficially similar in mode of action to the ‘pull’ border crop Napier grass, *Desmodium* is distinctly different, as it is preferred by larvae, not by adults[8,10]. In addition, *Desmodium* forms a mechanical barrier to dispersing larvae. Further field studies need to detail how oviposition preference, larval dispersal, development and survival on *Desmodium*, mechanical obstruction by *Desmodium*, and additional mechanisms such as parasitization and predation, interplays with crop phenology in suppressing various lepidopteran species across the cropping season. Knowing the exact interaction of mechanisms is critical if we for instance wish to substitute the fodder crop *Desmodium* with a food crop to enhance food security, or if we are to translate the concept of interceptive intercropping to other cropping systems.

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The surprising discovery that *Desmodium* hardly emits volatiles and does not repel herbivores contrasts strongly with the very large number of publications and the huge global attention that maize-*Desmodium* push-pull technology has garnered over more than two decades. Indeed, the narrative of the ‘push’ crop *Desmodium* repelling moths has been mentioned by numerous papers since its first mention around the year 2000. Astonishingly, however, close scrutiny of the literature revealed a total absence of primary data. Whereas the most cited paper from around 2000, Khan and colleagues[12], mentions some of the *Desmodium* volatiles and claims repellence of stemborers, no primary chemical analytical or behavioral data were presented in this paper, nor in any preceding or ensuing paper. Equally remarkable is how, in spite of thousands of citations and an abundance of efforts to emulate push-pull in other cropping systems, this crucial detail has collectively slipped the attention of the scientific community.

Further research should study how pest suppression in interceptive intercropping is affected by factors such as pest species, natural enemies, crop phenology, insect population dynamics, and abiotic factors including soil and climate, and others. This will be pivotal for improving the current maize intercropping strategy, tailoring it to the needs of local smallholder farmers and other ecosystem services sought after (e.g. replacing *Desmodium* with food crops with similar properties[34–36,41-43]), as well as rationally translating the concept to other cropping systems.

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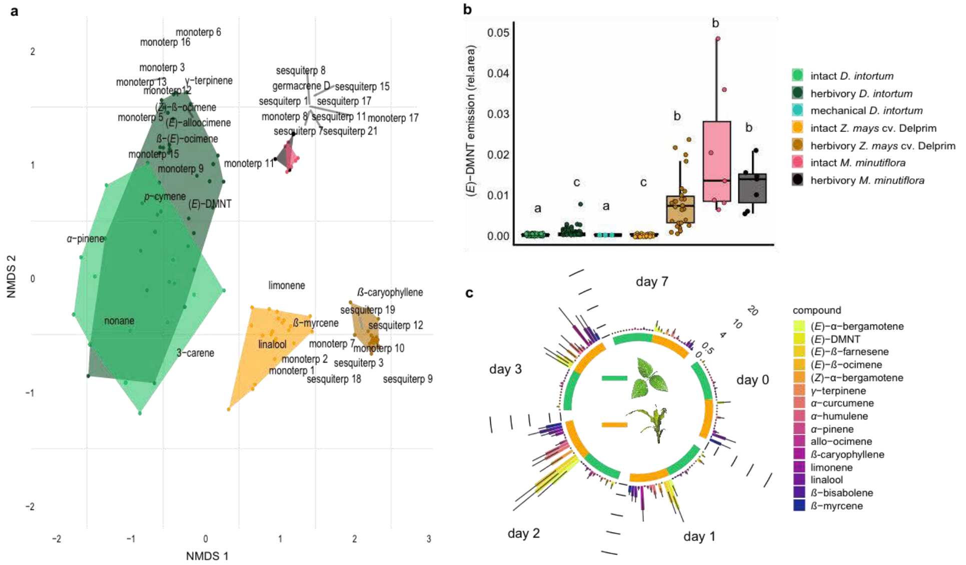

Fig. 1: *Desmodium intortum* does not constitutively release terpene volatiles, and hardly following larval feeding.

a, Nonmetric multidimensional scaling (NMDS) analysis of volatiles emitted by *D. intortum*, *Z. mays* cv. Delprim and *M. minutiflora* plants, intact and 48 hrs following *S. frugiperda* feeding (stress value = 0.138). (*E*)-4,8-dimethyl-1,3,7-nonatriene ((*E*)-DMNT), (*Z*)-*β*-ocimene, (*E*)-*β*-ocimene and (*E*)-alloocimene were not constitutively released, and only in low quantities in response to herbivory. Volatiles emitted by intact and herbivore-induced *D. intortum* (F_{model} = 15.597, R^2 = 0.132, p_{adj} = 0.021) and *Z. mays* plants (F_{model} = 50.521, R^2 = 0.512, p_{adj} = 0.021) were significantly different in PERMANOVA and pairwise comparison, but emissions from intact and herbivore induced *M. minutiflora* plants (F_{model} = 1.469, R^2 = 0.109, p_{adj} = 1) were not. **b**, (*E*)-DMNT emission before and 48 hrs following herbivory (n = 8, ± SE). The absolute peak areas were divided by the peak area of the internal standard and divided by the sum of monoterpenoids across all laboratory volatile collections for normalization. Treatments with different letters are different (Kruskal-Wallis with Benjamini and Hochberg *p* value correction, $\chi^2 = 57.315$, $p = 1.578\ 10^{-10}$). **c**, Emission of volatile monoterpenoids and sesquiterpenoids from *D. intortum* and *Z. mays* before, during and after *S. frugiperda* larval feeding (n = 5, ± SE). Peak areas of each terpenoid were divided by the area of the internal standard and divided by the sum of monoterpenoids or sesquiterpenoids across all laboratory volatile collections. Error-bars show the standard error for relative volatile emission of each group. Day 0 - volatile emission before herbivory, Day 1 - 24 hrs after herbivory, Day 2 after 48 hrs, and so on. Larvae were removed after 48 hrs.

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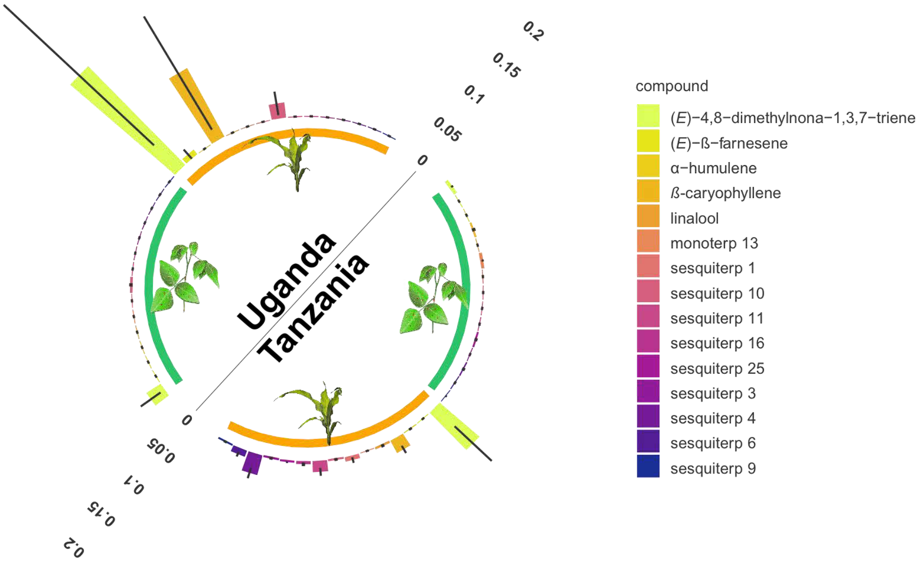

Fig. 2: Monoterpenoid and sesquiterpenoid emission by *D. intortum* and *Zea mays* plants under field conditions at several locations in Tanzania and Uganda.

The absolute peak area of each peak was divided by the sum of the area of monoterpenoids or sesquiterpenoid emission across all samples from the same location. Error bars represent ± SE on the scale of the relative volatile emission. Minor terpenoid compounds were not identified to species level as this was not the focus of the study, and was further hindered by the vast diversity of compounds and the lack of synthetic standards.

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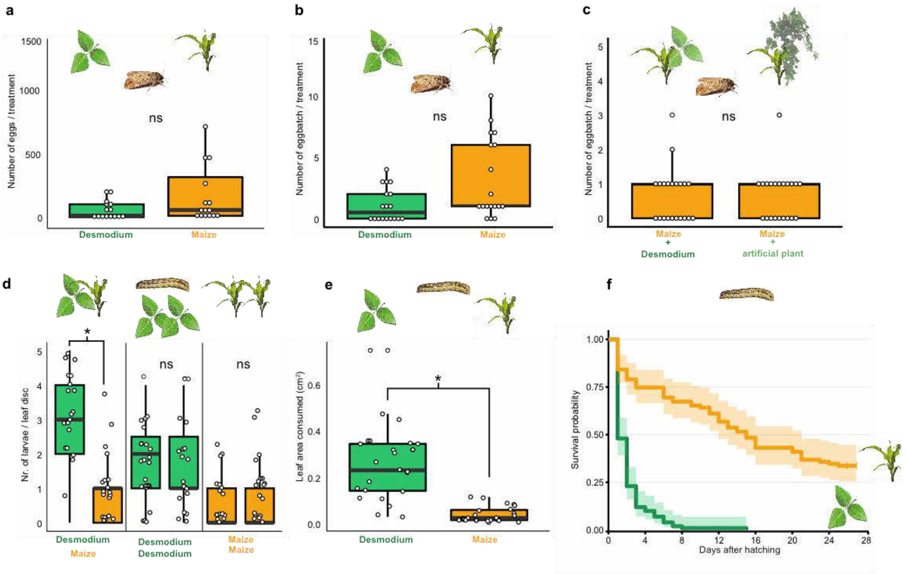

Fig. 3: *D. intortum* does not repel ovipositing *S. frugiperda*. Instead it is prefered by larvae but truncates their development.
a, The number of eggs laid on *D. intortum* or *Z. mays* plants in choice-experiments in cages (n = 25) did not differ (Wilcoxon signed rank exact test, $p = 0.055$). **b**, Number of egg batches laid on *D. intortum* or *Z. mays* plants (n = 25, Wilcoxon signed rank exact test, $p = 0.075$). **c**, Number of egg batches on *Z. mays* plants in a background of either *D. intortum* plant or a plastic plant mimic did not differ in wind tunnel oviposition assays (n = 21, Wilcoxon signed rank exact test, $p = 0.825$). **d**, First instar *S. frugiperda* larvae preferred *D. intortum* against *Z. mays* in two choice leaf disc bioassays (n = 25, Wilcoxon signed rank exact test, $p = 2.73*10^{-3}$). **e**, First instar *S. frugiperda* larvae consumed more *D. intortum* than *Z. mays* (20 hrs, two-choice leaf disc bioassays, n = 25, Wilcoxon signed rank exact test, $p = 3.338*10^{-6}$). **f**, Survival probability of *S. frugiperda* on diets consisting of *D. intortum* (greenleaf *Desmodium*) was lower than on *Z. mays*, with no larvae surviving on *D. intortum*. (Kaplan-Meier survival analysis, $p = 2.000*10^{-16}$). Error bars, ± SE.

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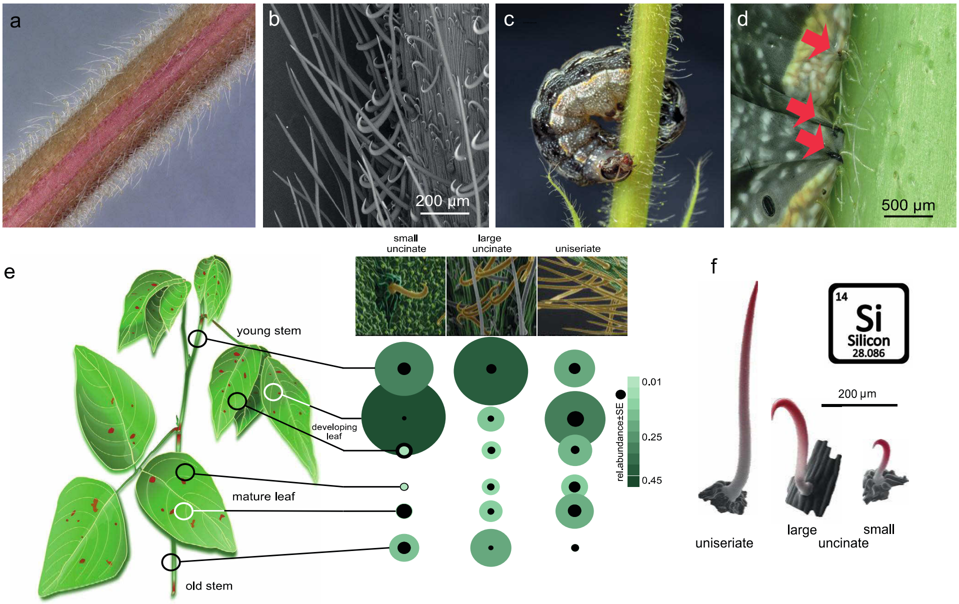

Fig. 4: Non-glandular trichomes on *Desmodium intortum* act as a physical barrier for herbivores.

a, Light microscopy image of a section of a young *D. intortum* stem densely covered with trichomes. **b**, Scanning electron microscopy (SEM) image of a young *D. intortum* stem. Straight uniseriate hairs (up to 2 mm long) extended beyond the large (0.2 - 0.4 mm) and small (0.05 - 0.2 mm) hooked uncinate trichomes (scale bar: 200 µm). **c,** A fifth instar *S. frugiperda* larva impaled and immobilized on a stem of *D. intortum* by both large and small uncinate trichomes. **d**, Fourth instar *S. frugiperda* larva pierced by uncinate trichomes (red arrows). Trichomes either immobilized larvae or broke off from the basal cell with the tip remaining in the larval body causing severe wounds. **e**, Distribution of non-glandular trichomes on different parts of the *D. intortum* plant. The relative abundance was calculated as the mean of trichome count divided by the sum of trichomes per trichome type across samples. Black circles indicate the standard error of relative trichome abundance (n = 5). **f**, SEM images combining EDX element topography images indicate relative surface silica (Si) distribution (red) of uniseriate, large and small uncinate trichomes (n = 5).

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METHODS

Plants

Seeds of the most common intercrop species in push-pull farming (*Desmodium intortum*, greenleaf *Desmodium*, and *Desmodium uncinatum*, silverleaf desmodium) were acquired from Simlaw seeds Co. Ltd, Nairobi, Kenya). *M. minutiflora* seeds were obtained from the South African Sugarcane Research Institute (SASRI, Mount Edgecombe, South Africa). Maize seeds (*Zea mays* cv. Delprim) were provided by the laboratory of Ted Turlings at University of Neuchâtel, Switzerland. The cultivar is a European commercial hybrid and long-time standard whose volatile emission patterns have been thoroughly studied[44].

Desmodium spp. seeds were sterilized by using 3% NaOCl and rinsed in distilled water and germinated on wet filter paper, and transferred to seedling trays with live or autoclaved soil (121 ºC for 20 min). After 21 days the plants were transferred to 18 cm diameter pots containing live or autoclaved soil and were grown for 8 weeks in a greenhouse (22 – 25 ºC, light cycle 16:8 hrs, RH 65%). Another set of plants were raised from cuttings of mature stem parts of *D. intortum* and rooted in distilled water. Rooted cuttings were then planted in pots containing autoclaved soil with different inoculants: 200 g soil of a Tanzanian push-pull field per each pot, autoclaved soil with 60 mg of *Rhizobium leguminosarum*, *Bradyrhizobium japonicum* mixture per each pot (equal portions of *Rhizobia* inoculant for *Phaseolus* beans, and soy beans from Samenfest GmbH., Freiburg, Germany) or autoclaved soil with 120 mg of mycorrhizal fungi inoculate per each pot (mixture of *Glomus intraradices*, *G. etunicatum*, *G. monosporum*, *G. deserticola*, *G. clarum*, *Paraglomus brasilianum*, *Gigaspora margarita*, *Rhizopogon villosulus*, *R. lutcolus*, *R. amylopogon*, *R. fulvigleba*, *Pisolithus tinctorius*, *Scleroderma cepa* and *S. citrinum*, Wildroot Organic Inc., Texas). The microbial inoculants were premixed in autoclaved soil before plant inoculation. Plants from cuttings grown on autoclaved soil were used as control. *M. minutiflora* seeds were germinated in live soil in plastic trays, and the seedlings were transferred into pots with live soil after two sets of leaves appeared. Eight weeks old *M. minutiflora* and *Desmodium spp.* plants were used in the experiments. Maize seeds were planted directly into live or autoclaved soil in pots and maintained in the greenhouse for 6 weeks.

For the cage oviposition experiments, maize seeds were sown next to 5 weeks old *D. intortum* plants in 12 cm pots and grown together for three weeks. For the wind tunnel experiments, maize and *D. intortum* plants were grown in separate pots and four to five weeks old maize and nine to eleven weeks old *D. intortum* plants were used.

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Insect rearing

S. frugiperda were obtained from the Ted Turlings laboratory at University of Neuchâtel, Switzerland, and were raised on a soybean based semi artificial diet supplemented maize whorls. The third instar larvae were separated into groups of ten individuals in plastic boxes. Pupae were sexed and separated in rearing cages. Adults were provided with a 5 % sucrose solution and 6 days old adults were mated for 6 hrs and used in oviposition experiments.

Volatile collections

The plants grown in the greenhouse were enclosed in a 60 cm x 20 cm polyethylene (PET) oven bag (Toppits ® 'Bratschlauch', Melitta, Minden, Germany) above ground for 24 hrs to saturate the headspace. Prior to sampling, 2 µl of 250 ng/ul nonane solution in hexane was injected onto a piece of filter paper into the oven bag 40 minutes prior to sampling. Solid phase microextraction (SPME) fibers (DVB/CAR/PDMS 50/30 µm, Supelco, Sigma-Aldrich, Bellefonte, PA, USA) were conditioned at 250 ºC in the split/splitless injector of the GC-MS in split mode for 10 minutes. The SPME fibers were exposed to the closed headspace for 30 minutes. The volatile emission of intact, mechanically damaged and herbivore-damaged plants were sampled. *D. intortum* plants were mechanically damaged by cutting ten randomly selected leaflets in half, perpendicularly to the midrib. For herbivore-treatment, eight fourth to fifth instar and 12 hrs starved *S. frugiperda* larvae were put on the plants. In the first sets of experiments the feeding period lasted for 48 hrs before volatile sampling.

A time series experiment of volatile terpenoid emission following herbivory was performed on *D. intortum* and *Z. mays* cv. Delprim plants grown on autoclaved soil inoculated with Tanzanian soil. Eight fourth instar larvae were put on each plant after 12 hrs of starving and removed after 48 hrs of feeding. The plants were sampled before herbivory and after 24 hrs, 48 hrs of herbivory. Larvae were removed from the plants after 48 hrs and plants were resampled 72 hrs and one week after the start of the experiment. The volatile headspace was closed for 24 hrs before each sampling and the SPME sampling procedure was the same as described above.

Field volatile samples of *D. intortum* (greenleaf *Desmodium*) and *Z. mays* were collected on farmer fields in Tarime and Musoma districts in Mara region, Tanzania, and Rural Community in Development (RUCID) center, in Mityana district, Uganda. Healthy *D. intortum* plants and maize plants with visible herbivore damage were selected and enclosed in 60 cm x 20 cm polyethylene (PET) oven bags for 18 hrs overnight. The use of standard and the SPME volatile sampling procedure was the same as described above.

Gas chromatography coupled mass spectrometry (GC-MS)

A GC-MS (Agilent technologies, 7890B GC coupled with 5975 MSD) was used for SPME analysis. Fibers were inserted into a 250 °C splitless injection port with The split valve closed for 1 min. The GC was equipped with a DB-WAX column (60 m x 250 µm x 0.25 µm). The carrier gas was helium and the total column flow was 34.883 mL/min. The oven temperature was programmed as follows: 50 °C/min, 10 °C/min to 220 °C, 20 °C/min to 250 °C. The final temperature was held for 1 min. The mass spectrometer was used in electron ionization mode 70 eV and the detector scanned in the 29-400 m/z range. Samples were also injected on a GC-MS equipped with an HP-5 column (Agilent technologies, 6890 GC coupled with 5977A MSD, column: 60 m x 250 µm x 0.25 µm), with similar inlet settings and carrier gas (helium). The oven

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program was as follows: 40 °C/2 min, 8 °C/min to 230 °C. The solvent delay and mass spectrometry settings were the same as described above.

GC-MS results were analyzed using Agilent Mass Hunter B.08.00, the peaks were auto integrated with agile integrator and manual integration. Compounds were tentatively identified by matching their mass spectra with those found in MS Libraries (NIST11 and Wiley12). The identification was verified by comparing calculated Kovats retention indices (RI) to those published in the NIST WebBook database and PubChem database and comparisons with analytical standards (See list of synthetic compounds in Table S1).

Oviposition choice experiments

We conducted two experiments to study the short-range/multimodal oviposition repellency and long-range/olfactory oviposition repellency of *D. intortum* for *S. frugiperda* females.

Short-range/multimodal oviposition repellency experiments

In short-range/multimodal oviposition repellency experiments, maize seeds (*Z. mays* cv. Delprim) and *D. intortum* cuttings were co-planted. The experiments were conducted three weeks after co-planting, when the biomass of each plant were roughly similar. Plants were placed in 30 x 30 x 30 cm net cages (Bugdorm, Megaview, Taiwan) in a climate chamber set to 25±2 °C, 65%±5% relative humidity and 16:8 h L:D light cycle. Six days old virgin *S. frugiperda*, one female and one male, were mated for 6 hrs and females were let to oviposit for 48 hrs. A cotton ball soaked in 5% sucrose solution was placed between the plants for adult feeding. The egg batches and the number of eggs per each batch were counted at the end of the second day on both plants and the cage surfaces.

Long-range/olfactory oviposition repellency experiments

To score for spatial repellency of *D. intortum*, a modified wind tunnel (180 cm x 80 cm x 60 cm, 30 cm/s airflow) was used (Extended data, Figure 1). At the furthest upwind part of the flight section of the tunnel, two six-weeks old maize plants (*Z. mays* cv. Delprim) were positioned at 60 cm from each other. Directly upwind and separated by a stainless steel gauze (100 mesh) an eight-weeks old *D. intortum* or artificial plastic plant was placed directly upwind from the maize plants. In both sections a 20 cm plexiglass sheet was placed in line with the airflow to separate the airflow of the two sides (Extended data, Figure 1). Two six days old females and one six days old male were released in the chamber 1 hr prior to scotophase. A cotton ball soaked in 5% sucrose solution was placed in the chamber at the release side as a source of food. The position of the female and the number of egg batches laid on each side of the chamber were recorded after scotophase, 12 hrs following the start of the experiment.

Larval choice experiments

We conducted two-choice feeding bioassays to determine the feeding preference of the first larval instar of *S. frugiperda*. We cut 8 mm diameter leaf discs from young leaves of 6-7 weeks old maize plants and leaves of 10-12 weeks old *D. intortum* plants. We put the leaf discs on wet filter paper discs 60 mm apart from each other in 100 mm x 20 mm plastic Petri-dishes. Ten one-day old *S. frugiperda* larvae were placed in each arena and the position of larvae was recorded after 1 h, 2 h and 20 h periods. After 20 h feeding each leaf disk was photographed and the

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consumed surface area of each disk was determined by image analysis using ImageJ (version 1.53)[45].

Larval survival experiments

Larval survival on maize and *D. intortum* scored in plastic petri-dishes (100 mm x 20 mm), which were lined with wet filter paper to increase humidity. Five first instar *S. frugiperda* larvae were moved to each arena on the day of egg-hatching and fed daily with an excess amount of freshly cut *D. intortum* leaves or leaf blades of 4-5 weeks old maize (*Z. mays* cv. Delprim). After reaching the fourth instar stage, the maize diet was supplemented with the ligule, leaf sheets and young stems of maize and the larvae were separated into individual plastic cups to prevent cannibalism. The growth of the larvae was monitored daily and we determined the larval stage based on body coloration and the diameter of head capsules. We terminated the experiment after the insects pupated.

Light microscopy of *Desmodium* spp.

Upper and mid stem branches as well as the leaves of healthy 8 weeks old *D. intortum* plants were sampled for light microscopy. In addition, *S. littoralis* larvae that were immobilized on *D. uncinatum* and *D. intortum* stems and leaves were observed and photographed with a digital light microscope (Keyence VHX-5000, Keyence Corporation, Osaka, Japan) equipped with standard zoom lens (VH-Z20R magnification: 20-200x and VH-Z100R magnification: 100-1000x). For detailed, high depth-of-field images, photo stacking technique was used. Series of images were captured (50-100 depending on the size of the examined larvae) at different focus distances (step size, 20 - 40 µm). Subsequently, partially focused images were combined with Helicon Focus software (Helicon Soft Ltd., Kharkiv, Ukraine) into a high depth of field image.

Scanning electron microscopy of *Desmodium* spp.

To get further insights in the structure of the *D. intortum* trichomes, scanning electron microscopy (SEM) was performed on leaf and stem samples. Healthy leaves and stems were collected from eight-weeks old and one-year old plants from the greenhouse, and scanned using a FEI Quanta 3D scanning electron microscope operating with a field emission gun (FEG) electron source, equipped with SE (LVSED/ETD), BSE (vCD) and EDAX SDD EDS detectors. Low vacuum mode (50-80 Pa specimen chamber pressure) was used in order to avoid sample charging, and allowed us to use plant material without sample fixation, dehydration and sample coating. The accelerating voltage was 10-20kV with 40-480 pA beam current.
Furthermore the elemental composition of trichomes was studied using energy-dispersive X-ray spectroscopy (EDX), acquisition time: 50 sec. Measurements were taken in four regions (base, lower and higher middle and tip) on the longer type of trichomes and from three regions in case of small uncinate trichomes.

Statistical analysis

In case of each volatile sample the absolute peak areas were divided by the area of the internal standard peak to account for differences in volatile sampling efficiency. The volatile components were categorized into four compound groups: monoterpenoids, sesquiterpenoids, green leaf volatiles and other volatiles. We calculated the total sum of peak areas for these

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volatile groups across samples for the laboratory volatile collections and field volatile collections by location. The volatile collections were further normalized across samples by dividing the absolute peak areas by the sum of the total area of the volatile group from the corresponding dataset.

The clustered heatmaps of volatile emission profiles were generated from z-scores calculated from the normalized volatile data using package pheatmap[46]. Jaccard dissimilarity indices were calculated from binary (presence/absence) standardized volatile data and non-metric multidimensional scaling (NMDS) was completed using the metaMDS function of package vegan in R[47]. Permutational multivariate analysis of variance (PERMANOVA) was completed on Jaccard dissimilarity indices using the adonis function of the vegan package. For assessing differences in the normalized volatile peak areas for (*E*)-DMNT and (*E*)-*β*-ocimene between groups Kruskal- Wallis tests and Wilcoxon rank sum tests were used from package stats with Benjamini and Hochberg *p* value correction[48].

We used Wilcoxon paired rank sum tests with a null hypothesis of random choice using package stats for two-choice oviposition experiments and larval choice experiments[48]. As the statistical power of Wilcoxon paired rank sum tests are limited, we also fitted generalized linear mixed models (GLMM) by maximum likelihood with fixed factor for choice and random factor for replication on the two-choice oviposition data using package lme4[49]. We used the simulation-based test from package DHARMa[50] to assess the goodness of fit for the complete model. The post hoc tests were completed with the emmeans package using Tukey's comparisons[51].

Survival probabilities were calculated with Kaplan–Meier survival analysis[52] and the survival curves were compared using a log-rank test between diets in package survival[53]. Survival curves were visualized using package survminer[54].

Data availability statement

Volatile analysis data associated with volatile analysis and behavioral bioassays are available in figshare with the identifier(s) [10.6084/m9.figshare.19297730] and GC-MS raw data from the authors upon reasonable request.

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ACKNOWLEDGEMENT

We are grateful to Mr. Samuel Nyanzi at Rural Community in Development (RUCID) center, Mityana, and Dr. Fred Kabi of Makerere University for support with volatile collections in Uganda. We thank Ms. Eva Svensson from Lund University for help with soil autoclave sterilization. We are thankful to Alex Berg for pictures of *Desmodium* spp. highlighting their trichomes as well as immobilized insect larvae and Ábel Szabó for his support in scanning electron microscopy. Special thanks to Prof. Ted Turlings' lab for providing maize (*Zea mays*) seeds and *Spodoptera frugiperda* colonies and Prof. Peter Anderson for *Spodoptera littoralis* used in some pictures. We thank Prof. Marie Bengtsson, SLU, Sweden for providing standards for identification purposes. The following funding agencies are acknowledged for making this project possible: Sida, Food Security Program (ABD, TD), Ekhagastifelsen (ALE), SLU Global (TD), the EU Erasmus Program (ES), János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BPM).

AUTHOR CONTRIBUTIONS

ALE, ABD and TD conceived the idea and designed the experiments. All the authors contributed at different stages to performing the experiments, data analysis and writing of the manuscript.

COMPETING INTEREST DECLARATION

The authors declare no competing interests.

ADDITIONAL INFORMATION

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EXTENDED DATA

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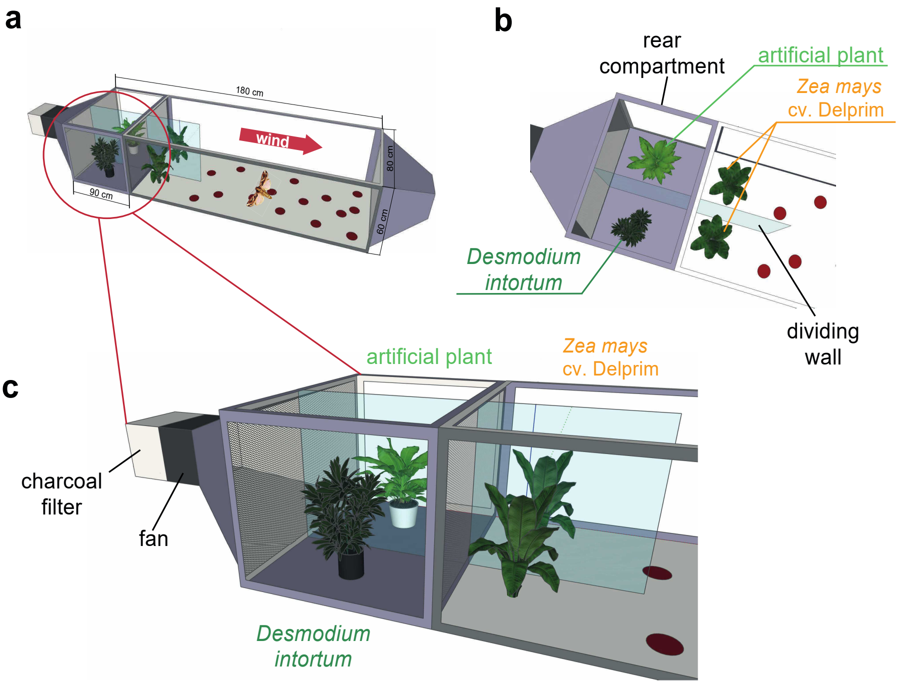

Fig. 1: Wind tunnel setup to study the oviposition repellency of *Desmodium intortum* volatiles. Two *Zea mays* cv. Delprim plants were placed in laminar filtered air flow with *D. intortum* (greenleaf *Desmodium*) or a plastic mimic plant directly upwind from the flight chamber containing two maize plants. A gravid *Spodoptera frugiperda* female was released in the wind tunnel. The number of egg batches laid on both maize plants were counted and the position of mimic plants and *D. intortum* plants were randomized.

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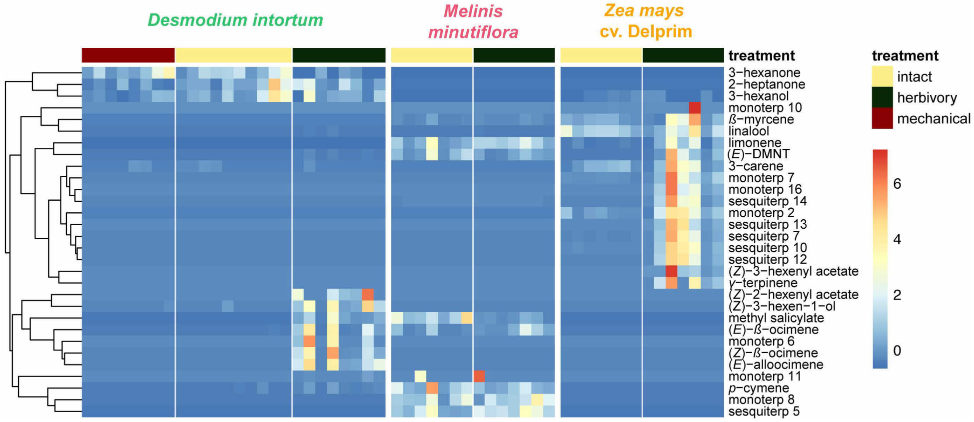

Fig. 2: Heatmap showing relative amounts of headspace volatile compounds emitted from intact, herbivore induced and mechanically damaged *Desmodium intortum*, *Zea mays* cv. Delprim and *Melinis minutiflora* plants grown in a greenhouse. The absolute peak areas were divided by the area of the internal standard peak and z-score was calculated (peak area - mean peak area/standard deviation of peak). The dendrogram of compounds was constructed via hierarchical clustering based on Euclidean distances. The major volatile constituents of intact *D. intortum* headspace were 2-heptanone and 3-heptanone. Monoterpenoids were only detectable after 48 hrs of *S. frugiperda* feeding, when (*E*)-4,8-dimethyl-nona-1,3,7-triene ((*E*)-DMNT), (*Z*)-β-ocimene, (*E*)-β-ocimene and (*E*)-alloocimene were emitted. The relative (*E*)-DMNT emission, (*E*)-β-ocimene emission and total monoterpenoid emission of intact and herbivore induced *D. intortum* were significantly different in pairwise comparisons with Kruskal-Wallis tests and pairwise comparisons with Wilcoxon rank sum test with Benjamini and Hochberg p-correction ($\chi^2 = 57.315$, $p = 0.00012$, $\chi^2 = 52.321$, $p = 8.5*10^{-5}$, and $\chi^2 = 52.904$, $p = 7.74*10^{-4}$). Linalool, β-myrcene were present in the headspace of intact maize. In response to 48 hrs of larval feeding (*E*)-DMNT, (*Z*)-α-bergamotene, β-caryophyllene, (*Z*)-β-farnesene, humulene and β-bisabolene were emitted.The relative (*E*)-DMNT emission and total sesquiterpenoid emission of intact and herbivore induced *Z. mays* cv. Delprim was significantly different using the same statistical tests ($\chi^2 = 57.315$, $p = 3.1*10^{-4}$ and $\chi^2 = 59.163$, $p = 8.2*10^{-4}$). The volatile headspace of the both intact and herbivore-induced *M. minutiflora* is composed of a variety of monoterpenoid and sesquiterpenoid compounds, such as (*E*)-DMNT, limonene, germacrene-D. Neither the relative (*E*)-DMNT emission nor the total monoterpenoid emission nor the total sesquiterpenoid emission of intact and herbivore induced *M. minutiflora* were significantly different in the same statistical tests ($\chi^2 = 57.315$, $p = 0.62$, $\chi^2 = 52.904$, $p = 0.63$ and $\chi^2 = 59.163$, $p = 0.12$).

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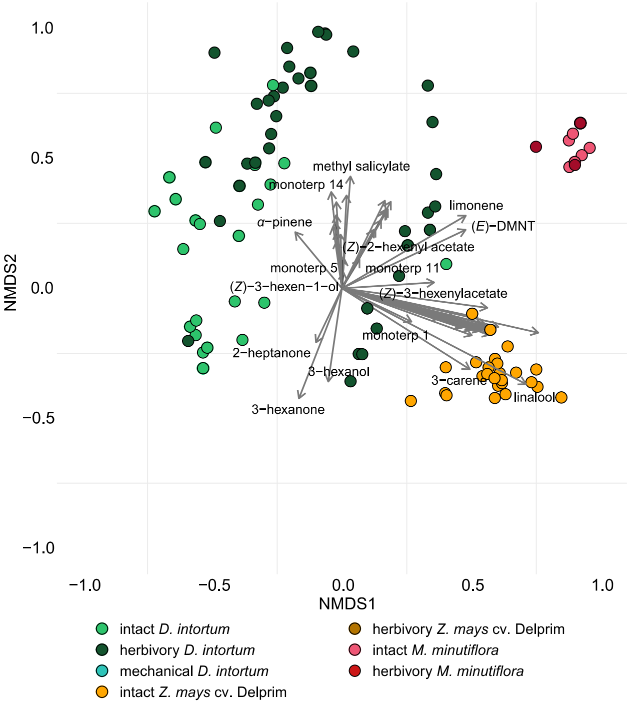

Fig. 3: Ordination of volatile samples from intact, herbivore damaged and mechanically damaged *Desmodium intortum*, *Zea mays* cv. Delprim and *Melinis minutiflora* plants based on non-metric multidimensional scaling (NMDS). The NMDS plots were based on presence-absence values and calculation of Jaccard-dissimilarity indices. The stress value of the plot is 0.138. Vectors represent correlations of volatile features with distribution of plant samples along the NMDS1 and NMDS2 axes.

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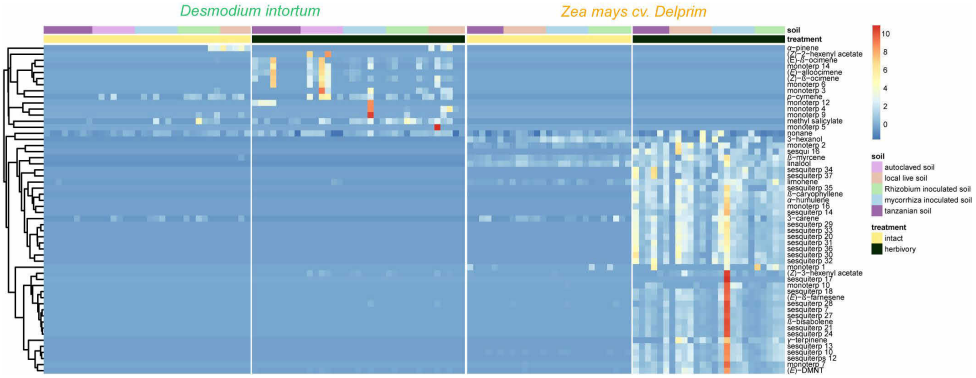

Fig. 4: Volatile emission profile of intact and herbivore damaged *Desmodium intortum* and *Zea mays* grown in soils with different microbial composition. The absolute peak areas were divided by the area of the internal standard peak and z-score was calculated (peak area - mean peak area/standard deviation of peak). The dendrogram of compounds was constructed via hierarchical clustering based on Euclidean distances.

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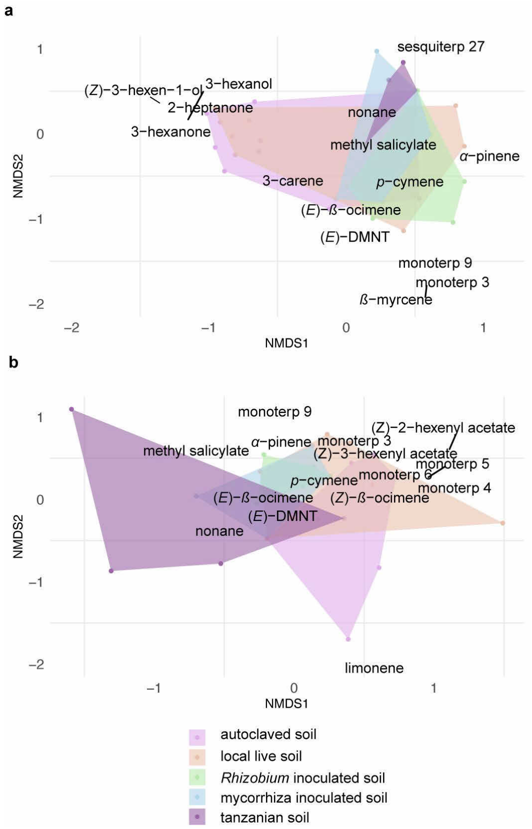

Fig. 5: The absence of volatile terpenoids in intact *Desmodium intortum* does not result from poor soil microbiota and insufficient nodulation. a, Non-metric multidimensional scaling (NMDS) ordination of volatile profiles from headspace of intact plants. **b,** NMDS ordination of herbivore-damaged *D. intortum* plants grown in different soils in a greenhouse. The stress values of NMDS ordination were 0.146 for intact and 0.120 for herbivore induced plants. The volatile profile of intact *D. intortum* on different soil treatments largely overlap while upon herbivory, some differentiation is observed. Scaling is based on Jaccard-distance matrix calculated from centered area values for each compound. The stress values are 0.146 and 0.120 for NMDS ordination of intact and herbivore-induced samples. Based on PERMANOVA and pairwise comparison of plants grown in different soil treatments the volatile profile of intact ($F_{model} = 3.260$, $R^2 = 0.189$, $p_{adj} = 0.615$) and herbivore-induced *D. intortum* ($F_{model} = 7.268$, $R^2 = 0.326$, $p_{adj} = 0.090$) did not cluster separately.

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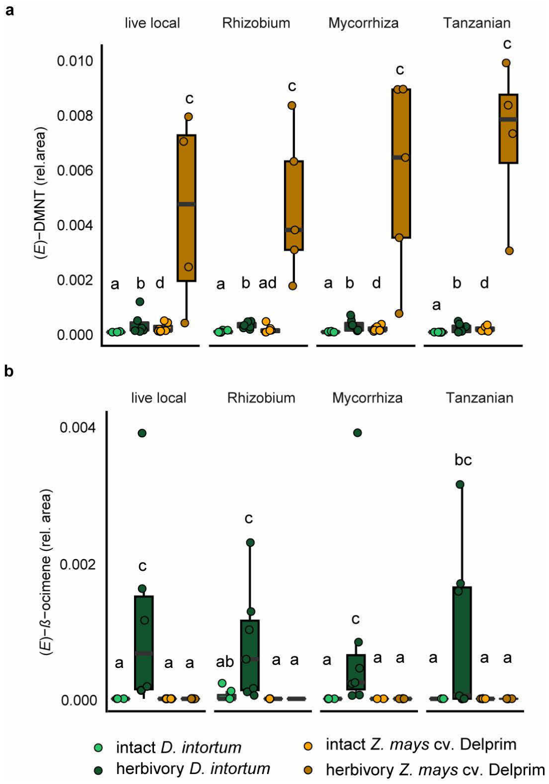

Fig. 6: The emission profile of *Desmodium intortum* and *Zea mays* cv. Delprim was not significantly altered by soil microbial treatments. a, The relative (*E*)-4,8-dimethyl-nona-1,3,7-triene ((*E*)-DMNT) emission and (*E*)-*ß*-ocimene emission of *D. intortum* and *Z. mays* cv. Delprim plants grown in soils containing *Rhizobium* spp., mixture of mycorrhizal fungi and soil of push-pull fields. The absolute peak areas were divided by the area of the internal standard peak to calculate relative values. The error bars show the standard error in relative emission units. Inoculation did not alter significantly the relative (*E*)-DMNT (χ^2 = 80.156, p = 0.303). **b,** Neither did inoculation affect the (*E*)-ß-ocimene (χ^2 = 7.688, p = 0.103) emissions of intact *D. intortum* plants based on pairwise comparisons with Kruskal-Wallis test with Wilcoxon rank sum test with Benjamini and Hochberg p-correction. Herbivore induced *D. intortum* plants grown in different soils were also not significantly different from each other in the relative (*E*)-DMNT (χ^2 = 5.153, p = 0.272) and (*E*)-*ß*-ocimene (χ^2 = 80.395, p = 0.268) emissions.

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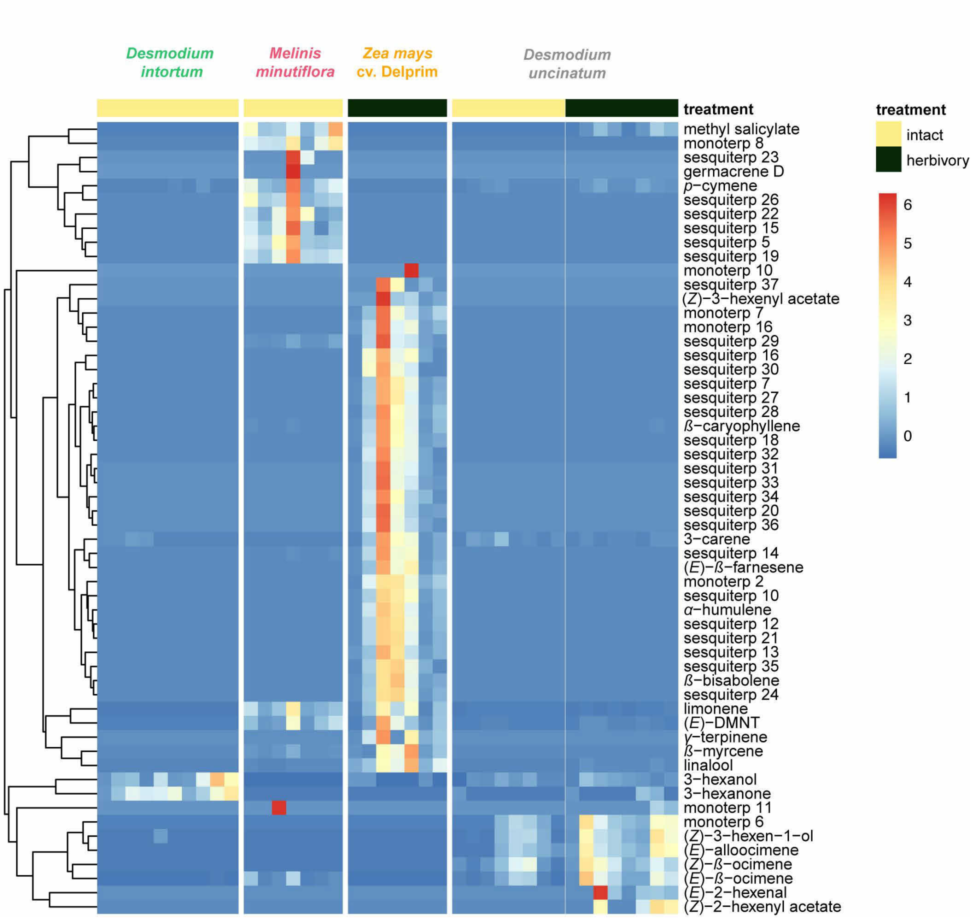

Fig. 7: Volatile emission of *Desmodium uncinatum* and *Desmodium intortum* compared to *Melinis minutiflora* and *Zea mays* cv. Delprim. The heatmap shows the relative amounts of volatile compounds emitted from intact *D. intortum* (greenleaf *Desmodium*), *M. minutiflora* and *D. uncinatum* (silverleaf *Desmodium*) as well as herbivore-damaged *Z. mays* (maize) and *D. uncinatum* plants. The absolute peak areas were divided by the area of the internal standard peak and z-score was calculated (peak area - mean peak area/standard deviation of peak). The dendrogram of compounds was constructed via hierarchical clustering based on Euclidean distances.

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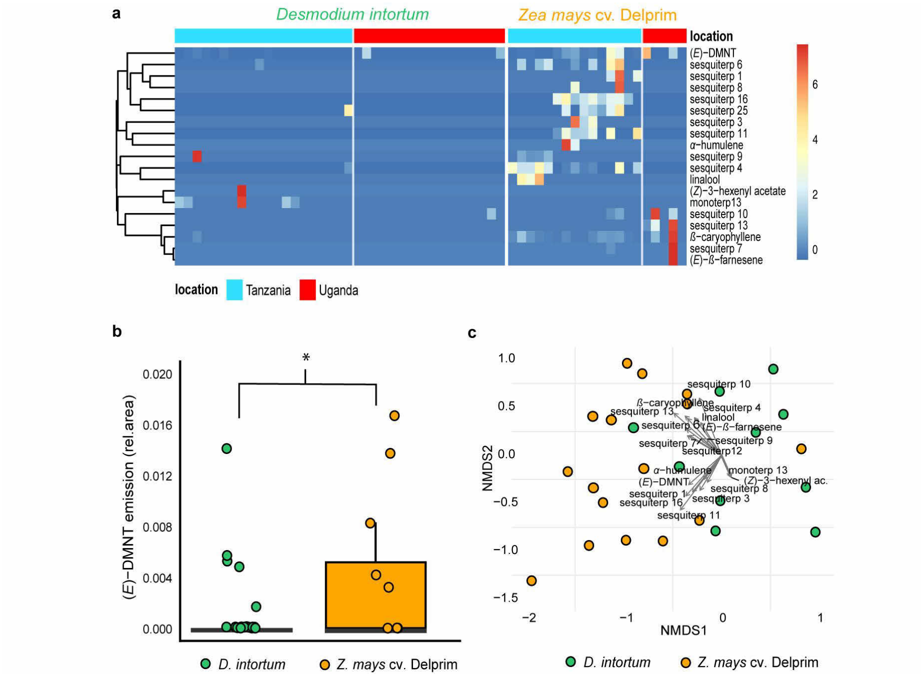

Fig. 8: Volatile emission of field grown *Desmodium intortum* and *Zea mays* plants from two locations. a, Heatmap volatile emissions of *D. intortum* (greenleaf *Desmodium*) and *Z. mays* plants at locations in Tanzania and Uganda. The absolute peak areas were divided by the total area of compounds belonging to monoterpenoids, sesquiterpenoids or green leaf volatiles per location and z-score was calculated (peak area - mean peak area/standard deviation of peak). The dendrogram of compounds was constructed via hierarchical clustering based on Euclidean distances. **b,** Similarly to greenhouse experiment, the constitutive emission of monoterpenoids, such as (*E*)-4,8-dimethyl-nona-1,3,7-triene ((*E*)-DMNT) and (*E*)-*ß*-ocimene were not detectable in case of *D. intortum* plants, due to possible underlying biotic and abiotic stressors emission of (*E*)-DMNT was visible in a small fraction of *D. intortum* samples. Based on Kruskal-Wallis tests and Wilcoxon rank sum test with Benjamini and Hochberg p-correction the relative (*E*)-DMNT abundance of *Z. mays* volatile samples was significantly higher than that of *D. intortum* volatile samples (χ^2 = 15.310, p = 2*10^{-3}). **c,** Non-metric multidimensional scaling (NMDS) of the volatile profile of *D. intortum* and *Z. mays* plants from field locations. The vectors represent the correlation of volatile features with the distribution of plant samples along the NMDS1 and NMDS2 axes. The stress value of the NMDS plot is 0.116. Based on PERMANOVA and pairwise comparison the volatile profile of *D. intortum* and *Z. mays* were significantly different (F_{model} = 8.816, R^2 = 0.149, p_{adj} =1*10^{-3}).

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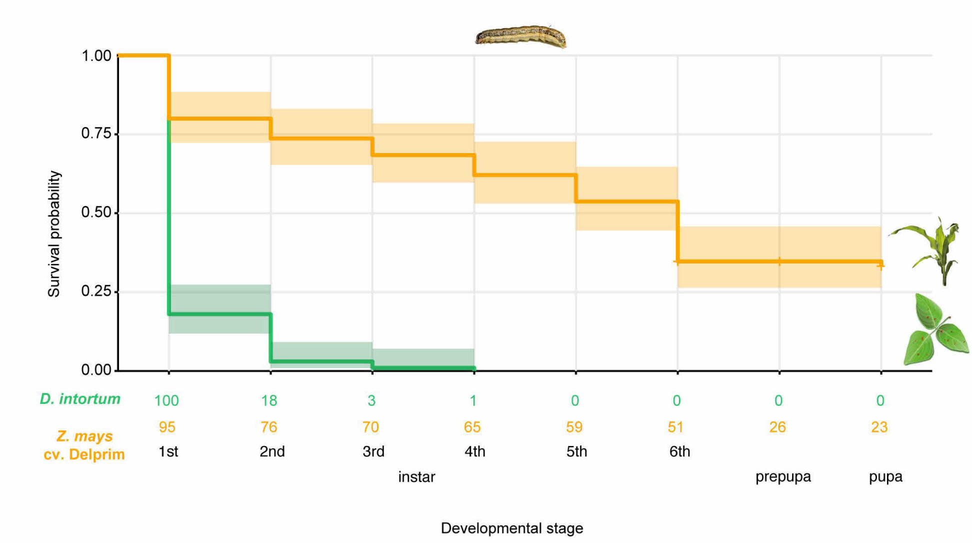

Fig. 9: The survival probability of *Spodoptera frugiperda* on diets consisting of *Desmodium intortum* (greenleaf *Desmodium*) or *Zea mays* cv. Delprim (maize) leaves. The Kaplan-Meier survival curves show that larvae on *D. intortum* diet had significantly higher mortality than larvae on *Z. mays* diet ($p = 2*10^{-16}$). The *D. intortum* diet resulted in a total mortality by the 4th instar larval stage. The inset below the plot shows the number of specimens reaching each developmental stage on the two types of diets.

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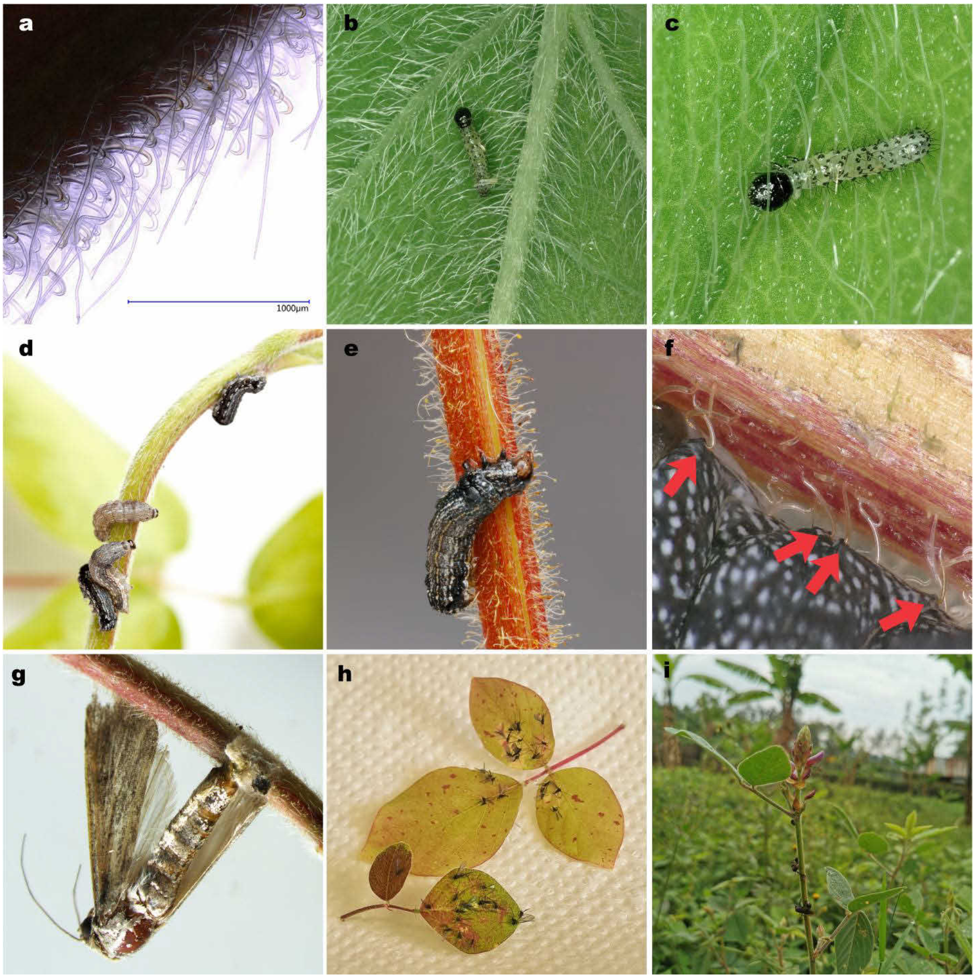

Fig. 10: *Spodoptera littoralis* larvae and adult *Spodoptera frugiperda* immobilized on *Desmodium intortum* and *Desmodium uncinatum* stems. a, Light microscopic picture of trichomes on the stem of *D. intortum.* **b-c,** Despite the dense network of sharp, straight and hooked trichomes, neonate larvae of *Spodoptera* spp. are able to graze and easily navigate through the leaf surfaces of *D. intortum*. **d-e**, Immobilized *S. littoralis* larvae on stems of *D. uncinatum* and on *D. intortum* stems. **f,** The cuticle of an *S. littoralis* larva pierced by uncinate trichomes, the red arrows indicate puncture sites. **g,** Ovipositing *S. frugiperda* female immobilized on *D. intortum*. **h,** *Bradysia sp.* immobilized on *D. intortum* leaves. **i,** *Hymenopteran* insects immobilized on *D. intortum* stems at a volatile collection site in Mwanza, Tanzania.

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SUPPLEMENTARY INFORMATION

Chemical analysis of GC-MS samples

Compounds were tentatively identified by matching their mass spectra with those found in MS Libraries (NIST11 and Wiley). The identification was verified by synthetic standards and matching Kovats retention indices found in literature for DB-WAX and HP-5 capillary columns.

Retention indices of volatile components, GC-MS raw data, the list of synthetic standards (suppliers and purity information) that were injected on DB-WAX and HP-5 columns to verify library based identification of headspace volatile components and behavioral bioassay data are available on FigShare (https://figshare.com/account/projects/134051/articles/19297730).

Volatile collections site selection

Samples were collected from Tarime and Musoma districts in Mara region in Tanzania. A small survey of the farmers practicing push-pull farming and/or growing *Desmodium* spp. was conducted to identify suitable sampling sites. Four locations were selected for *Desmodium intortum* volatile collection, three of them being *D. intortum* (greenleaf desmodium) monoculture and one of them a push-pull plot. One farm was selected for collection of volatiles also from herbivore infested maize plants.
Desmodium intortum volatiles samples were also collected in Uganda during the rainy season from Rural Community in Development (RUCID) centre, in Mityana district, from a *D. intortum* monoculture. The plots had been growing for four years at the time of sampling and were managed by trimming *Desmodium* about six times a year to feed animals.

Sampling site	Practice	GPS coordinates	Relative humidity	Temperature
Kitagasembe village, Gwitiriyo ward, Tarime district, Mara region, Tanzania	*D. intortum* monoculture, Maize with common beans as intercrop (*Phaseolus vulgaris*)	-1.3, 34.4792	70%	20 - 22° C
Gwitiriyo, Gwitiriyo ward, Tarime district, Mara region	*D. intortum* monoculture	-1.266661, 34.488133	68%	20 - 22° C
Kyoruba village, Pemba Ward, Tarime District.	*D. intortum* monoculture	-1.318, 34.520	75%	20 - 22° C

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Vi Agroforestry center, Lubango Ward, Musoma District, Mara region	Push-pull farming with *D. intortum* intercropping	-1.53054, 33.857955	81%	20-22^{o} C
RUCID centre, Mityana district, Uganda	*D. intortum* monoculture and maize monoculture	0.437941, 32.042500	68%	20- 26° C

Visualization of wind tunnel oviposition bioassay

The three dimensional model of wind tunnel assays (Extended Data Fig.1) was prepared in SketchUp (version 20.0).

ACTA UNIVERSITATIS AGRICULTURAE SUECIAE
DOCTORAL THESIS NO. 2022:35

The maize push-pull technology is an effective and ecological approach for cereal pest management used by smallholder farmers in east and southern Africa. In this thesis we show impact of the technology on soil microorganisms after long-term usage as well as re-examine its mechanisms of pest control.

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SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

Online publication of thesis summary: https://pub.epsilon.slu.se

ISSN 1652-6880
ISBN (print version) 978-91-7760-945-2
ISBN (electronic version) 978-91-7760-946-9