SOIL LEGACY EFFECTS ALTER PLANT VOLATILE EMISSIONS IN RESPONSE TO DIVERSIFIED CROPPING SYSTEMS

by

Shealyn Chelsea Malone

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DEDICATION

For my Mom and Dad,

who taught me that when you chase your passions, good things will follow.

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TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

LIST OF FIGURES

Figure Page

LIST OF FIGURES CONTINUED

Figure Page

ABSTRACT

Soil microbes can influence the emissions of plant volatile organic compounds (VOCs) that serve as host-location cues for insects and their natural enemies. The influence of the soil microbial community on the plasticity of plant VOC synthesis and emissions is particularly important in agricultural settings where crop rotations and management practices cause significant shifts in the soil microbiome. Studies have shown agricultural soils to influence plant-insect interactions through changes in foliar chemistry, but their potential to alter VOC emissions is unknown.

To determine the effect of diversified agricultural practices on crop VOC emissions through microbe-mediated soil legacy effects, I measured VOCs from wheat (*Triticum aestivum* L.) in a series of field and greenhouse experiments. In Chapter II, I determined the effect of the soil microbiome on VOCs in the greenhouse by first measuring VOCs from wheat plants grown in sterilized soil or soil with added inoculum from an agricultural field. Next, to determine the effect of diversified agricultural practices on VOC phenotypes, I measured VOCs from wheat plants in the field in rotation with either fallow or a mixture of cover crops that was terminated by grazing cows. Finally, in Chapter III, I explored the interactive effect of herbivory and the soil microbiome on VOC emissions in a full factorial experiment in which wheat grown in soil inoculum from wheat-fallow or wheat-cover crop rotation that was subjected to larval feeding by the wheat stem sawfly (WSS; *Cephus cinctus* Norton), a major pest of wheat.

Across all studies I found that soils associated with a higher microbial diversity—cover crop soils and inoculated soils—tended to emit more total VOCs and blends that would likely increase pest resistance to the WSS through 1) shifts in key bioactive compounds and 2) enhanced herbivore-induced VOC emissions. Results also suggest that soil microbes may be more likely to alter plant VOCs when plants experience abiotic or biotic stressors. Together, these results suggest that agricultural practices may indirectly influence plant resistance through microbe-altered VOCs, and these effects are more likely to occur when plants experience additional stressors, such as herbivory or drought.

CHAPTER ONE

INTRODUCTION

Plant Volatile Organic Compounds

Plants produce a vast diversity of secondary metabolites known as volatile organic compounds (VOCs). These lightweight non-polar compounds have high vapor pressures, all of which allow them to freely cross the cell membrane and volatilize into the atmosphere (Niinemets et al. 2004). Given their physical properties, diversity, and ubiquity, plant VOCs mediate a variety of intra- and interspecific ecological interactions (Dudareva et al. 2013; Pichersky et al. 2006), including the attraction of pollinators, natural enemies, and eavesdropping herbivores (Bouwmeester et al. 2019; Kessler and Kalske 2018; Muhlemann et al. 2014; Parachnowitsch and Mason 2015), plant-plant communication (Heil and Karban 2010), and protection against pathogens and herbivores (Hammerbacher et al. 2019; Unsicker et al. 2009).

The production and emission of plant VOCs is highly plastic in response to environmental factors. VOC emissions are highly sensitive to prevailing environmental conditions including light intensity, temperature, and water availability (Gouinguené and Turlings 2002; Timmusk et al. 2017). Because their synthesis is dependent on substrates produced through photosynthesis and cell respiration, the production of volatile compounds can be tightly linked to changes in primary metabolism (Dudareva et al. 2013). Further, some classes of volatile compounds play an important role in the protection against a variety of abiotic stressors (Sharkey and Yeh 2001; Vickers et al.

2009). While environmental factors such as resource availability and abiotic conditions can impact emission rates, genetics also dictate VOC synthesis and results in compositional variation among tissue types, across ontogenetic stages, and in response to biotic challenges, such as herbivore damage (Barton and Koricheva 2010; Cole 1980; Venkatesan 2015). In response to herbivory, plants emit altered, or induced, VOC blends that may be quantitatively and qualitatively different from the constitutive blend emitted when the plant is not experiencing herbivore challenge (Dicke and Loon 2000; Heil 2014). The production of herbivore-induced VOC blends is dependent upon cell membrane damage and the presence of elicitors from insect herbivores, which together modify gene expression and trigger volatile release (De Moraes et al. 1998; Gange et al. 2003; Moreira et al. 2018). While environmental and genetic factors can independently influence VOC emissions, they can also interact with one another in complex ways that may be synergistic or antagonistic, depending on the severity of stress and evolutionary history of the system.

Despite being one of the most variable aspects of a plant’s phenotype, plant VOCs convey important information about plant status that can be perceived by organisms in the environment (Heil and Karban 2010; Kessler and Kalske 2018). For example, herbivore-induced VOCs relay information regarding host location and quality to foraging insect predators, cuing these insects to the presence of an insect host (DeMoraes et al. 1998; Dicke and Loon 2000; Fatouros et al. 2005). While some plant VOCs have toxic properties that deter phytophagous insects, many specialist insects exploit constitutive plant VOCs to locate appropriate hosts for foraging and oviposition (Bruce et al. 2005; War et al. 2011). Plants emit many of the same vegetative volatile compounds; therefore, insects have developed sophisticated techniques to perceive VOC blends and specific ratios of compounds to accurately recognize host specific information (Bruce et al. 2005; Junker et al. 2018). However, pollution and a rapidly changing climate can alter VOC blends as well as the ability of insects to recognize plants signals on the landscape (Glenny et al. 2018; Fuentes et al. 2016). This has important consequences for ecosystem dynamics that are influenced by plants and their insect symbionts (Glenny et al. 2018; Meiners 2015).

Soil Microbe-Induced Plant Volatiles

Just as the emission of plant VOCs are ubiquitous across plant species, so are plant associations with soil microbes. Mutualistic soil microbes, including arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR), have traditionally been recognized for their ability to increase the growth and stress tolerance of plants through improved resource acquisition and growth promoting hormones (Goswami et al. 2016; Smith and Read 2010). However, more recent work has elucidated additional mechanisms by which soil microbes enhance plant performance through modulation of defense pathways and increased resistance to herbivores and pathogens (Conrath et al. 2006; Jung et al. 2012).

During establishment and maintenance of the symbioses, soil microbes interact with plant defensive signaling pathways, altering the expression of key hormones, including jasmonic acid (JA) and salicylic acid (SA), which play important roles in the synthesis and regulation of secondary compounds, including VOCs (Jung et al. 2012;

Van Wees et al. 2008). JA and SA pathways are known to exhibit crosstalk and generally are antagonistic to one another (Pieterse et al. 2008). As such, microbes that suppress SA-pathways, for example—such as AMF—allow for the upregulation of JA-pathways, which may influence constitutive production of secondary compounds or alternatively, 'prime' plant defenses, influencing induced secondary compounds (Pieterse et al. 2014). Primed plants are able to elicit a greater and more rapid expression of defense genes after pathogen or herbivore damage (Conrath et al. 2006; Pieterse et al. 2014; Pozo and Azcón-Aguilar 2007), resulting in higher resistance to pathogens and herbivory. Thus, through altered regulation of defense hormones, root colonization by beneficial microbes initiates substantial physiological changes in the host plant that influence the production of VOCs and this has major consequences for multitrophic plant-insect interactions (Grunseich et al. 2020; Jung et al. 2012; Pineda et al. 2010; Sharifi et al. 2018; Shikano et al. 2017).

Microbial colonization of plants may increase (Fontana et al. 2009) or decrease (Babikova et al. 2014) emissions of VOCs, which can enhance (Babikova et al. 2014; Ballhorn et al. 2013) or reduce (Brock et al. 2018) the attraction of herbivores. Altered plant VOCs also impact natural enemies of herbivores; while plants inoculated with microbes are generally more attractive to natural enemies following herbivory (Hempel et al. 2009; Hoffmann et al. 2011; Schausberger et al. 2012; Pangest et al. 2015), they can also exhibit reduced attractiveness (Gange et al. 2003; Pineda et al. 2013). Furthermore, microbial associations may alter constitutive volatiles to be more like that of plants under herbivore attack (Fontana et al. 2009; Guerrieri et al. 2004), which may serve as false signals to foraging natural enemies. In summary, plant-microbe interactions can reduce, enhance, or have no effect on plant resistance depending on whether the plant VOCs affected impact herbivore behavior or the behavior of their natural enemies. These complex interactions can have important implications for how microbes influence multitrophic plant-insect interactions in natural and agricultural systems.

Pest Resistance in Agroecosystems through Plant-Soil Feedbacks

Given our knowledge of microbe-induced plant volatiles, there exists a clear opportunity for agriculture to enhance pest resistance of crops through the use beneficial microbes that alter the VOC signaling (Babalola 2010; Gadhave et al. 2016; Timmusk et al. 2017). Plant volatile-based insect pest management has already proven successful through other mechanisms that alter the emissions of VOCs across the agricultural landscape (Shrivastava et al. 2010; Maura 2020; Pickett and Khan 2016). For example, trap crops, or unharvested crops planted around the field to be protected, emit VOCs that are more favorable to pests than those emitted by the cash crop, thus ‘intercepting’ pests and reducing field damage (Adler and Hazzard 2009; Buteler et al. 2010; Morrill et al. 2001). Additionally, cultivars of crops vary in VOC blends, making some varieties less attractive to pests relative to other varieties (Buteler and Weaver 2012; Weaver et al. 2009). Given that soil microbes can alter VOC emissions and subsequent insect behavior, there is potential to apply knowledge of microbe-induced plant volatiles to agricultural settings where beneficial soil microbes might increase pest resistance to crops through altered VOC emissions.

Most of our knowledge of microbe-induced plant volatiles involves inoculation of plants with a single strain of microbes, and while many beneficial strains have been identified in the laboratory, their application in the field yields varied results (Gadhave 2016). This is likely because of variability in field environments that prevents successful establishment of microbial inoculants (Timmusk et al. 2017). For microbial applications to be successful, they must be able to establish in the presence of indigenous microbes and local abiotic conditions, colonize plants, and persist in the soil among changing seasonal conditions (Babalola 2010). Thus, inoculation success requires thoughtful inoculant development and management practices, many of which are still not well understood (Babalola 2010; Verbruggen et al. 2013) and constraining the widespread commercial use of microbial inoculants for improved pest resistance.

In lieu of microbial inoculums, an alternative approach is to promote the presence and activity of existing beneficial soil microbes through the ecological concept of plant-soil feedbacks (Mariotte et al. 2018; Pineda et al. 2017; Putten et al. 2013). Plant-soil feedbacks occur when plants influence soil microbes via root exudates, and soil microbes, in turn, alter the performance of plants grown later in the same soil through mutualistic or pathogenic interactions (Bever 1994; Kulmatiski et al. 2008;). While much of this work has focused on how plant-soil feedbacks influence the biomass of subsequent plants grown in the same soil, plant-soil feedback studies have shown that plants can steer soil microbiomes to improve plant resistance of the subsequent crop through shifts in foliar defense compounds (Badri et al. 2013; Blundell et al. 2019; Howard et al. 2020; Hu et al. 2018; Hubbard et al. 2019; Huberty et al. 2020a; Huberty et al. 2020b; Pineda et al. 2020; Wang et al. 2019; Zhu et al. 2018), suggesting that plant-soil feedbacks may provide a novel means to shape the soil microbiome to enhance crop performance and protection.

Plant-soil feedbacks are inherent features of existing agricultural practices, particularly the use of cover crops. Unlike cash crops which are planted for harvest, cover crops were traditionally planted to manage soil erosion, soil fertility, soil water, and abate weeds, pests, and diseases (Fageria et al. 2005). However, cover crops may provide additional modes of pest management through their impact on the soil microbiome (Kaplan et al. 2018). Cover crops—and the way in which they are managed—steer distinct soil microbe communities, recruiting and inhibiting different bacteria and fungi through plant-specific root exudates (Babin et al. 2019; Frasier et al. 2016; Kim 2020). Indeed, diversified crop rotations and nutrient management practices have been shown to enhance microbial biomass and function, and soil enzyme activity (Borase et al. 2020; Kim et al. 2020). Therefore, cover crops and management practices have the potential to influence the performance of the subsequent cash crop through microbe-mediated plant-soil feedbacks (Kaplan et al. 2018; Pineda et al. 2017). Though it has long been recognized that cover crops may decrease negative plant-soil feedbacks (i.e. 'soil sicknesses'), recent work has focused on using plant-soil feedbacks to promote the presence and activity of beneficial soil microbes to improve plant resistance (Huang et al. 2013; Kaplan et al. 2018; Pineda et al. 2017). Indeed, both the use of cover crops (Blundell et al. 2019) and diversified management practices (Murrell et al. 2019) have been shown to decrease pest populations in agricultural systems through increased foliar chemical defense. While these and other studies (Carrillo et al. 2018; Ingerslew & Kaplan 2018) demonstrate that agricultural soils can influence plant-insect interactions through changes to foliar chemistry, how they may alter VOC emissions it is still unknown.

Experimental Questions

Understanding the extent to which farming management systems influence crop VOC emissions will help determine the potential of farming management decisions to impact pest resistance in crops through soil legacy effects. The primary goals of the research are as follows:

i) To characterize the effects of agriculturally-derived soil microbes and diversified cropping systems on VOC emissions by wheat through abiotic and biotic soil legacy effects (chapter 2)
ii) To examine the effects of agricultural soil inoculation on constitutive- and herbivore-induced VOC emissions by wheat (chapter 3)
iii) To summarize soil effects on crop VOC emission among farming management systems and discuss implications for future research (chapter 4)

The research objectives were addressed with a combination of field and laboratory studies using wheat production in the northern Great Plains (NGP) as a model system. In the semi-arid NGP, cash crops are traditionally grown in rotation with fallow to preserve soil moisture (Padbury et al. 2002).However, improved moisture storage through management techniques has provided an opportunity for more diversified rotations, which offer numerous below- and aboveground benefits (Fageria et al. 2005; Sarrantonio and Gallandt 2003), including increased diversity and abundance of the soil microbiome (Kim et al. 2020). Therefore, diversified farming management strategies may improve pest resistance of crops through altered microbe-induced plant VOC emissions. In the

NGP, altered emissions by wheat will have important implications for insect pests, particularly the wheat stem sawfly (*Cephus cinctus*), the major pest of wheat in the NGP (Beres et al. 2011; Buteler and Weaver 2012; Piesik et al. 2008; Weaver et al. 2009). The larvae of the wheat stem sawfly are parasitized by braconid parasitoids (*Braconid lissogaster and B. cephi*) (Buteler et al. 2015; Buteler et al. 2008; Peterson et al. 2011), which also use volatile cues to locate appropriate hosts (Perez 2009).

It is unknown weather changes in farming management system are able to impact plant volatiles through changes to the soil microbiome. Thus, it is critical to understand the volatile-plasticity of crops in response to management-driven soil effects to improve volatile signaling that deters pests and strengthens cues for parasitoids. This research aims to better understand the complex interactions between plants and soils in driving insect interactions in agroecosystems.

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CHAPTER TWO

CROPPING SYSTEMS ALTER CROP VOLATILE CUES IMPORTANT FOR INSECT PESTS THROUGH SOIL LEGACY EFFECTS

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

Author: Shealyn C. Malone
Contributions: Conducted the experiments; analyzed the data; drafted the initial manuscript; revised the manuscript

Co-Author: David K. Weaver
Contributions: Conceived the project; revised the manuscript

Co-Author: Lila Hamberg
Contributions: Conducted the experiments

Co-Author: Fabian D. Menalled
Contributions: Conceived the project; revised the manuscript

Co-Author: Tim F. Seipel
Contributions: Conceived the project; assisted with data analysis; revised the manuscript

Co-Author: Justin B. Runyon
Contributions: Revised the manuscript

Co-Author: Megan L. Hofland
Contributions: Assisted with experiments

Co-Author: Amy M. Trowbridge
Contributions: Conceived the project; revised the manuscript

Manuscript Information

Shealyn C. Malone[1,4], David K. Weaver[1], Fabian Menalled[1], Tim Seipel[1], Justin B. Runyon[2], Lila Hamburg[1], Megan L. Hofland[1], Amy M. Trowbridge[3]

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Cropping systems alter crop volatile cues important for insect pests through soil legacy effects

Shealyn C. Malone[1,4], David K. Weaver[1], Fabian Menalled[1], Tim Seipel[1], Justin B. Runyon[2], Lila Hamburg[1], Megan L. Hofland[1], Amy M. Trowbridge[3]

[1] Department of Land Resources & Environmental Sciences, Montana State University, Bozeman, MT 59717, USA

[2] USDA Forest Service, Rocky Mountain Research Station, Forest Sciences Laboratory, Bozeman, MT 59717, USA

[3] Department of Entomology, University of Wisconsin, Madison, WI 53715, USA

[4] Author to whom any correspondence should be addressed

Email: shealyn.malone@gmail.com; Phone: 303-918-3367; Fax: 406-994-3933

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Abstract

Plant volatile organic compounds (VOCs) serve many important ecological roles, including the mediation of plant-insect interactions. VOC emissions can change in response to environmental conditions and shifts in soil features, including water availability and soil microbial communities. Soil-mediated plasticity of plant VOCs is particularly important in agricultural settings where management practices, like crop rotation, can cause significant shifts in the soil microbiome. While studies have shown that soil microbiomes can alter plant growth and foliar chemistry, effects on VOC emissions remain largely unknown, despite potentially important implications for crop-pest dynamics. To determine how agricultural practices affect VOC emissions through soil microbe effects, we measured VOC emissions from wheat (*Triticum aestivum* L.) in both greenhouse and field experiments. To determine whether the presence of soil microbes from agricultural fields can modify volatile emission of crops, we collected VOCs from wheat plants grown in either sterilized soil or sterilized soil inoculated with live soil inoculum collected from the field. Next, to assess the effect of cropping systems on plant VOC phenotypes, VOC emissions were measured in the field from wheat plants grown in rotation with either fallow or a mixture of cover crops that was terminated by grazing cows. In the greenhouse, we found that the presence of soil microbes from an agricultural field caused shifts in the relative amount of individual compounds and increased the emissions of total volatiles. In field conditions, wheat grown in cover crop soils emitted more total volatiles compared to wheat grown in fallow soils. Wheat grown in cover crops also emitted less *Z*-3-hexenyl acetate and β-ocimene, both known host-

location cues for the wheat stem sawfly (WSS; *Cephus cinctus* Norton), a wheat pest, suggesting the indirect role of crop rotations and soil microbes in plant resistance against pests. One of the salient features of our work was that in both the greenhouse and field study, we found strong negative correlations between emissions of saturated aldehyde compounds and plant biomass. Wheat grown in the greenhouse in inoculated soil and in the field in cover crop rotations may have experienced soil-related stressors that contributed to the trends observed, highlighting the complexity of interactions between soil microbes and other soil properties. These results suggest that agricultural practices can indirectly influence crop VOC emissions through the soil microbiome, and their impacts may be governed by interactions with abiotic soil properties. Further, the observed differences in VOC emissions suggest that cropping management strategies may alter host plant attractiveness for insect pests.

Introduction

Plants produce unique blends of volatile organic compounds (VOCs), many of which play important roles in mediating plant-insect interactions (Bouwmeester et al. 2019; Heil 2014; Muhlemann et al. 2014). For example, plant VOCs may deter phytophagous insects, or alternatively, be exploited by these organisms for host recognition (Bruce et al. 2005; Chiu et al. 2017; Gray et al. 2015; Paré and Tumlinson 1999). The functionality of VOCs is enhanced by their plastic nature; VOCs can change with great specificity in response to pathogen infection, abiotic stress, insect feeding and oviposition, and these altered blends often have fitness enhancing properties (Sharifi et al. 2018; Venkatesan

2015; Vickers et al. 2009). For example, herbivore-damaged plants can selectively signal to the appropriate species of parasitoid through highly specific shifts in volatile blends (De Moraes et al. 1998; Gange et al. 2003) while plants under heat stress improve membrane stabalization through increased production of key volatile compounds (Sharkey et al. 2001; Vickers et al. 2009). Plant VOCs change also in response to a number of environmental conditions, including the soil in which they grow (Gouinguené and Turlings 2002; Timmusk et al. 2014). Soil properties, including water availability (Gouinguené and Turlings 2002), as well as the presence and composition of microbial communities (Rasmann et al. 2017; Sharifi et al. 2018; Shikano et al. 2017), can initiate systemic changes within the plant that ultimately influence VOC synthesis and emissions (Paszkowski 2006; Pozo and Azcón-Aguilar 2007). The important role of VOCs in mediating plant-insect interactions underscores the need to assess the degree to which belowground soil properties shape aboveground community dynamics through altered VOC emissions.

Studies have shown that soil microbes can modify plant VOC emissions and alter insect behavior. For example, inoculation with either arbuscular mycorrhizae (AMF) or plant growth promoting rhizobacteria (PGPR) alters plant VOCs, increasing (Babikova et al. 2014) or decreasing (Brock et al. 2018) attraction of herbivores (Schausberger et al. 2012) and their natural enemies (Pineda et al. 2013). While many laboratory studies have examined the effect of individual soil microbial strains on VOC emissions, *in situ* soils harbor thousands of microbial taxa with positive, negative, and neutral impacts on plants. Thus, in natural systems, plant VOCs will likely be shaped by the net effect of the entire soil microbial community, not just a single strain (Kaplan et al. 2018). Studies have shown that whole soil microbiomes influence the foliar chemistry of plants (Blundell et al. 2019; Howard et al. 2020; Hubbard et al. 2019; Huberty et al. 2020a; Huberty et al. 2020b; Pineda et al. 2020; Zhu et al. 2018), but to our knowledge, no studies have investigated their effect specifically on volatile emissions.

Agricultural practices, such as crop rotations, off-farm chemical inputs, mechanical and cultural tactics, and reliance on biological processes (collectively called cropping systems) can cause significant shifts in biotic and abiotic soil properties (Dabney et al. 2001; Lapsansky 2016; Navarro-Noya et al. 2013) with potential implications for microbe-plant interactions and VOC emissions. Cover crops, for example, help prevent ‘soil-sickness’, or the accumulation of species-specific autotoxins and pathogenic soil microbes that result from repeated planting of the same crop or related species (Huang et al. 2013). Cover crops can also increase beneficial microbes within the soil, enhancing crop resistance against insect pests in the following season through improved crop nutrient uptake and increased induced activity of herbivore-defense genes (Murrell et al. 2019). Importantly, the effects of cover crops on the soil microbiome depend strongly on the way in which they are managed. For example, when cover crops are terminated by herbicides rather than with mechanical or biological methods (i.e. mowing or grazing), the abundance, activity, and diversity of the soil microbiome are significantly reduced (Kim et al. 2020). Cover crops not only influence biotic soil properties, they also improve abiotic properties through organic matter enrichment, reduction of soil compaction, and increased nutrient availability (Fageria et al. 2005). Thus, cover crops are important tools for building soils with improved physical, chemical, and biological properties (Fageria et al. 2005; Sarrantonio and Gallandt 2003), including increased microbial diversity (Kim et al. 2020).

Crop production in the semi-arid northern Great Plains (NGP) is dominated by monocultures of drought-resistance crops, especially wheat (*Triticum aestivum* L.) (Padbury et al. 2002). In addition to low precipitation, wheat grown in the NGP faces intense pressures from the wheat stem sawfly (WSS; *Cephus cinctus* Norton), the most economically important insect pest of wheat in the region (Beres et al. 2011). Females WSS use volatiles cues released by plants, including the green leaf volatile *Z*-3-hexenyl acetate and the monoterpene β-ocimene, to locate appropriate hosts for oviposition (Buteler and Weaver 2012; Piesik et al. 2008; Weaver et al. 2009). Given the importance of VOCs for mediating wheat-WSS interactions, it is possible that wheat plants emitting less or unattractive VOC blends or blends that cannot be recognized by ovipositing females could experience enhanced pest resistance.

The adult stage of the WSS is short-lived (less than one week), and the larval stages are found within the stem of the host plant (Ainslie 1920). As such, the wheat stem sawfly cannot be managed with insecticides and although solid-stem cultivars increase larval mortality, they are only partially resistant (Beres et al. 2017; Sherman et al. 2010; Varella et al. 2015; Weaver et al. 2004). Alternatively, the use of soil microbially-altered VOC emissions through diversified cropping systems may be a viable approach for influencing adult WSS behavior and increasing crop protection. Studies have shown that diversification of cropping systems through the use of cover crops and organic management decreases insect pest pressure (Koch et al. 2015; Murrell et al. 2019; Koch et al. 2012; Blundell et al. 2019). Specifically, Adhikari et al. (2018) determined that organic fields had less wheat stems cut by WSS and supported more parasitoids than conventional fields. Therefore, we expect the integration of cover crops and livestock grazing could lead to reduced pest pressure of wheat through altered emissions of volatile compounds known to be important to adult WSS.

In this study, we assessed the effect of cropping system on the plasticity of wheat VOCs by coupling a greenhouse and a field study. First, to determine whether the presence of soil microbes from an agricultural field modify plant VOCs, we conducted a greenhouse study in which VOCs were collected from wheat grown in sterile soil or soil inoculated with aliquots of soil from an agricultural field. Second, to determine effect of cropping systems—and the soil microbes associated with them—on plant VOCs, VOCs were collected from wheat grown in the NGP from two unique rotations 1) wheat-fallow or 2) wheat-cover crop, where a seven-species cover crop mixture was terminated by cattle. We hypothesized that, in the greenhouse study, the presence of soil microbes would alter the emission of plant VOCs due to plant-microbe interactions that were not experienced by the plants grown in sterile soil. In the field study, we hypothesized that cropping history (i.e. cover crop or fallow) and concomitant shifts in the soil microbiome would modify VOC blends of wheat, including key shifts in key volatile compounds known to be important for WSS resistance.

Methods

Study site and cropping systems

The greenhouse experiment was conducted with soil collected from the Montana State University Northern Agriculture Research Center located south of Havre, MT (48°29'48.8"N, 109°48'10.4"W), and this is also the location where the field experiment took place. The site is a water-limited agroecosystem of the NGP with an average annual precipitation of 305 mm. Average annual high and low temperatures at the site are 13.6 °C and 0.0 °C, respectively (Western Regional Climate Center, 2020). Since 2012, two replicate fields (40 m x 360 m) have been planted in an alternating two-year rotation of winter wheat (Judee variety) with either cover crop mixtures or fallow. Within each field, treatments were randomly repeated in 3 separate plots (8 m x 14 m; Fig. S1). The location of each treatment was randomized in 2012 and has been maintained through time.

This work focused on two cropping systems: 1) winter wheat rotated fallow and 2) winter wheat rotated with a 7-species mixture of cover crops (radish (*Raphanus raphanistrum* L.), lentil (*Lens culinaris* Medikus), field pea (*Pisum sativum* L.), oat (*Avena sativa* L.), turnip (*Brassica rapa* L.), sorghum and sudan grass (*Sorghum* x *drummondii* Nees ex. Steud.), and soybean (*Glycine max* L.) (Fig. S1). The soils from these treatments differed in bacterial (Ouverson et al. in prep) and fungal (Dupre et al. in prep) community composition and diversity. Cover crop species were selected based on United States Department of Agriculture-Agricultural Research Service (USDA-ARS) recommendations for the NGP and represent a range of functional groups with potential for the provision of various ecosystem services (https://www.usda.gov/plains-area-mandan-nd/ngprl/docs/cover-crop-chart/). Cover crop plots were planted mid-May and terminated late-June to protect soil moisture, as practiced in the region (O’Dea et al. 2013). Termination of cover crops was achieved by targeted cattle grazing, an ecologically based management approach to enhance economic and environmental sustainability of growing cover crops (McKenzie et al. 2017; Thiessen Martens and Entz 2011). Both cropping systems were spot treated with glyphosate to manage postharvest weeds during July and September.

Greenhouse study

Soil collection

Soil was collected from the cover crop rotations at the Northern Agriculture Research Center (48°29'48.8"N, 109°48'10.4"W) on July 4, 2018 just before cover crop termination, to attain peak season microbe diversity (Ishaq et al. 2020). Soil was collected from plots that were currently planted in cover crops and would be planted with wheat later that fall. Surface litter was cleared and approximately 2,000 g of soil was collected to a depth of 30 cm with a sterilized shovel at three random locations within each plot. Bags of soil were stored on ice and transported back to Montana State University (Bozeman, MT) where all samples were homogenized and stored at -20 °C until later use in the greenhouse.

Soil conditioning and response phase plant growth

Establishment and proliferation of soil microbe inoculations relies on plant growth and carbon inputs to fully establish microbial communities in greenhouse soil (Brinkman et al. 2010). Therefore, in the soil inoculated treatment, a field inoculum of 15% by mass was added to a 1:1 mixture of autoclaved Sunshine Mix #1 soil (Gro Horticulture Inc., Bellevue, WA) and MSU soil (see below) and wheat was allowed to grow for two four-week conditioning periods before planting wheat for the response phase (Fig. S2).. Sunshine soil is comprised of Canadian sphagnum peat moss, perlite, vermiculite, starter nutrient charge, wetting agent, and dolomitic lime. MSU soil is equal parts of Bozeman silt loam soil, washed concrete sand, and Canadian sphagnum peat moss. AquaGro 2000 G wetting agent (Aquatrols, Paulsboro, NJ) was blended in at one pound per cubic yard of soil mix and aerated steam pasteurized at 80°C for 45 minutes. The live soil inoculum was used to introduce microbe communities from the field while minimizing nutrient discrepancies between soils. This technique is used to elucidate the contribution of soil biota to soil legacy effects on plant performance (Brinkman et al. 2010) and has been successfully applied to assess the impact of agricultural soil microbial communities on plant growth and competitive interactions (Johnson et al. 2017; Miller and Menalled 2015), and overall agroecosystem resilience (Seipel et al. 2019). The soil inoculum treatments had distinctive bacterial (Ouverson et al. in prep) and fungal (Dupre et al. in prep) community composition and diversity.

Prior to the experiment, wheat seeds of the cultivar Reeder were collected from a single spike of wheat collected in Big Sandy, MT during Fall of 2018 and grown to maturity in the Montana State University Plant Growth Center. We used genetically identical seeds in order to control for chemotypic variation between plants (Gouinguené et al. 2001). Before each planting, we surface sterilized the wheat seeds in 5% bleach and rinsed twice with deionized water. Single seeds were sown in individual square pots (10 x 9 x 9 cm) that had been washed and sterilized. During each condition phase, plants were allowed to grow for four weeks, at which point the aboveground biomass was harvested. After one week of fallow, we planted pots for a second condition phase. During conditioning, plants were watered *ad libitum* and not fertilized as our purpose was to support establishment of microbial community, not optimize plant growth. At the conclusion of the two phases, soil had been conditioned for a total of eight weeks. To reduce any effect of variation in plant development stage on VOC emissions, we planted one cohort of plants each week for the first condition phase during March 2019.

After the second condition phase and one week of fallow, single wheat seeds that had been surface sterilized were planted in each pot for the response phase from which VOCs would be collected (Fig. S2). In addition to the pots with inoculated soil, new pots were also planted with sterilized Sunshine Mix/MSU soil in order to compare plant VOCs in the absence of soil microbial communities. All plants were fertilized within a day of planting and this treatment continued biweekly. While fertilizers are known to affect microbial community structure and biomass, especially for fungal species (Donnison et al. 2000; Sarathchandra et al. 2001), low rates have been shown to preserve their functional diversity (Marschner et al. 2003). Therefore, to preserve the functional diversity inherent in the field inoculum while also ensuring plants received nutrients necessary for development, we fertilized at a 50% rate with 60 ml of 20-20-20 (N-P-K, 50 ppm) fertilizer (Peters General Purpose Fertilizer, Allentown, PA) and watered *ad libitum*. During the conditioning and response phases, plants were grown in a greenhouse with supplemental light (GE Multi-Vapor MVR1000/C/U, GE Lighting, Cleveland, OH) under a 15:9 light:dark photoperiod. Mean daytime temperatures were 22 °C (±1.5°) and mean night temperatures were 20 °C (±1.5°).

Volatile organic compound (VOC) collections in the greenhouse

To determine whether soil microbes influence wheat VOCs, volatiles were collected from plants grown in inoculated soil and sterile soil. Because the sampling system allowed us to collect VOCs from six plants each day, each sample consisted of three plants from each soil treatment. We collected VOCs three times a week from mid-June to mid-July 2019, always during stem elongation (Zadok 32–34; Zadoks et al. 1974) for a total of 72 plants sampled. Of these, three plants were dropped due to sampling errors (n_{sterile} = 36, $n_{\text{inoculated}}$ = 33).

To collect wheat VOCs, a push-pull sampling technique was used in which volatiles were collected from the headspace of chambers constructed from Teflon bags (48.26 x 54.61 cm; ClearBags, El Dorado Hills, CA) that were secured at the base of the plants with twist ties. To flush ambient VOCs from the chamber prior to sampling, ambient air—filtered by two solid-phase adsorbent traps containing 30 mg of Super-Q (Alltech Associates, Inc., Deerfield, Illinois)—was delivered into the chamber at 500 mL min^{-1} for 10 minutes. After flushing ambient air from chambers, we continued to deliver filtered air into the chamber at 500 mL min^{-1} and collected sampled air on single solid-phase adsorbent traps at a flow rate of 475mL min^{-1} while still pushing in 500 mL min^{-1} of filtered air. We collected volatiles for 4 h from approximately 10:00 to 14:00 each day.

Collection times were recorded and VOC amounts reported on a per hour basis. After sampling, volatile collection traps were removed, wrapped in aluminum foil, and eluted within 24 hours (see below). In order to standardize VOC emissions by plant biomass, wheat was harvested for fresh weight immediately after VOC collections.

To account for the effects of environmental variables on VOC emissions, Thermochron temperature ibuttons (Maxim Integrated, San Jose, CA) were placed in each chamber and set to measure temperature every minute. Photosynthetically active radiation (PAR), measured every hour, was acquired from the local Montana State University meteorological station. Both temperature data (from the start to end of the sample period) and PAR data (from start of the day until the end of the sampling period) were averaged to yield a cumulative value for each of the variables per sample day. To take into account the light and temperature dependence of these emissions, we used these cumulative values to calculate basal emission VOC rates standardized to 30 °C according to Guenther (1997).

Field study

The field experiment was conducted at the Montana State University Northern Agriculture Research Center located south of Havre, MT (48°29'48.8"N, 109°48'10.4"W) (see above). The Judee variety of winter wheat used in both rotations was selected for its host-plant resistance to WSS due to its solid stem, which impedes larval development, and short stature, which makes it less attractive to ovipositing females (Wallace and McNeal, 1996; Varella et al. 2017). Wheat was monitored for WSS infestation throughout the course of the study. Given that no infestation was found, changes in VOC emissions reported here were not in response to the presence of WSS, and it is assumed they were induced by soil biological and physical characteristics.

Volatile organic compound (VOC) collections in the field

To characterize the effect of diversified cropping systems on VOC emissions, wheat volatiles were collected during four sampling periods over two seasons: summer of 2018 (June 20 and July 4) and summer of 2019 (June 11 and June 25). For each sample period, VOCs were collected from four ~10 cm sections of a row of wheat plants within each plot for a total of 24 samples (n_{cover} = 12, n_{fallow} = 12).

To collect VOCs, we used the same a push-pull sampling technique used in the greenhouse in which volatiles were collected from the headspace of chambers constructed from Teflon bags (48.26 x 54.61 cm; ClearBags, El Dorado Hills, CA) that were secured at the base of the plants with twist ties. After flushing ambient air from chambers, we delivered filtered air into the chamber at a flow rate of 225 mL min^{-1} and collected sampled air on single solid-phase adsorbent traps at a flow rate of 225 min^{-1}. In summer 2019, we increased inlet and outlet flow rates to 500 mL min^{-1} and 475 mL min^{-1}, respectively, to increase sample concentrations. Samples were collected for 2 to 3.5 hours between the hours of 9:00 and 14:00 on mostly sunny days to avoid excessive afternoon heat. Similar to VOC collections in the greenhouse, the temperature of each chamber was collected using ibuttons and PAR was collected using the local NARC meteorological station in order to calculate basal volatile emission rates.

Other factors influencing VOC emissions: Biomass and water availability

During the summer of 2019, we observed smaller wheat plants with greater leaf senescence for plants grown in rotation with cover crops (Fig. S3). Given that volatile production requires substrates from primary metabolism, we quantified wheat plant biomass from both field seasons by harvesting aboveground biomass immediately before wheat harvest in mid-July from quadrats (0.75 x 0.75 m) within each plot. Plants were cut at the soil surface and dried at 50 °C for 48 hours before being weighed.

Cover crops can reduce water reserves in the soil, which can impact subsequent crop growth and yield (Powers, 1990). To determine the impact of cropping system on soil water, volumetric soil water content was measured in 2018 (the season before we measured wheat volatiles), while the field was planted with cover crop or laid fallow. Soil water content was measured four times over the growing season in 2018: May 25 (immediately after cover crop seeding), June 4, June 20, and July 2 (immediately before cover crop termination). At each time period, volumetric soil water content (%) was measured at six depths, 10, 20, 30, 40, 60, and 1000 cm, using a PR2/6 Profile Probe (Delta-T Devices Ltd, Burwell, Cambridge, UK). Soil water content was not measured during the rotation year (fallow or cover crop) in 2017. Thus, we do not have soil water content measurements for the field in which 2018 wheat volatile measurements were collected.

Analysis and identification of VOCs

We determined the identities and relative amounts of VOCs from both greenhouse and field experiments using gas chromatography-mass spectrometry (GC-MS). To do this, volatile traps were eluted with 200 µL of methylene chloride (Fisher Scientific, Fair Lawn, NJ). 10 µL of a 30 ng μL^{-1} solution of *n*-nonyl acetate (Sigma-Aldrich, Saint Louis, MO) in methylene chloride was added as an internal standard following elution and samples were stored at -70 °C until analysis. For chemical analysis, 3 µL of each sample was injected onto an Agilent Technologies 6890 GC-5973 MS fitted with an HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness; J&W Scientific, Folsom, CA). Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1} and injector temperature was set to 250 °C. The oven profile consisted of an initial temperature of 50 °C followed by a ramp of 5 °C min^{-1} to 200 °C, then a second ramp of 25 °C min^{-1} to 275 °C. Volatile compounds were identified by comparing retention times and mass spectra with commercial standards using ChemStation software (Agilent Technologies, Wilmington, DE) and the NIST Mass Spectral Library (National Institute of Standards and Technology, Gaithersburg, MD). All standards were purchased from Sigma Aldrich (Saint Louis, MO) with the exception of decanal and β-ocimene (The Good Scents Company, Oak Creek, WI). We quantified the relative concentrations of each compound by comparing its peak area with that of the internal standard, *n*-nonyl acetate. Concentrations of compounds were standardized for the number of hours for which they were collected and for aboveground plant biomass (ng g^{-1} h^{-1}).

Data analysis

We performed all analyses in R version 3.5.2 (R Core Team, 2018). Raw emission rates were converted to basal emission rates standardized to 30 °C using cumulative temperature and PAR from each VOC collection (Guenther 1997).

For the greenhouse study in which wheat plants were grown in either sterile soil or soil conditioned with inoculations of field soil, we evaluated differences in the VOC community composition in response to soil inoculations using a permutational multivariate ANOVA (Anderson et al. 2014; Oksanen et al. 2019). To do so, we calculated dissimilarities among samples using the Bray-Curtis metric using the ‘vegdist’ function in the ‘vegan’ package (Oksanen et al. 2019). We used non-metric multidimensional scaling (NMDS) through the ‘vegan’ package to visualize VOC composition by plotting the first two axes. Homogeneity of dispersion tests (‘betadisper’) were used to evaluate the differences among treatments in VOC variability followed by ANOVAs to compare the mean distance-to-centroid of VOCs among each treatment (Anderson 2006; Anderson et al 2006). To compare the effects of soil inoculation on VOC emissions for individual compounds, families of compounds (alkanes, aldehydes, terpenes, etc.), and the sum of all measured compounds, hereafter called ‘total’, we fit linear mixed models using the ‘lmer’ function in the ‘lm4’ package (Bates et al. 2015). Measures of individual, family, or total VOCs were the dependent variables and soil treatment was the fixed effect. Sample day was used as a random effect to account for day-to-day variability in other unmeasured environmental factors from each trial. All response variables were either log or square root transformed to meet the assumption of normality and homoscedasticity. To assess if inoculum presence accounted for variation in individual, family, or total VOCs, we performed Type III ANOVA using the ‘anova’ function in the ‘lmerTest’ package (Kuznetsova et al. 2017). To determine the effect of inoculum presence on plant biomass, we fit linear mixed model with soil treatment as the fixed effect and day as the random effect. Finally, to evaluate the relationship between plant biomass and VOC emissions across inoculum presence, we built linear mixed models. We started with models with total VOCs and families of VOCs as the dependent variable, biomass, soil type, and their interaction as the independent variables, and sample day as a random effect. We used stepwise p-value elimination to determine the final model for emissions of total VOCs and families of VOCs.

We applied similar statistical approaches to the analysis of our field study in which VOCs were measured from wheat grown in rotation with either fallow or cover crops. Similar to greenhouse VOCs, we evaluated differences in the VOC community composition among cropping systems using PERMANOVA and visualized VOC composition using NMDS. VOC variability among treatments was evaluated using homogeneity of dispersion tests followed by ANOVAs to compare mean distance-to-the centroid of VOCs among each rotation. To compare the effect of cropping system on VOC emissions in the field, we fit linear mixed models and assessed their significance using the ‘anova’ function for individual VOCs, families of VOCs, and the sum of all measured VOCs (‘total’ VOCs). Measures of individual or total VOCs were the dependent variables and cropping systems was the fixed effect. Sample day was used as a random effect to account for plot-to-plot variability in other unmeasured environmental factors from each sample day. All response variables were either log or square root transformed as needed to meet the assumption of normality and homoskedasticity. For our analysis of field VOCs, we found no significant difference between sample days within a year; therefore, field VOCs were aggregated by year.

Because we observed differences in the size and quality of wheat by crop rotation during the summer of 2019 and to account for the role of soil water content on crop productivity, we modeled the effect of cropping system on biomass and soil water content. Harvest biomass from 0.75 x 0.75 m quadrats was modeled by fitting a linear mixed model using the ‘lmer’ function (Bates et al. 2015) with cropping system and year as the fixed effect and plot as a random effect. To determine the relationship between cropping system and soil water content, percent soil water content was averaged over the entire growing season for each depth and modeled as a function of depth. We used the ‘lmer’ function (Bates et al. 2015), with soil water content as the response variable, cropping system, depth (as a quadratic orthogonal polynomial), and their interaction as the predictor variables and plot as a random effect.

Results

Greenhouse results

A total of 22 volatile compounds were quantified from wheat plants grown in inoculated or sterile soil. Volatile compounds quantified include four monoterpenes, four sesquiterpenes, two alkanes/alkenes, three ketones and esters, one GLV, and six aldehydes, in addition to 2-ethyl-1-hexanol and methyl jasmonate (Table 1).

The relative abundances of compounds differed (Fig. 2; PERMANOVA, $F_{1,67}$ = 8.43, $P < 0.0001$) between plants grown in inoculated and sterile soil. Wheat grown in inoculated soil emitted two-fold more total VOCs than wheat grown in sterile soil (Fig. 3A and Table 1; $F_{1, 55.5}$ = 41.23, $P < 0.0001$) and this trend was observed for all compound families, except terpenes. For example, wheat grown in inoculated soil emitted four times more aldehydes than wheat grown in sterile soil (Fig. 3B; $F_{1,55.6}$ = 128.2, $P <$ 0.0001), but half the amount of terpene compounds (Fig. 3C and Table 1; $F_{1,56.5}$ = 14.0, P = 0.0004).

We observed a correlation between VOC emissions and plant biomass (Fig. 4). Plants grown in inoculated soil were significantly smaller than those grown in sterile soil with a mean biomass of 1.99 g (95% CI from 1.37 to 2.60 g) compared to 6.10 g (95% CI from 5.49 to 6.70 g), respectively ($F_{1,57.4}$ = 250, $P < 0.0001$). We found that total volatile emissions were negatively correlated with plant biomass, independent of soil treatment (Fig. 4A; $F_{1,56.3}$ = 57.2, $P < 0.0001$, $R^2 = 0.88$) and this trend was driven by aldehydes (Fig. 4B; $F_{1, 56.6}$ = 140.3, $P < 0.0001$, $R^2 = 0.88$). Additionally, we found that terpene emission was positively correlated to biomass, but only when plants were grown in inoculated soil (Fig. 4C; $F_{1, 56.3}$ = 5.2, $P = 0.03$, $R^2 = 0.70$).

Field results

A total of 33 volatile compounds were quantified from wheat plants grown in rotation with fallow or cover crops. Plants grown in different cropping systems emitted the same 33 compounds, but in different relative amounts. Volatile compounds quantified include five monoterpenes, two sesquiterpenes, eleven alkanes/alkenes, five ketones, one green leaf volatile (GLV), and nine aldehydes (Table 2).

During summer 2018, we found no difference in total VOC emissions ($F_{1,4}$ = 1.99, $P = 0.23$) or the overall blends emitted by wheat among cropping system, as estimated by PERMANOVA ($F_{1,22} = 1.57$, $P = 0.1$). However, during summer 2019, we found differences in VOC emissions between cropping systems with all plants emitting the same 33 compounds, but in different relative amounts (Fig. 5; PERMANOVA, $F_{1,22} = 9.62$, $P < 0.001$, $R^2 = 0.30$). We also found that the VOC composition emitted by wheat plants grown in rotation with cover crops was more variable than the composition emitted by wheat following fallow (Fig. 5; $F_{1,22} = 14.87$, $P < 0.001$). There was no difference in total volatile emissions by cropping system (Fig. 6A; $F_{1,4} = 2.91$, $P = 0.2$ however, the emissions of total aldehydes (Fig. 6B; $F_{1,4} = 19.98$, $P = 0.01$) and total terpene compounds (Fig. 6C; $F_{1,4} = 4.66$, $P = 0.04$) did vary among cropping system. Wheat grown in rotation with cover crops emitted four-fold more aldehydes but nearly half the amount of terpenes than wheat grown in rotation with fallow (Table 2). Additionally, wheat grown in rotation with fallow released two-fold more *Z*-3-hexenyl acetate and six-fold more β-ocimene, both known attractants for WSS adults (Fig. 7; Table 2).

In summer 2018, when the field was planted in either cover crops or fallow, we found that soil water content varied with the interaction of rotation and depth (Fig. S4; $F_{2,133.0} = 30.43$, $P < 0.001$). Soil water content did not vary between cropping systems at 10, 20, 40, and 100 cm, but at 30 and 60 cm, soil water content was nearly two-fold (+9.26%, SEM = 4.24% df = 22, t = 2.19, $P = 0.040$) and four-fold (+18.70%, SEM = 5.27% df =

22, t = 3.55, *P* = 0.024) greater in fallow rotations when compared to cover crop rotations. In 2019, we observed that wheat plants were generally smaller in the cover crop rotations (Fig. S3). Mean wheat biomass, sampled immediately before harvest from 0.75 x 0.75 m quadrats, was found to be 50% less in cover crop rotations with a mean biomass of 149 g (95% CI from 50 to 248 g) compared to 288 g (95% CI from 189 to 387 g) for wheat grown in rotation with fallow.

Discussion

Understanding how agricultural soils and cropping systems influence crop VOCs through plant-soil feedbacks will inform the potential of diversified systems to enhance the pest resistance of crops (Kaplan et al. 2018; Pineda et al. 2010). We hypothesized that 1) in the greenhouse, the presence of soil microbes would alter the emission of plant VOCs due to plant-microbe interactions that were not experienced by the plants grown in sterile soil and 2) in the field, cropping systems would differentially modify VOC blends of wheat, including shifts in key volatile compounds known to be important for the WSS. In greenhouse conditions, volatile phenotypes of wheat varied when plants were grown with and without soil microbes from agricultural fields, suggesting that agricultural soil microbes do have the potential to modify emissions of crops. Further, in field conditions wheat grown in rotation with cover crops emitted altered VOC blends compared to wheat grown in rotation with fallow with plants growing in cover crop rotations emitting lower amounts of compounds known to attract adult female WSS (Buteler and Weaver 2012; Weaver et al. 2009). In both the greenhouse and the field study, we found strong correlations between treatment and plant biomass, suggesting that wheat grown in inoculated soil (in the greenhouse) and in cover crop rotations (in the field) may have experienced soil-related stressors that contributed to the VOC trends we observed (Giron-Calva et al. 2017; Huang et al. 2013; Timmusk et al. 2014; Wildt et al. 2003), illustrating the complex interaction of microbial composition and soil properties in driving plant-soil interactions (Wang et al. 2018).

In the field, we found that wheat grown in both rotations emitted the same 33 compounds, however, the variability of the relative compound abundances was greater in plants grown in rotation with cover crops with higher associated microbe diversity (Dupre et al. in prep; Ouverson et al. in prep). Though the biological significance of variability in VOC abundance is poorly understood (Wetzel and Whitehead 2020), plants producing more variable blends have a higher probability of shifting emissions of key bioactive compounds that may enhance fitness (Jones et al. 1991). To this end, we found that wheat grown in rotation with cover crops emitted VOC blends with lower amounts of compounds known to be attractive to ovipositing female WSS such as *Z*-3-hexenyl acetate and β-ocimene. Wheat cultivars emitting lower amounts of *Z*-3-hexenyl acetate are less attractive to ovipositing WSS females, receiving fewer eggs and incurring less damage (Buteler and Weaver 2012; Weaver et al. 2009). Thus, our results suggest that wheat grown in rotation with cover crops emitting lower amounts of these compounds may be less apparent to ovipositing females and could experience reduced WSS pressure.

Wheat grown in inoculated soil and in rotation with cover crops emitted higher amounts of saturated aldehydes. Though wheat is known to release C6 aldehydes

(Hamilton-Kemp et al. 1987), which are a type of GLV, the only GLV we observed was *Z*-3-hexenyl acetate. Instead, we found that aldehyde emissions consisted primarily of aliphatic saturated aldehydes with eight or more carbons. Interestingly, many of the saturated aldehydes we observed, including nonanal, decanal, dodecanal, tridecanal, and tetradecanal, have been identified in volatile blends collected from adult WSS (Bartelt et al. 2002). Coupled gas chromatography-electroantennographic detection (GC-EAD) revealed that these compounds stimulated the antennae of male and female adults. In follow up insect bioassays, however, none of these compounds could account for adult recognition individually (Cossé et al. 2002), highlighting the sophisticated mechanisms used by insects to interpret chemical cues. While saturated aldehydes showing EAD-activity may not individually influence adult WSS behavior, they may be important components of volatile blends (e.g. synergists) that collectively elicit behavior (Bruce et al. 2005). More work is necessary to elucidate how saturated aldehyde compounds influence adult behavior in the context of the more complex chemical environment.

In both greenhouse and field experiments, soil inoculum treatments and cropping management practice differentially influence VOC emissions of plants. While previous work on soil legacy effects and microbe-induced plant volatiles suggests the role of soil microbes on the plant metabolome (Huberty et al. 2020a; Pineda et al. 2020; Pineda et al. 2010; Shikano et al. 2017), it is unlikely that the changes we detected in volatile emissions were due to soil microbes alone. One line of support for this includes the drastic relationship we observed between aldehyde emissions and plant biomass among treatments. High emissions of saturated aldehydes from plants is commonly reported in response to physiological stress (Wildt et al. 2003; Giron-Calva et al. 2017), as is low plant biomass (Timmusk et al. 2014). In the greenhouse study, we found that wheat grown in inoculated soil produced three-fold less biomass than wheat grown in sterile soil. Similarly, during the 2019 field season, wheat grown in cover crop rotations appeared less healthy and produced nearly half the wheat biomass as wheat grown in rotation with fallow. Though management-driven soil microbial effects likely played a role in the VOC emissions we observed, the large difference in plant biomass among treatments suggests that some plants may have experienced soil-related stressors that contributed to the trends observed in our study.

We posit that in the greenhouse, plants grown in inoculated soil experienced stress due to repeated planting of the same species during the two condition phases and response phase, causing negative plant-soil feedbacks. Plant-soil feedbacks occur when plants alter the soil biota, which subsequently modifies plant growth in positive or negative ways (Mariotte et al. 2018; Putten et al. 2013). Negative plant-soil feedbacks are caused by repeated planting of the same species and subsequent accumulation of autotoxins and species-specific pathogens in the soil, which have negative impacts on crop growth and productivity (Huang et al. 2013; Wang et al. 2018). In wheat, repeated planting can cause the build-up of autotoxins including ferulic and *p*-coumaric acids, which upon accumulation in the soil can be oxidized by pathogenic soil microbes to form additional autotoxins such as vanillic acid and 4-hydroxybenzoic acid (Lodhi et al. 1987; Turner and Rice 1975). In this way, autotoxins and soil-borne pathogens can exert synergistic negative plant-soil feedbacks crops (Lodhi et al. 1987; Ye et al. 2006), with visible symptoms such as reduced growth (Huang et al. 2013). We observed a three-fold decrease in biomass among wheat grown in inoculated soil. Such distinct variability between treatments provides evidence that plants grown in inoculated soil experienced negative plant-soil feedback-stress not experienced by plants grown in sterile soil.

We posit that in the field, wheat grown in rotation with cover crops experienced some degree of drought stress. In semi-arid agricultural systems, water limitation is the primary constraint to crop productivity, and thus, cash crops are traditionally grown in rotation with fallow to preserve soil moisture (Padbury et al. 2002). Nonetheless, the adoption of cover crop rotations across the NGP has increased in recent years due to improved management techniques that curtail soil water usage, including the identification of optimum termination dates for cover crops (Miller et al. 2011; O’Dea et al. 2013). However, growing climate variability makes it increasingly difficult to identify the optimum termination date, raising the risk that cover crops will diminish soil water stores for the following cash crop (Alonso-Ayuso et al. 2018). Indeed, in 2018, the summer before we sampled wheat VOCs, soil water content was lower in cover crop rotations than fallow, suggesting that cover crops grown in 2018 reduced soil water stores, leading to the observed differences in wheat biomass and yield in 2019 (Dupre et al. in prep).

All of the stressors described above – water limitation and accumulation of autotoxins and pathogenic microbes – have profound effects not only on growth and plant primary metabolism, but also on secondary metabolism, including the emission of VOCs. Across greenhouse and field studies, we observed that plants with less biomass emitted more total VOCs. Plant VOC emissions tend to be strongly correlated with stress severity (Timmusk et al. 2014), and this has been observed in response to a range of biotic and abiotic stressors including pathogens, herbivores, allelochemicals, nutrient availability, and drought (Beauchamp et al. 2005; Gouinguené and Turlings 2002; Lerdau and Coley 2002; Trowbridge et al. 2014). It has been estimated that the emission of VOCs by terrestrial plants is typically 1-2% of whole photosynthesis, but this percentage may increase to 10%, or even 50%, under stress conditions (Kesselmeier et al. 2002), diverting significant amounts of photosynthetic substrates away from growth. Consistent with our observations, Timmusk et al. (2014) observed that wheat grown under water limitation had elevated emissions of VOCs and less growth compared to control conditions.

Trends in total VOC emission were driven predominantly by aldehyde compounds which made up 59% and 34%of total volatiles emissions of wheat grown in inoculated soil and cover crop rotation, respectively. Contrary to the C6 aldehyde species commonly reported in wheat emissions (Hamilton-Kemp et al. 1987), we observed a large amount of C9-C14 aliphatic saturated aldehydes including nonanal, octanal, and decanal. While C6 aldehydes are products of the lipoxygenase/hydroperoxide lyase (LOX/HPL) pathway (Matsui 2006), saturated aldehydes are more commonly understood to be natural oxidation products of lipid peroxides (Shahidi 2001). Lipid peroxides form when membranes are damaged through accumulation of reactive oxygen species (ROS), which occurs concomitantly with biotic and abiotic stressors including drought and exposure to pathogens and autotoxins (Selote et al. 2004; Shi et al. 2017). Indeed, greater aldehyde emission has been observed in response to pathogen attack, mechanical wounding, herbivory, and ozone stress (Wildt et al. 2003; Giron-Calva et al. 2017).

Contrary to aldehyde emission, which was negatively correlated with plant biomass, terpene emission was positively correlated with plant biomass. We observed lower biomass and lower terpene emissions when wheat was grown in inoculated soil and in rotation with cover crops. Thus, both of these treatments likely experienced higher levels of stress compared to their counterpart treatment. Plants experiencing stress-induced lipid peroxidation not only have reduced photosynthetic activity due to chlorophyll membrane failure (Apel and Hirt 2004; Kanagendran et al. 2018; Prochazkova et al. 2001), but also necessitate ongoing maintenance respiration, compounding the constraint of carbon for other primary and secondary metabolic needs such as growth and synthesis of chemical defense compounds. While terpenes display a wide range of biological activities, including important roles in plant defense, they are costly molecules to synthesize because they require significant reduction of carbons (Gershenzon 1994). Thus, it is possible that lower carbon availability limited terpene production in inoculated and cover crop treatments. Given the role of terpenes in plant defense, wheat grown in cover crop rotations with lower emissions of terpenes may exhibit reduced resistance to pathogen- and herbivore-attack in some cases.

Our work aimed to elucidate the role of soil microbes and cropping system on soil legacy effects on plant VOCs. Multiple soil properties likely influenced the VOC dynamics we observed, highlighting the complex nature of teasing apart the role of different soil characteristics and how they interact to influence soil legacy effects

(Bennett and Klironomos 2019). Plant-microbe associations in the rhizosphere exist on a spectrum from beneficial to parasitic, and their nature can change depending on environmental conditions and stressors (Bennett and Klironomos 2019; Fry et al. 2018; Ulrich et al. 2019). For agricultural crops that receive ample resources, rhizosphere associations may lean toward parasitic and form less frequently (Bennett and Klironomos 2019; Morgan et al. 2005). Alternatively, for crops experiencing stress, rhizosphere associations may become beneficial, enhancing plant functional traits to deal with the stressor. As such, an amalgam of biotic and abiotic factors will influence the ability of soil legacy effects to influence crop resistance, not just the soil microbiome. While cover crops and termination practices may promote unique soil microbe communities with different potential functionalities, it is their interaction with other environmental factors that will ultimately shape the way soil microbiomes influence plant VOCs. There is a need to continue to understand how plant-soil feedbacks will be shaped by the abiotic and biotic soil properties, and their interactions.

The patterns we report here regarding the influence of soil legacy effects on VOC emissions lay a solid foundation for future work aimed at exploring WSS management through the use of semiochemicals. Results enhance our understanding how cropping systems alter soils properties, and in turn, VOC emissions and insect behavior. Our data point towards a need to untangle the relative importance of soil microbes on VOCs when considering the interactive effects with other factors influenced by management. We also observed interesting trends in terms of saturated aldehyde compounds being emitted in response to our treatments. The emissions of saturated aldehydes have not been well described and the ecological impacts are virtually unknown. Our research highlights the need to study the potential biological activity of these compounds and their potential roles in mediating inter- and intraspecific interactions.

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Fig. 1

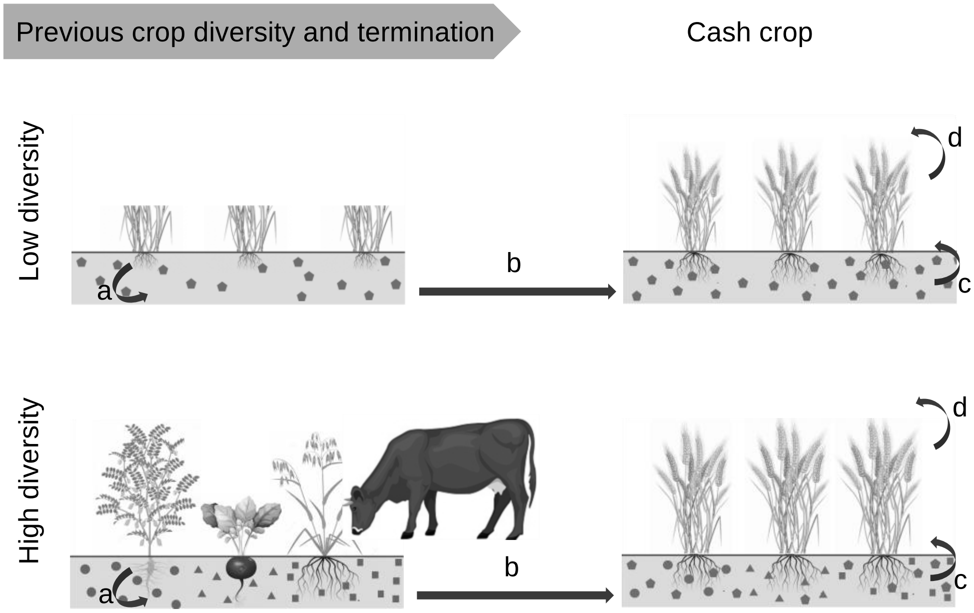

Figure 1. Experimental framework and overarching hypothesis. Cropping management practices have the potential to modify crop-pest interactions through soil legacy effects. a) High diversity systems influence soil microbe communities differentially than low diversity systems through plant-specific root exudates and manure. b) Soil microbe communities persist into the next growing season where they c) interact with subsequent crops, potentially modifying volatile organic compounds (VOC) emissions. d) Altered VOCs may differentially attract pests and their natural enemies, conferring different levels of pest resistance to crops.

Fig. 2

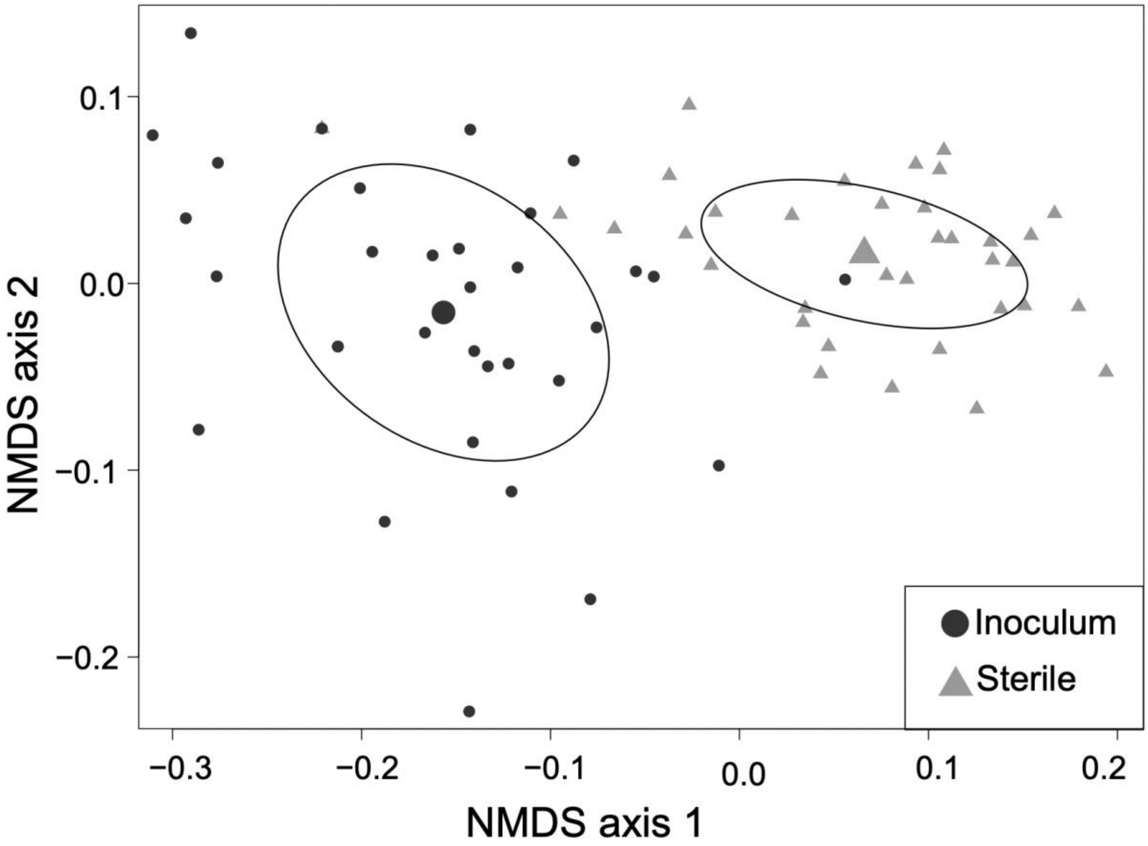

Figure 2. Nonmetric multidimensional scaling (NMDS) of soil inoculum effect on the composition of volatile organic compounds emissions by wheat grown with a field-collected soil inoculum or in sterile soil. Black dots represent plants grown in inoculated soil and gray dots represent plants grown in sterile soil. Ellipses encircle the relative volatile composition of all the plant individuals from the same soil inoculation.

Fig. 3

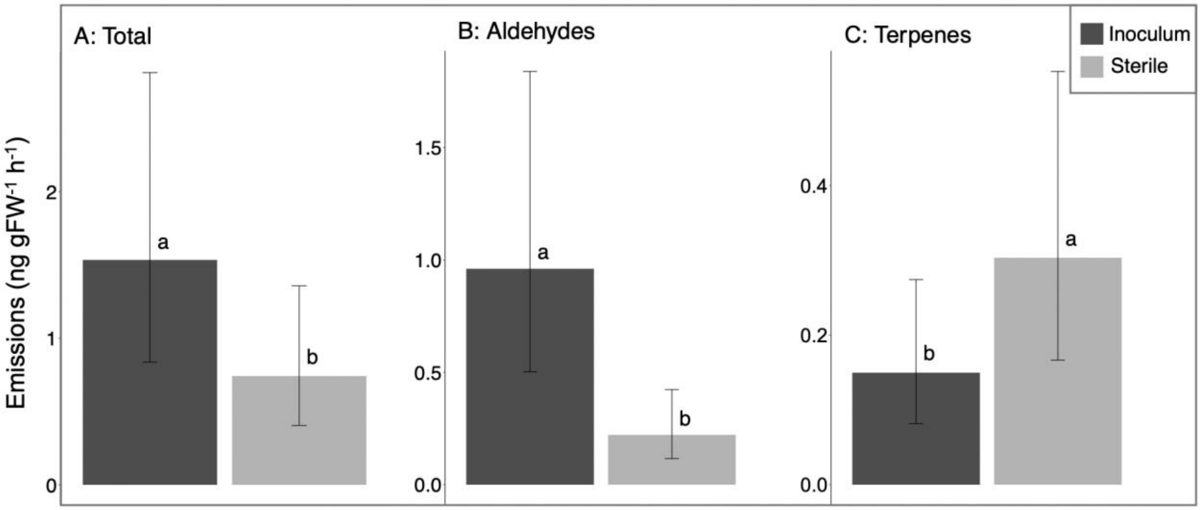

Figure 3. Total volatile organic compound (VOC) emissions (panel A), emissions of aldehydes (panel B), and emissions of terpenes (panel C)) as a function of soil inoculation. Means ± SEM are shown and significance between treatments is expressed using different lower-case letters (α <0.05).

Fig. 4

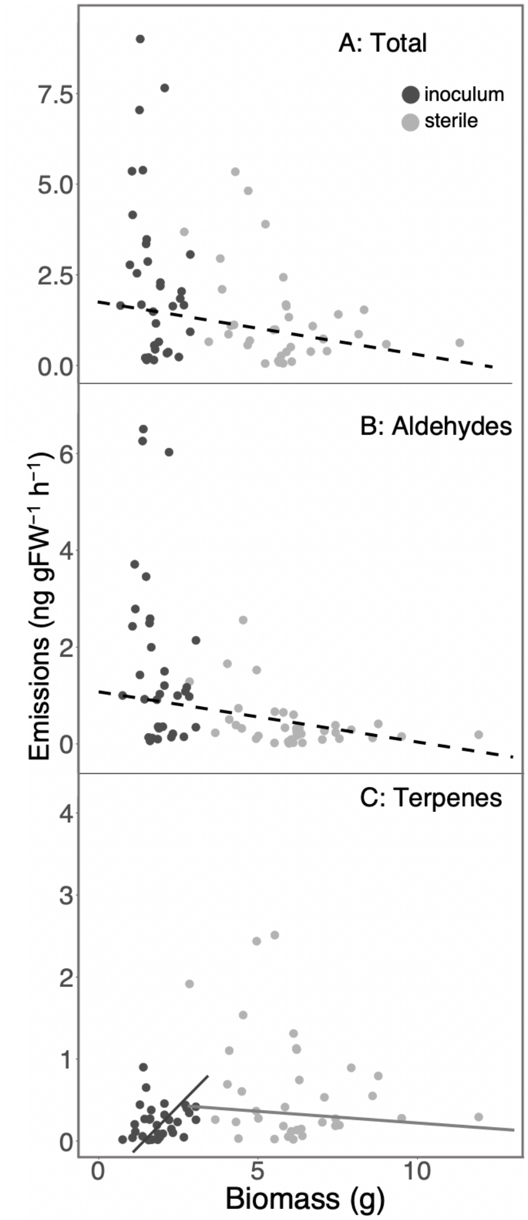

Figure 4. Correlations between emissions of total volatiles organic compounds (panel A; $R^2 = 0.88$) and aldehydes (panel B; $R^2 = 0.88$) as a function of biomass. Dashed black lines represent the best fit linear model for the data. Terpene emissions were correlated with biomass when wheat was grown in inoculated soil ($R^2 = 0.70$; solid black line), but there was no relationship when wheat was grown in sterile soil (solid gray line; panel C). Black dots represent plants grown in inoculated soil and gray dots represent plants grown in sterile soil.

Fig. 5

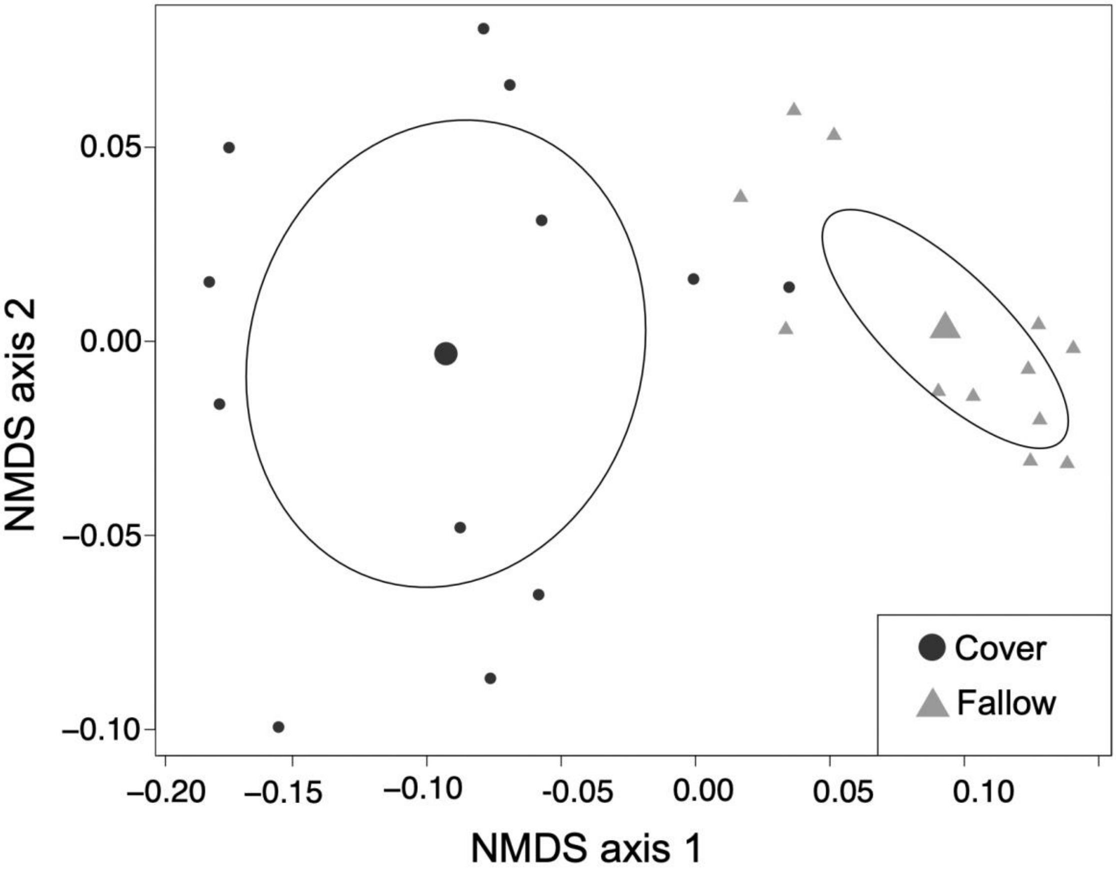

Figure 5. Nonmetric multidimensional scaling (NMDS) of cropping system effect on composition of volatile organic compounds emissions by wheat averaged over two sampling periods during the summer of 2019. Black dots represent wheat grown in rotation with cover crops and gray dots those grown in rotation with fallow. Ellipses encircle the relative volatile composition of all the plant individuals from the same cropping system.

Fig. 6

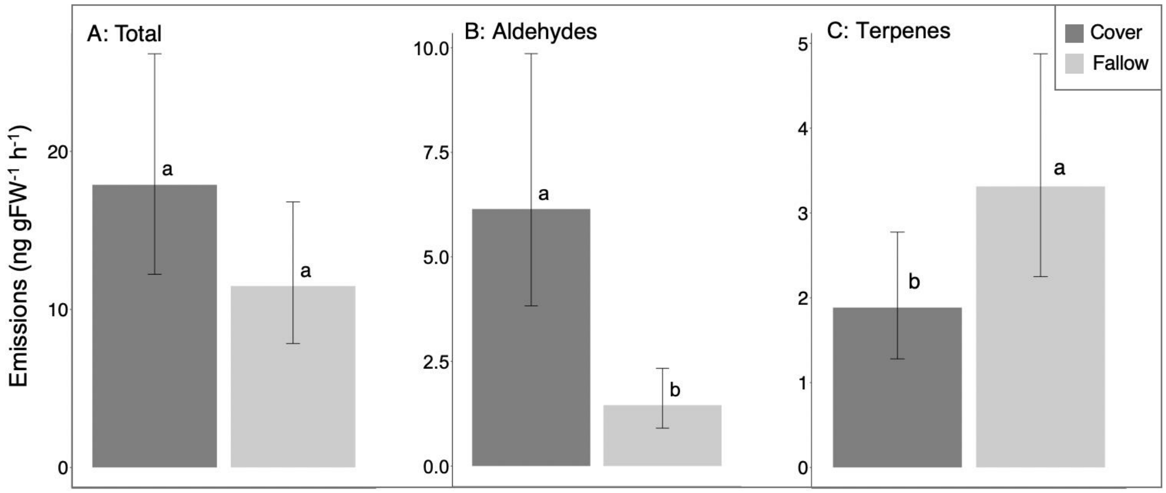

Figure 6. Total volatile organic compound (VOC) emissions (panel A), emissions of aldehydes (panel B), and emissions of terpenes (panel C) as a function of cropping system. Means ± SEM are shown and significance between treatments is expressed using different lower-case letters (α <0.05).

Fig. 7

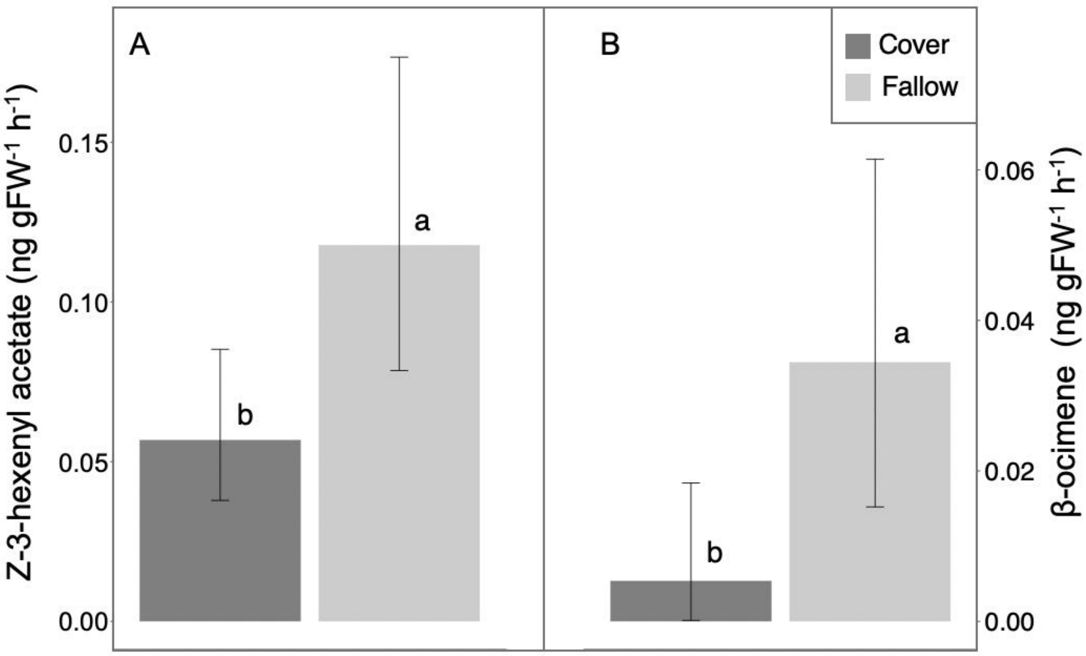

Figure 7. Emissions of Z-3-hexenyl acetate (panel A) and emissions β-ocimene (panel B) as a function of cropping system. Means ± SEM are shown and significance between treatments is expressed using different lower-case letters ($\alpha < 0.1$).

Tables

Table 1. Mean emission rates (ng nonyl acetate equivalents gFW^{-1} h^{-1}) of total compounds, families of compounds, and individual compounds of wheat plant grown with a field collected soil inoculum or in sterile soils.

Compounds	**ID^1**	**ANOVA**		**Mean emission rates (lower, upper 95% CI)**	
		F-value	**P-value**	**Inoculum**	**Sterile**
Total volatiles		41.2	< 0.0001*	1.54 (0.84, 2.82)	0.75 (0.41, 1.32)
All terpenes		14.0	0.0004*	0.16 (0.09, 0.28)	0.31 (0.17, 0.56)
Monoterpenes		14.0	0.0004*	0.14 (0.07, 0.25)	0.31 (0.19, 0.46)
α-pinene	SS	23.2	< 0.0001*	0.001 (3.9e-5, 0.004)	0.01 (0.01, 0.02)
β-ocimene	SS	15.8	0.0002*	0.09 (0.04, 0.16)	0.22 (0.14, 0.33)
Linalool	SS	8.5	0.005*	0.03 (0.01, 0.06)	0.06 (0.03, 0.09)
α-terpineol	NIST$_{>50}$	1.2	0.277	0.01 (0.006, 0.02)	0.01 (0.008, 0.02)
Sesquiterpenes		18.1	< 0.0001*	0.03 (0.02, 0.06)	0.08 (0.04, 0.15)
Caryophyllene		12.6	0.0007*	0.008 (0.002, 0.02)	0.04 (0.02, 0.05)
β-farnesene	NIST$_{>50}$	32.4	< 0.0001*	0.0003 (0.0001, 0.002)	0.015 (0.001, 0.02)
Unknown sesquiterpene 1	NIST	21.8	< 0.0001*	0.008 (0.002, 0.2)	0.04 (0.02, 0.06)
Unknown sesquiterpene 2	NIST	0.2	0.635	0.01 (0.006, 0;03)	0.02 (0.01, 0.03)
Alkanes and alkenes		72.0	< 0.0001*	0.03 (0.01, 0.06)	0.01 (0.007, 0.02)
Tetradecane	NIST	60.8	< 0.0001*	0.01 (0.005, 0.02)	0.004 (0.002, 0.008)
Hexadecane	NIST	62.9	< 0.0001*	0.02 (0.01, 0.04)	0.01 (0.005, 0.02)
Ketones and esters		32.4	< 0.0001*	0.08 (0.04, 0.15)	0.03 (0.01, 0.06)
6-methyl-5-heptene-2-one	SS	13.3	0.001*	0.08 (0.04, 0.12)	0.03 (0.01, 0.06)
2-tridecanone	NIST$_{>50}$	27.2	< 0.0001*	0.01 (0.006, 0.02)	0.006 (0.003, 0.01)
Hexahydrofarnesyl acetone	SS	4.7	0.034*	0.006 (0.002, 0.01)	0.004 (0.001, 0.008)

Aldehydes		128.2	< 0.0001*	0.96 (0.50, 1.84)	0.22 (0.12, 0.42)
Octanal	SS	148.5	< 0.0001*	0.09 (0.05, 0.17)	0.02 (0.01, 0.04)
Nonanal	SS	150.7	< 0.0001*	0.26 (0.14, 0.50)	0.06 (0.03, 0.12)
Decanal	SS	108.4	< 0.0001*	0.49 (0.25, 0.94)	0.11 (0.06, 0.21)
Undecanal	NIST$_{>50}$	132.5	< 0.0001*	0.05 (0.02, 0.09)	0.01 (0.005, 0.02)
2-undecenal	NIST	64.6	< 0.0001*	0.02 (0.01, 0.03)	0.006 (0.003, 0.01)
Dodecanal	NIST	141.9	< 0.0001*	0.04 (0.02, 0.08)	0.01 (0.005, 0.02)
Green leaf volatiles (GLV)		0.03	0.856	0.08 (0.04, 0.13)	0.08 (0.05, 0.13)
Z-3-hexenyl acetate	SS	0.03	0.856	0.08 (0.04, 0.13)	0.08 (0.05, 0.13)
Other		29.4	< 0.0001*	0.10 (0.05, 0.20)	0.04 (0.02, 0.08)
2-ethyl-1-hexanol	SS	12.0	0.001*	0.06 (0.03, 0.08)	0.03 (0.01, 0.05)
Methyl jasmonate	SS	88.0	< 0.0001*	0.05 (0.01, 0.11)	0.01 (0.005, 0.04)

*Denotes differences between treatments ($\alpha < 0.05$)

[1] Identification (ID) of compounds based upon comparison of retention time and mass spectra with synthetic standards (SS) or comparison of mass spectra using NIST Mass Spectral Search Program (NIST); NIST$_{>50}$ = Denotes NIST match probability greater than 50%

Table 2. Mean emission rates (ng nonyl acetate equivalents gFW-1 h-1) of total compounds, families of compounds, and individual compounds in response to cropping system.

Compounds	**ID[1]**	**ANOVA**		**Mean emission rates (lower, upper 95% CI)**	
		F-value	**P-value**	**Cover**	**Fallow**
Total volatiles		2.9	0.163	17.88 (12.22, 26.18)	11.47 (7.84, 16.80)
All terpenes		4.6	0.044*	1.88 1.28, 2.77)	3.31 (2.25, 4.88)
Monoterpenes		4.7	0.041*	0.34 (0.25, 0.40)	0.54 (0.40, 0.73)
α-pinene	SS	8.0	0.047*	0.05 (0.04, 0.08	0.02 (0.015, 0.04)
β-pinene	SS	43.3	0.003*	0.004 (0.0002, 0.01)	0.08 (0.06, 0.11)
Limonene	SS	1.6	0.223	0.03 (0.02, 0.06)	0.06 (0.03, 0.11)
β-ocimene	SS	7.0	0.057	0.005 (0.0001, 0.018)	0.03 (0.02, 0.06)
Linalool	SS	6.3	0.020*	0.19 (0.14, 0.26)	0.33 (0.24, 0.45)
Sesquiterpenes		4.5	0.046*	1.5 (0.99, 2.28)	2.75 (1.81, 4.18)
Unknown sesquiterpene	NIST	0.5	0.473	1.43 (0.95, 2.15)	1.74 (1.16, 2.62)
β-farnesene	NIST$_{>50}$	16.3	0.016*	0.07 (0.0004, 0.29)	1.05 (0.56, 1.70)
Alkanes and alkenes		0.3	0.612	2.95 (2.218, 4.00)	3.28 (2.42, 4.45)
Decane	SS	0.1	0.750	0.03 (0.01, 0.05)	0.02 (0.01, 0.04)
Cyclohexane	NIST	8.7	0.007*	0.19 (0.13, 0.27)	0.40 (0.28, 0.58)
3-methylundecane	NIST$_{>50}$	5.2	0.084	0.16 (0.11, 0.25)	0.31 (0.21, 0.47)
Dodecene	NIST	4.0	0.058*	0.13 (0.09, 0.17)	0.08 (0.06, 0.11)
Dodecane	NIST	0.3	0.593	0.16 (0.12, 0.23)	0.15 (0.11, 0.20)
Trimethyltetradecane	NIST	0.3	0.641	0.06 (0.03, 0.10)	0.07 (0.04, 0.12)
Tridecane	SS	0.2	0.665	0.37 (0.26, 0.52)	0.34 (0.24, 0.47)
1-tridecene	NIST	0.01	0.921	0.02 (0.001, 0.06)	0.02 (0.001, 0.07)
3-methyltridecane	NIST	1.9	0.187	0.87	1.18

				(0.64, 1.20)	(0.86, 1.62)
Tetradecane	NIST	0.7	0.401	0.24 (0.18, 0.33)	0.20 (0.15, 0.28)
Pentadecane	NIST	1.3	0.320	0.63 (0.45, 0.89)	0.48 (0.34, 0.68)
Ketones and esters		2.3	0.201	5.66 (2.94, 10.90)	2.85 (1.48, 5.50)
6-methyl-5-heptene-2-one	SS	14.9	0.018*	0.24 (0.21, 0.53)	0.10 (0.06, 0.16)
2-undecanone	$\text{NIST}_{>50}$	7.2	0.055	0.24 (0.17, 0.65)	0.10 (0.05, 0.19)
2-tridecanone	$\text{NIST}_{>50}$	1.4	0.306	3.53 (1.78, 7.01)	2.04 (1.03, 4.05)
2-pentadecanone	$\text{NIST}_{>50}$	2.1	0.220	0.96 (0.50, 1.84)	0.50 (0.26, 0.96)
Hexahydrofarnesyl acetone	SS	5.1	0.088	0.29 (0.13, 0.64)	0.08 (0.04, 0.19)
Green leaf volatiles (GLV)		7.0	0.015*	0.06 (0.04, 0.09)	0.12 (0.08, 0.18)
Z-3-hexenyl acetate	SS	7.0	0.015*	0.06 (0.04, 0.09)	0.12 (0.08, 0.18)
Aldehydes		20.0	0.011	6.14 (3.83, 9.86)	1.45 (0.91, 2.33)
Benzaldehyde	SS	14.0	0.020*	0.19 (0.11, 0.34)	0.04 (0.02, 0.08)
Octanal	SS	26.9	0.007*	0.48 (0.30, 0.75)	0.10 (0.06, 0.15)
Nonanal	SS	33.4	0.048*	1.64 (1.09, 2.47)	0.32 (0.22, 0.49)
Ethylbenzaldehyde	SS	1.1	0.352	0.17 (0.01, 0.52)	0.04 (0.01, 0.25)
Decanal	SS	20.2	0.011*	1.62 (0.94, 2.80)	0.30 (0.18, 0.52)
p-acetylethylbenzene	NIST	20.8	0.010*	0.99 (0.60, 1.62)	0.21 (0.13, 0.35)
Undecanal	$\text{NIST}_{>50}$	22.0	0.009*	0.26 (0.16, 0.41)	0.06 (0.04, 0.09)
Tridecanal	NIST	5.3	0.084	0.36 (0.25, 0.52)	0.20 (0.14, 0.29)
Tetradecanal	NIST	1.4	0.308	0.14 (0.08, 0.23)	0.09 (0.06, 0.15)

*Denotes significant differences between treatments ($\alpha < 0.05$)

[1] Identification (ID) of compounds based upon comparison of retention time and mass spectra with synthetic standards (SS) or comparison of mass spectra using NIST Mass Spectral Search Program (NIST); $\text{NIST}_{>50}$ = Denotes NIST match probability greater than 50%

Supplemental Figures

Fig. S1

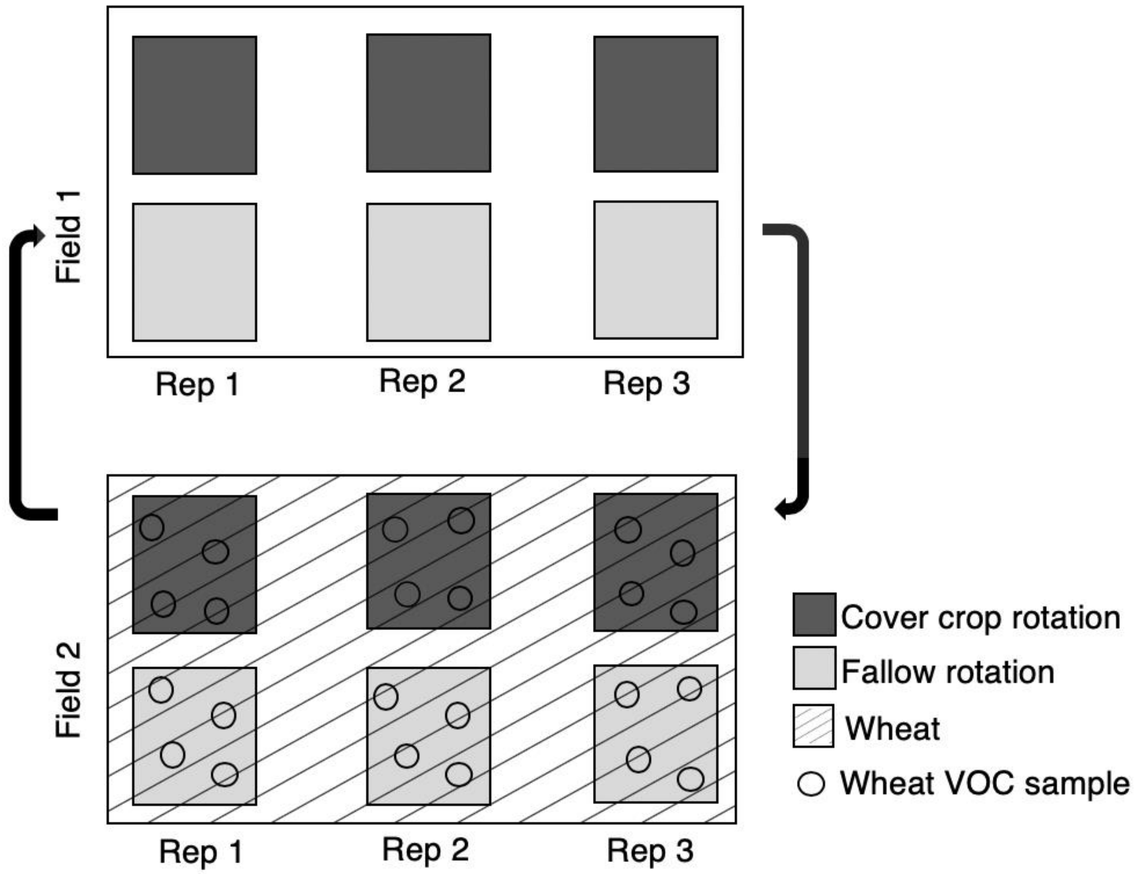

Figure S1. Sample design to determine whether diversified cropping systems influence wheat volatile organic compounds (VOC) emissions soil legacy effects. Our field study focused on two cropping systems representing low and high diversity systems: wheat rotated with fallow (yellow) and wheat rotated with a 7-species mixture of cover crop which was terminated by grazing cows (green). Plots were approximately 8 x 14 m. VOC emissions were collected over four sampling periods during the summer of 2018 and 2019. For each collection period, VOCs were collected from twenty-four 10 cm-sections of wheat (n=24). Of the 96 samples collected over two summers, four were dropped from the analysis due to sampling errors.

Fig. S2

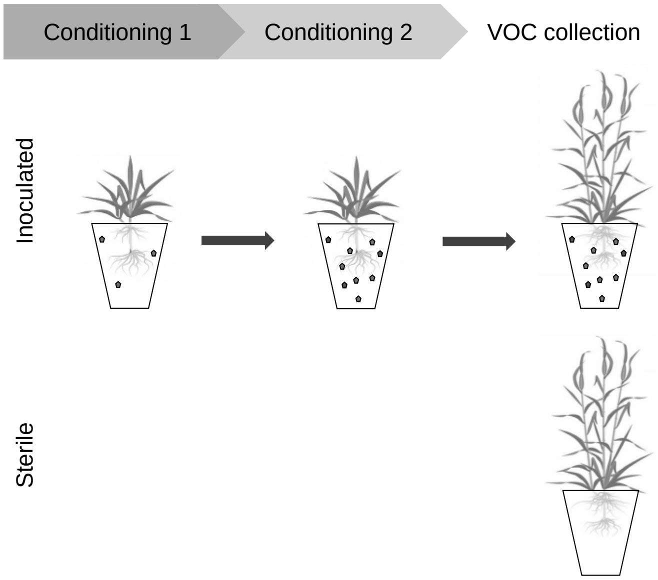

Figure S2. Sample design to isolate the effect of soil microbial communities on volatile organic compound (VOC) emissions. To establish microbial communities from the field while minimizing the effect of other soil properties, aliquots of field soil (collected from cover crop rotations) were added to sterilized greenhouse soil. Soil was conditioned by growing wheat for two four-week periods with each conditioning phase followed by aboveground harvest of plant biomass and a week of fallow. For the response phase in which VOCs emissions were measured, inoculated pots and additional pots containing 100% sterilize greenhouse soil were planted with wheat and grown to stem elongation, at which point they were sampled for emissions of VOCs.

Fig. S3

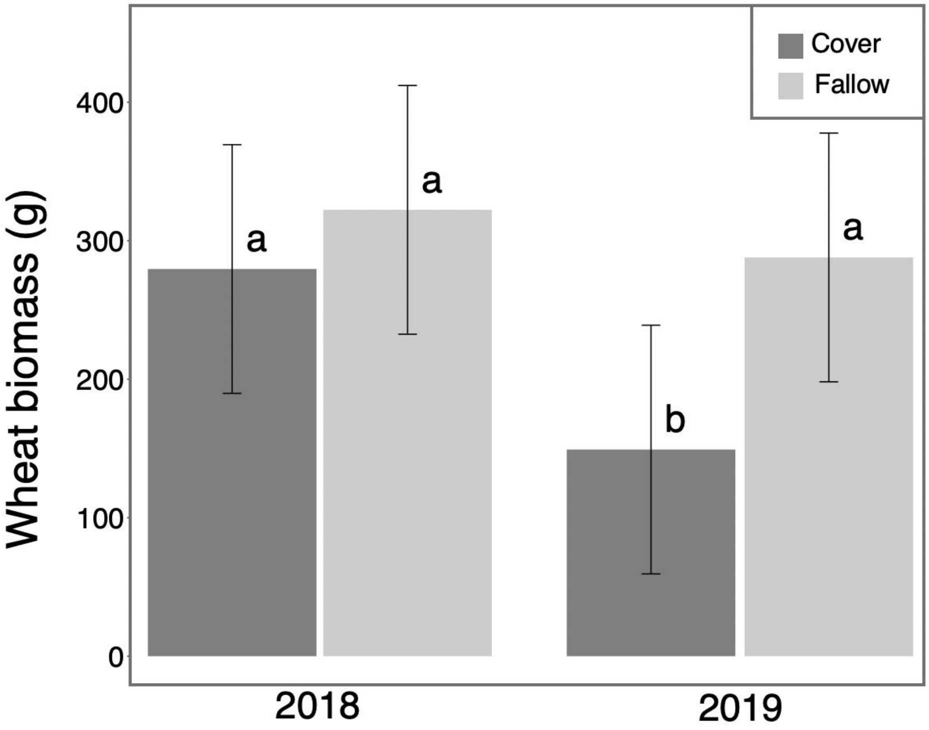

Figure S3. Mean wheat aboveground biomass (sampled immediately before harvest from 0.75 x 0.75 m quadrats) in response to cropping system. Means ± SEM are shown and significance between treatments is expressed using different lower-case letters ($\alpha < 0.05$).

Fig. S4

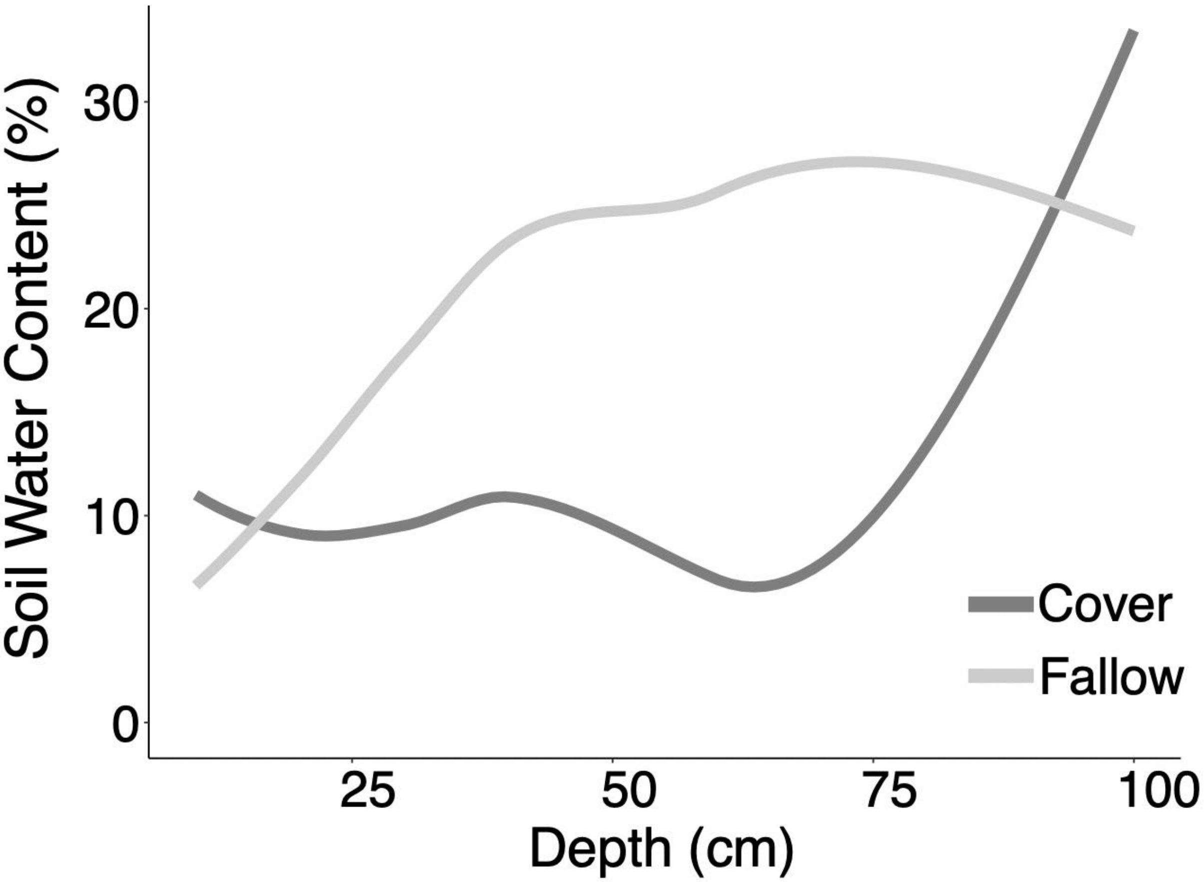

Figure S4. Soil water content (%) in cover crop (dark gray) and fallow (light gray) rotations averaged over four sample days (May 2018-July 2018) as a function of soil depth (cm). Lines represent the best fit polynomial model for the data.

CHAPTER THREE

SOIL MICROBES ALTER HERBIVORE-INDUCED VOLATILE EMISSIONS IN RESPONSE TO CEREAL CROPPING SYSTEMS

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

Author: Shealyn C. Malone
Contributions: Conducted the experiments; analyzed the data; drafted the initial manuscript; revised, read and approved the final manuscript

Co-Author: David K. Weaver
Contributions: Conceived the project; revised, read, and approved the final manuscript

Co-Author: Tim F. Seipel
Contributions: Conceived the project; assisted with data analysis; revised, read, and approved the final manuscript.

Co-Author: Fabian D. Menalled
Contributions: Conceived the project; revised, read, and approved the final manuscript

Co-Author: Megan L. Hofland
Contributions: Assisted with experiments; revised, read, and approved the final manuscript

Co-Author: Justin B. Runyon
Contributions: Revised, read, and approved the final manuscript

Co-Author: Amy M. Trowbridge
Contributions: Conceived the project; revised, read, and approved the final manuscript

Manuscript Information

Shealyn C. Malone, David K. Weaver, Tim F. Seipel, Fabian D. Menalled, Megan L. Hofland, Justin B. Runyon, Amy M. Trowbridge

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Soil microbes alter herbivore-induced volatile emissions in response to cereal cropping systems

Shealyn C. Malone[1,4], David K. Weaver[1], Tim F. Seipel[1], Fabian D. Menalled[1], Megan L. Hofland[1], Justin B. Runyon[2], Amy M. Trowbridge[3]

[1] Department of Land Resources & Environmental Sciences, Montana State University, Bozeman, MT 59717, USA

[2] USDA Forest Service, Rocky Mountain Research Station, Forest Sciences Laboratory, Bozeman, MT 59717, USA

[3] Department of Entomology, University of Wisconsin, Madison, WI 53715, USA

[4] Author to whom any correspondence should be addressed

Email: shealyn.malone@gmail.com; Phone: 303-918-3367; Fax: 406-994-3933

Keywords

crop rotations, microbe-plant-insect interactions, indirect defense, *Triticum aestivum, Cephus cinctus,* pest management

Abstract

Aims (main purpose and research question)

Soil microbes can influence the emissions of plant volatile organic compounds (VOCs), which serve as host-location cues for insect pests and their natural enemies. Agricultural practices steer unique soil microbial communities, but how this influences crop VOCs and subsequent insect attraction remains unknown. The aim of this study was to investigate the effect of diversified agricultural practices on constitutive and herbivore-induced VOC emissions by crops through soil-microbe legacy effects.

Methods

In a full factorial experiment, we measured VOC emissions by wheat (*Triticum aestivum*) grown in soil inoculum from wheat-fallow or wheat-cover crop rotations that was subjected to feeding by larval wheat stem sawflies (*Cephus cinctus*).

Results (main findings)

Under herbivory, plants grown in cover crop inoculum emitted greater total VOCs, including higher concentrations of 2-pentadecanone, a known insect repellent, and nonanal, a compound important in the recruitment of natural enemies. Plants grown in

fallow inoculum showed no differences in emissions whether under herbivory or not. Soil inoculum did not influence VOC emissions of wheat plants in the absence of larval feeding.

Conclusions

These results suggest that agricultural practices influence crop VOC emissions through microbe-mediated soil effects. Additionally, crops grown in wheat-fallow rotations may be less successful recruiting natural enemies of pests through herbivore-induced VOC signaling.

Abbreviations

Volatile organic compounds (VOCs); arbuscular mycorrhizae fungi (AMF); green leaf volatiles (GLVs); plant growth promoting rhizobacteria (PGPR); northern Great Plains (NGP), wheat stem sawfly (WSS); gas chromatography-mass spectrometry (GC-MS); non-metric multidimensional scaling (NMDS); generalized linear mixed-effects model (GLMM); jasmonic acid (JA)

Introduction

Plant volatile organic compounds (VOCs) have multiple ecological roles including attracting pollinators, acting as cues for foraging herbivores, and functioning as direct and indirect defense against herbivores (Dudareva et al. 2013; Heil 2014; Heil and Karban 2010; Parachnowitsch and Manson 2015). Given their central role in mediating plant-insect interactions, it is no surprise that many forms of pest management employ plant VOCs to influence the behavior of pests and their natural enemies (Pickett and Khan 2016; Shrivastava et al. 2010). For example, trap crops, or unharvested crops planted around the field to be protected, emit VOCs that are more favorable to pests than those emitted by the cash crop, thus ‘intercepting’ pests and reducing field damage (Adler and Hazzard 2009; Buteler et al. 2010; Morrill et al. 2001). Intercropping, the simultaneous cultivation of multiple plant species, can lower pest pressure by masking volatile cues necessary for pests to locate appropriate hosts (Lopes et al. 2016; Tahvanainen and Root 1972). Additionally, specific crop varieties may differ in constitutive, or always present VOC emissions, making them less attractive to pests compared to other varieties of the same crop species (Buteler and Weaver 2012; Weaver et al. 2009). Similarly, crop varieties may vary in VOCs emitted upon herbivore attack, also known as herbivore-induced VOCs, influencing their ability to recruit natural enemies (Kappers et al. 2011; Rasmann et al. 2005).

The manipulation of plant VOCs through beneficial soil microbes is another, but far less understood, form of plant volatile pest management. Beneficial microbes can enhance plant resistance against herbivorous insects through a cascade of molecular and chemical changes that occur in the plant during microbe colonization of the rhizosphere (Jung et al. 2012; Sharifi et al. 2018). Microbe modulation of hormonal defense pathways may ultimately lead to altered emission of constitutive and herbivore-induced plant VOCs, altering attraction of pests and their natural enemies, both of which rely on volatile cues to locate hosts. For example, plants inoculated with arbuscular mycorrhizae fungi (AMF) have been shown to elevate constitutive emissions of common defense VOCs, including terpenes and green leaf volatiles (GLVSs), with altered attraction of herbivores and their predators (Fontana et al. 2009; Meier and Hunter 2019; Rapparini et al. 2008). Alternatively, in some cases, the beneficial effect of soil microbes may become apparent only after herbivore challenge through a process called 'priming'. Primed plants are able to respond faster and more effectively upon herbivore attack (Conrath et al. 2006; Pozo and Azcón-Aguilar 2007), though they may show no chemotypic differences in constitutive defenses. For example, *Arabidopsis thaliana* inoculated with plant growth-promoting rhizobacteria (PGPR) emit the same constitutive volatiles as non-inoculated plants; however, following herbivore attack, primed inoculated plants emit altered herbivore-induced VOCs that are more attractive to natural enemies (Pangesti et al. 2015).

Despite the potential to enhance pest resistance of crops through microbe-altered plant VOCs, current knowledge of these microbe-plant-insect interactions is limited to one or a few microbial strains (Heinen et al. 2018, Kaplan et al. 2018; Pineda et al. 2017), which often do not demonstrate the same effects when applied as inoculums to the field (Gadhave et al. 2016; Timmusk et al. 2017). An alternative approach to enhance beneficial microbe-crop associations in agricultural is to promote the presence of existing beneficial microbes that already exist in the soil through the ecological concept of plant-soil feedbacks (Bever 1994; Kaplan et al. 2018; Mariotte et al. 2018; Putten et al. 2013). Plant-soil feedbacks occur when plants influence soil microbes via root exudates, and soil microbes, in turn, alter the performance of plants grown later in the same soil through mutualistic or pathogenic interactions (Bever 1994; Kulmatiski et al. 2008). While much of this work has focused on how plant-soil feedbacks influence the biomass of subsequent plants grown in the same soil, plant-soil feedback studies have shown that plants can steer whole soil microbiomes to improve plant resistance of the subsequent plant through shifts in foliar defense compounds (Badri et al. 2013; Pineda et al. 2020; Wang et al. 2019; Zhu et al. 2018). Thus, plant-soil feedbacks provide a novel means to shape the soil microbiome to enhance crop performance, but their influence on VOCs remains unclear.

Plant-soil feedbacks fit well in the context of existing agricultural farming practices, particularly the use of cover crops. Cover crops—and the way in which they are managed—steer distinct soil microbe communities, recruiting and inhibiting different bacteria and fungi through plant-specific root exudates (Babin et al. 2019; Frasier et al. 2016; Kim 2020). Compared to fallow, cover crops increase microbe diversity and minimize the accumulation of soil-borne pathogens (Lupwayi et al. 1998), leading to proliferation of beneficial microbes in the rhizosphere (Vukicevich et al. 2016). Diversified management practices, such as grazing termination of cover crops, are also likely to increase soil microbe diversity through nutrient-rich manure inputs that increase niche availability in the soil (Hartmann et al. 2015; Navarro-Noya et al. 2013). Interestingly, agricultural practices driving more diverse soil microbe communities have been shown to decrease pest populations: organically managed soils (Blundell et al. 2019) and mycorrhizal cover crops (Murrell et al. 2019) reduce plant attractiveness to pests through increased foliar chemical defense. While these and other studies (Carrillo et al. 2018) demonstrate that agricultural soils can influence plant-insect interactions through changes to foliar chemistry, it is still unknown how they may influence plant-insect interactions through altered VOC emissions.

Here, we evaluate how soil microbes from diversified agricultural practices alter constitutive and herbivore-induced VOC emissions by wheat (*Triticum aestivum* L.), a major crop of the northern Great Plains (NGP; Fig. 1). Wheat grown in the NGP faces intense pest pressure from the wheat stem sawfly (WSS; *Cephus cinctus* Norton), a stem-mining pest that causes $350 million dollars of lost revenue annually in North America (Beres et al. 2011). Females use volatile cues to locate appropriate plant hosts where they oviposit eggs into the stems of wheat and other grasses (Buteler and Weaver 2012; Perez-Mendoza et al. 2006; Piesik et al. 2008; Weaver et al. 2009). Upon hatching, the developing larvae mine and feed upon the parenchyma of the stem throughout the growing season, which impairs photosynthesis and movement of assimilate to the developing seed head (Delaney et al. 2010; Macedo et al. 2007; Macedo et al. 2006; Macedo et al. 2005). Destructive feeding increases via larval boring through stem nodes over the growing season resulting in reduced grain quality, protein content, and grain yield (Ainslie 1920; Morrill and Kushnak 1996). Like many plants that elicit herbivore-induced defense mechanisms, wheat stems infested with larvae generally emit altered VOCs (Peck 2004; Pérez 2009; but see Piesik *et al.*, 2009), some of which are used as host location cues by braconid parasitoids, natural enemies of the WSS (Pérez 2009). Braconid parasitoids can effectively reduce WSS populations and lower yield loss by providing high irreplaceable larval mortality (Buteler et al. 2015; Buteler et al. 2008;

Peterson et al. 2011); however, this is contingent upon adequate parasitoid abundance which can have significant spatial and management-driven variability (Peterson et al. 2011; Rand et al. 2014; Runyon et al. 2002; Weaver et al. 2004). Thus, agricultural practices have the potential to improve the biological control of WSS through improved VOC signaling and subsequent parasitoid recruitment.

Understanding the potential of cover crop rotations to influence crop VOCs will help inform whether agricultural practices might be leveraged to enhance pest-resistance in crops. In this study, we isolate the influence of the soil microbe community and herbivory on the chemical plasticity of VOC emissions by wheat in a full factorial greenhouse experiment. Wheat plants were grown in sterilized soil mixed with live soil inoculum from established rotations: 1) wheat-fallow or 2) wheat-cover crop rotations terminated by grazing cattle to represent low and high diversity cropping systems, respectively. To observe the effect of soil inoculum on constitutive and induced volatiles of wheat, we measured VOCs emitted by WSS-infested and uninfested wheat plants grown in soil from the two different cropping systems. We hypothesize that 1) larval feeding will increase VOC emission by wheat compared to constitutive VOCs. However, given that more diverse cropping systems increase the potential for priming of defenses through beneficial plant-microbe associations, we hypothesize that 2) wheat grown in cover crop inoculum will produce more constitutive and induced VOCs compared to wheat grown in fallow inoculum.

Materials and Methods

Soil collection

Soil was collected from the Northern Agriculture Research Center located south of Havre, MT (48°29'48.8"N, 109°48'10.4"W). The site is a water-limited agroecosystem of the Northern Great Plains (NGP) with an average annual precipitation of 305 mm. Average annual high and low temperatures at the site are 13.6 °C and 0.0 °C, respectively (Western Regional Climate Center, 2020). Since 2012, two replicate fields (40 m x 360 m, each) have been planted in an alternating two-year rotation of winter wheat (*Triticum aestivum* L., Judee variety) with either cover crop mixtures or fallow. Within each field, each treatment is randomly repeated in 3 separate plots (8 m x 14 m; Fig. 1). The location of each treatment was randomized in 2012 and has been maintained through time.

This study used inoculums of soil collected from low and high diversity cropping systems: 1) winter wheat rotated with fallow and 2) winter wheat rotated with a 7-species mixture of cover crops that was terminated by grazing cows. Hereon in, soil from these cropping systems will be referred to as ‘fallow inoculum’ and ‘cover crop inoculum’. The cover crop mixture consisted of radish (*Raphanus raphanistrum* L.), lentil (*Lens culinaris* Medikus), field pea (*Pisum sativum* L.), oat (*Avena sativa* L.), turnip (*Brassica rapa* L.), sorghum x sudan grass (*Sorghum drummondii* Nees ex. Steud.), and soybean (*Glycine max* L.). Species were selected based on USDA-ARS recommendations for the NGP and represent a range of functional groups with potential for the provision of various ecosystem services (https://www.ars.usda.gov/plains-area/mandan-nd/ngprl/docs/cover-crop-chart/). Cover crop plots were planted mid-May and terminated late-June to protect soil moisture, as practiced in the region (O'Dea et al. 2013). Termination of cover crops was achieved by targeted cattle grazing, an ecologically based management approach to enhance economic and environmental sustainability of growing cover crops (McKenzie et al. 2017; Thiessen Martens and Entz 2011). Both cropping systems were spot treated with glyphosate to manage postharvest weeds during July and September.

Soil collection occurred on July 4, 2018 immediately before cover crop termination to attain peak season microbe diversity (Ishaq et al. 2017). We collected soil that would be planted in wheat later that fall; thus, soil was collected from plots that were either planted in cover crops or laid fallow, depending on the rotation. Surface litter was cleared and approximately 2,000 g of soil was collected to a depth of 30 cm with a sterilized shovel at three random locations within each plot. Bags of soil were stored on ice and transported back to Montana State University (Bozeman, MT) where all samples of a single soil inoculum were homogenized and stored at -18 °C until later use in the greenhouse.

Plant growth

Wheat seeds of the commonly grown spring wheat cultivar Reeder were collected from a single spike of wheat grown in Big Sandy, MT during Fall of 2018 and grown to maturity in the Montana State University Plant Growth Center. We used these genetically identical seeds in order to control for chemotypic variation between plants (Gouinguené et al. 2001). Mature seeds were surface-sterilized in 5% bleach, rinsed twice with deionized water, allowed to dry completely, and stored at -18°C until planting. Upon planting, seeds were sown in washed and sterilized conical pots in a 1:1 mix of autoclaved Sunshine Mix #1 soil (Gro Horticulture Inc., Bellevue, WA) and MSU soil with a 15% by mass inoculum of live field soil. Sunshine Mix #1 soil is comprised of Canadian sphagnum peat moss, perlite, vermiculite, starter nutrient charge, wetting agent, and dolomitic lime. MSU soil is equal parts of Bozeman silt loam soil, washed concrete sand, and Canadian sphagnum peat moss. AquaGro 2000 G wetting agent (Aquatrols, Paulsboro, NJ) is blended in at one pound per cubic yard of soil mix and aerated steam pasteurized at 80°C for 45 minutes. The live soil inoculum was used to introduce microbe communities from the field while minimizing nutrient discrepancies between soils. This technique is used to elucidate the contribution of soil biota to plant-soil feedbacks on plant performance (Brinkman et al. 2010) and has been successfully applied to assess the impact of agricultural soil microbial communities on plant growth and competitive interactions (Johnson et al. 2017; Miller and Menalled 2015), and overall agroecosystem resilience (Seipel et al. 2019). The soil inoculum treatments differed in bacterial and fungal community composition and diversity (Dupre, unpublished; Ouverson, unpublished). To reduce any effect of variation in plant development stage on infestation and VOC emissions, we planted one cohort of plants each week from late March 2019 to early May 2019. During each planting, we homogenized 600 g of soil inoculum in 3400 g of sterilized soil and divided the soil equally between 30 conical pots (17.8 cm height x 6.9 cm diameter; Stuewe & Sons, Tangent, OR). This was repeated for the second soil type, always sterilizing equipment in between with 70% isopropyl alcohol.

Plants were fertilized biweekly at a 50% rate with 60 ml of 20-20-20 (N-P-K, 50 ppm) fertilizer (Peters General Purpose Fertilizer, Allentown, PA) starting 3 weeks after planting to preserve the functional diversity inherent in the field inoculum while also ensuring plants received nutrients necessary for development. While fertilizers are known to affect microbial community structure and biomass (Donnison et al. 2000; Sarathchandra et al. 2001), low fertilizer rates have been shown to preserve the functional diversity of soil microbial communities (Marschner et al. 2003). Plants were watered *ad libitum* and grown in a greenhouse with supplemental light (GE Multi-Vapor MVR1000/C/U, GE Lighting, Cleveland, OH) under a 15 L:9 D photoperiod. Mean daytime temperatures were 22°C and mean night temperatures were 20°C (±1.5°).

Wheat stem sawfly (WSS) collection and infestation

WSS larvae were collected from naturally occurring populations near Amsterdam-Churchill, MT (45°45'26.8"N 111°24'13.4"W). Wheat stubs—in which larvae undergo diapause—were collected from heavily infested fields in the Fall of 2018 and Spring of 2019. Stubs were stored in plastic Ziploc bags at 0 °C for a minimum of 100 days to facilitate completion of larval diapause. Approximately three weeks prior to infestation, stubs were transferred to aeriated plastic Tupperware where adults were held at room temperature (22–27 °C) until adult emergence began. To ensure newly emerged females were used to infest plants, we removed all adults from the Tupperware every 24 hours. Surplus adults were stored in Mason glass jars at 2 °C for up to two days for later use before they were discarded. Emerging adults are sexually mature (Holmes 1977).

We exposed plants to adults at stem elongation (Zadoks 32–34) (Zadoks et al. 1974). This stage was chosen because wheat is particularly susceptible to infestation at this stage under field conditions. Transparent plastic cylinders (60-65 cm x 3.8 cm) were placed over the plant to be infested and sterilized soil was placed around the bottom of the tube to prevent adults from escaping. Each cage had three round holes (3.8 cm diameter) covered with fine cloth-mesh distributed along the cylinder for air circulation and had an additional 1 cm diameter hole, plugged with removable cotton cloth, through which adults were introduced. One male and three female adults were added to each cylinder for a three-day oviposition period. Cylinders were also placed over control plants, but no adults were added. After infestation, cylindrical cages and adults were removed.

Volatile organic compound (VOC) collections

Volatiles were collected immediately after anthesis and the onset of milk development (Zadoks 71), which was approximately 21 days post-infestation. The sampling system allowed collection of VOCs from 12 plants each day. As such, each sample consisted of six plants in each soil inoculum, four of which had been exposed to adults and two of which had not. We chose to expose more than half of the plants because infestation is variable, and this ensured we had an adequate number of infested plants for analysis. We collected VOCs two to four times a week from a total of 240 plants. We sampled from May 22 through July 2, always infesting plants 19-22 days before measurement during stem elongation.

For each VOC collection, plants were placed in 1 L glass collection chambers in a volatile collection system (VCS, Analytical Research Systems, Inc., Gainesville, FL) as previously described by Piesik et al. (2006). The apparatus features 12 glass chambers attached to a volatile collection port on one end and open on the other end to enclose one individual plant per chamber. Chambers were placed atop Teflon guillotine stands, which separated plant roots and potting soil from the collection chamber. Gaps between the plant stem and Teflon guillotines were filled with cotton to prevent soil VOCs from entering the collection chamber. Purified, humidified air was delivered at a rate of 1.0 L min^{-1} over the plants and the flow and pressure were maintained by a regulated vacuum pump. Two volatile collector traps—one purge trap and one sample trap—containing 30 mg of Super-Q (Alltech Associates, Inc., Deerfield, Illinois) adsorbent was inserted into each volatile collection port. In order to clear ambient air from the chamber, air was initially collected on purge traps for 10 min before switching to the sample traps. Both the purge and the sample traps were set to a flow rate of 1.0 L min^{-1}. We collected volatiles on the sample traps for 8 h from approximately 9:00 to 17:00 each day. After sampling, volatile collection traps were removed, wrapped in aluminum foil, and eluted within 24 hours (see below).

To account for the effects of environmental variables on VOC emissions, Thermochron temperature ibuttons (Maxim Integrated, San Jose, CA) placed in each chamber and set to measure temperature every five minutes. Photosynthetically active radiation (PAR), measured every hour, was acquired from the local university weather station. Both temperature data (over the 8 h sampling period) and PAR data (from start of day until the end of the sampling period) were averaged to yield a cumulative value for each of the variables per sampling day. To normalize emissions for comparison, we used these cumulative values to calculate basal emission volatile rates standardized to 30°C according to Guenther (1997).

After VOC collections, plants were cut at the base of the stem to measure aboveground biomass, tiller lengths, and count the number of internodes per tiller. To determine whether plants were infested, stems were split along their length with a scalpel. For each infested stem, we measured larval weight, length of stem bored, and number of internodes bored. Plants exposed to adults that were not infested were initially characterized as 'exposed' but later aggregated with control plants due to no difference in total volatiles ($F_{1,113.6} = 0.027$, $P = 0.87$).

Analysis and identification of VOCs

We determined the identities and relative amounts of VOCs using gas chromatography-mass spectrometry (GC-MS). To do this, volatile traps were eluted with 200 µL of methylene chloride (Fisher Scientific, Fair Lawn, NJ). 10 µL of a 30 ng μL^{-1} solution of nonyl acetate (Sigma-Aldrich, Saint Louis, MO) in methylene chloride was added as an internal standard following elution and samples were stored at -70 °C until analysis. For chemical analysis, 3 µL of each sample was injected onto an Agilent Technologies 6890 GC-5973 MS fitted with an HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness; J&W Scientific, Folsom, CA). Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1} and injector temperature was set to 250 °C. The oven profile consisted of an initial temperature of 50 °C followed by a ramp of 5 °C min^{-1} to 200 °C, then a second ramp of 25 °C min^{-1} to 280 °C. Volatile compounds were identified by comparing retention times and mass spectra with commercial standards using ChemStation software (Agilent Technologies, Wilmington, DE) and the NIST Mass Spectral Library (National Institute of Standards and Technology, Gaithersburg, MD). All standards were purchased from Sigma Aldrich (Saint Louis, MO) with the exception of decanal and β-ocimene (The Good Scents Company, Oak Creek, WI). We quantified the concentrations of each compound by comparing its peak area with that of the internal standard, nonyl acetate. Concentrations of volatile compounds were standardized for the number of hours for which they were collected and for aboveground plant biomass (ng g^{-1} h^{-1}).

Data analysis

We performed all analyses in R version 3.5.2 (R Core Team, 2018). Raw emission rates were converted to basal emission rates standardized to 30°C using cumulative temperature and PAR from each volatile collection (Guenther 1997). To compare the effects of soil inoculum and infestation on induced VOC emissions, we fit linear mixed models using the ‘lmer’ function in the ‘lm4’ package (Bates et al. 2015). Measures of individual or total VOCs were the dependent variables and soil inoculum, infestation, and their interactions were fixed effects. Sample day was used a random effect to account for day-to-day variability in other unmeasured environmental factors from each trial. All response variables were transformed as needed to meet the assumption of normality and homoskedasticity. To assess if cropping systems and infestation and their interaction accounted for variation, we performed Type III ANOVA using the ‘anova’ function in the ‘lmerTest’ package (Kuznetsova et al. 2017). When cropping system, infestation, or their interaction accounted for variation, we compared post-hoc difference using pairwise Tukey comparisons using the ‘emmeans’ function (Lenth et al. 2020).

We evaluated differences in the volatile community composition among soil inoculum, infestation, and their interaction using permutational multivariate ANOVA (PERMANOVA; Anderson et al. 2014; Oksanen et al. 2019). To do so, we used the ‘vegdist’ function in the ‘vegan’ package (Oksanen et al. 2019) to calculate dissimilarities among samples using the Bray-Curtis metric. To visualize differences among treatment, we used non-metric multidimensional scaling (NMDS) and plotted the first two axes.

To evaluate the probability of infestation by soil inoculum for plants exposed to adults, we used a generalized linear mixed-effects model (GLMM) with soil inoculum as the fixed effect, sample day as the random effect, and infestation as the response variable. To do this, we calculated the mean odds of plant infestation when exposed to adults using the ‘glmer’ function in the ‘lme4’ package (Bates et al. 2015). Finally, plant biomass was modeled using a linear mixed model using the ‘lmer’ function in the ‘lm4’ package with soil, infestation, and their interaction as fixed effects and sample day as a random effect (Bates et al. 2015).

Results

A total of 26 volatile compounds were quantified using GC-MS analysis from the headspace of wheat plants. Volatile compounds quantified include five monoterpenes, two sesquiterpenes, nine alkanes, five ketones and esters, three aldehydes, one green leaf volatile (GLV) and one irregular terpene (Table 1). Plants grown in different soil inoculums emitted the same 26 compounds, but in different relative amounts. The overall VOC composition varied in response to the interaction of soil inoculum and larval feeding (PERMANOVA Infestation x Soil: $F_{1, 104} = 2.91$, $P = 0.034$), as did the quantity of total VOCs emitted (Fig. 2; Soil * Infestation: $F_{1, 211.8} = 5.78$, $P = 0.017$).

Constitutively, soil inoculum did not influence VOC emissions (df = 210, t = 0.62, $P = 0.926$) and regardless of soil inoculum, plants emitted an average of 13.53 ng gFW^{-1} h^{-1} total constitutive VOCs (95% CI: 11.04 to 16.60 ng gFW^{-1} h^{-1}). We found that in the absence of feeding injury, plants did not differ in their emissions of individual volatile compounds (Table 1). Consistent with our finding that soil inoculum did not influence quantity of total of individual constitutive VOCs, we observed no effect of soil inoculum on the probability of infestation for plants exposed to ovipositing females ($F_1 = 0.53$, $P = 0.469$). Infestation rates were 65% (95% CI: 54% to 75%) for wheat grown in fallow inoculum and 60% (95% CI: 48% to 70%) for wheat grown in cover crop inoculum.

Induced VOC emissions caused by larval feeding varied among soil inoculum. VOC emissions did not vary in fallow inoculum after feeding (df = 213, t = 1.01, $P = 0.744$), and plants emitted the same amounts of total and individual VOCs whether infested with larvae or not (Fig. 2; Table 1). Plants grown in cover crop inoculum, however, emitted 28% more total volatiles (3. 17 ng gFW^{-1} h^{-1}) when fed upon by larvae compared to constitutive levels (df = 212, t = -2.38, $P = 0.084$) and 34% more total volatiles than infested plants grown in fallow inoculum (df = 211, t = -2.66, $P = 0.041$).

Six of the 26 compounds differed in response to either soil inoculum, larval feeding (infestation), or their interaction. Plants grown in cover crop inoculum emitted 35% more nonanal when infested than when not infested (Fig. 3; Infestation: $F_{1, 210.2} = 6.65$, $P = 0.011$, df = 210, t = -2.87, $P = 0.024$). We also observed treatment effects on the emissions of 2-pentadecanone (Fig. 4; Soil x Infestation: $F_{1, 217.0} = 6.44$, $P = 0.012$) and four alkane compounds including 3-methyltetradecane (Fig. 5a; Soil: $F_{1, 208.8} = 10.16$, $P = 0.002$), tridecane (Fig. 5b; Soil: $F_{1, 208.8} = 16.72$, $P < 0.001$), tetradecane (Fig. 5c; Soil: $F_{1, 208.7} = 8.74$, $P = 0.003$), and 3-methylundecane (Fig. 5d; Soil: $F_{1, 208.8} = 13.94$, $P < 0.001$). Under larval feeding, these compounds were emitted at higher rates from plants grown in cover crop inoculum than those grown in fallow inoculum (Table 1). Finally, when we grouped all nine alkane compounds, we found that infested plants grown in cover crop inoculum emitted ~35% more alkanes (Table 1; Soil: $F_{1, 208.8} = 12.09$, $P = 0.001$, df = 211, t = -3.06, $P = 0.013$).

Soil inoculum had a small, albeit significant, influence on plant biomass (Soil: $F_{1, 209.7} = 3.93$, $P = 0.049$) with a mean 5.4% increase (+0.424 ± 0.216 g) in biomass for plants grown in cover crop inoculum. While three weeks post-infestation plants only trended towards lower biomass compared to non-infested plants (Infestation: $F_{1, 214.1} = 3.20$, $P = 0.075$), it is likely that this trend would have become more prominent had larval feeding progressed further into development. Overall, we conclude that all plants grew similarly among treatments.

Discussion

Despite the important role of the soil microbiome in agroecosystem functioning (Brussaard et al. 2007), we still have limited knowledge of its role in insect attraction through modification of plant VOCs. Understanding how diversified cropping systems influence VOC emissions through plant-microbe interactions will provide important insight into the effects of soil on crop signaling to pests and regulation of pests through natural enemies (Kaplan et al. 2018; Mariotte et al. 2018; Pineda et al. 2017). In this study, we found that inoculation of plants with soil microbes from two different cropping systems did not alter constitutive emissions of VOCs but did influence their emissions following larval feeding. VOC emissions by wheat grown in fallow inoculum did not vary whether experiencing feeding injury or not. In contrast, wheat grown in cover crop inoculum produced more total volatiles upon larval feeding, including increased emissions of individual compounds known to influence insect behavior. Our findings indicate that soil microbiomes from different agricultural practices have the potential to influence pest-resistance of crops through altered herbivore-induced VOC profiles, leading to enhanced direct defense through repellence of pests and enhanced indirect defense through attraction of their natural enemies.

Several studies have shown that individual microbial strains can alter constitutive VOC emissions by plants (Fontana et al. 2009; Meier and Hunter 2019; Rapparini et al.

2008). Though the effect of whole soil microbiomes on plant VOCs has not been previously described, is well-known that multiple microbial species influence plant chemical defenses in non-additive ways; combinations of microbial species may increase, decrease, or have no effect on pest resistance (Bennett et al. 2009; Gange and West; Shrivastava et al. 2015) compared to the effect of a single microbial strain. Contrary to our first hypothesis, we found that whole soil microbiomes from two different cropping had no effect on the quantity or quality of constitutive VOCs emitted by plants. This suggests that crop rotations will not confer different levels of pest resistance through altered VOC emissions and subsequent attraction of pests, nor will they falsely signal natural enemies, as has been suggested as a potential outcome of microbe-altered constitutive plant VOCs (Fontana et al. 2009; Guerrieri et al. 2004).

Contrary to observations of constitutive VOCs, VOC emissions did vary among soil inoculum when plants were infested with larvae. These patterns may be due to priming mechanisms by which microbes present in the cover crop inoculum induced systemic resistance in plants grown in cover crop soil but did not do so significantly in fallow soil (Conrath et al. 2006; Pozo and Azcón-Aguilar 2007). Beneficial microbes, upon colonization of roots, can cause the induction of a unique physiological state within the plant called “priming”. Primed plants display faster and stronger activation of defense pathways fallowing attack by herbivores (Conrath et al. 2006), including altered emissions of VOCs. Indeed, studies have observed microbially-driven differences in VOC emissions that were significant only after herbivore feeding (Pangesti et al. 2015; Pineda et al. 2013), suggesting that soil microbes primed plant defenses, altering herbivore-induced VOCs but not constitutive VOCs. In support of our second hypothesis, we found that wheat grown with soil from the cover crop rotation was able to induce emissions of VOCs under infestation while wheat grown with soil from wheat-fallow rotation was not. Other studies measuring the effect of soil type and herbivory on foliar plant defenses show similar results: upon insect feeding, some soil treatments show no induced response to herbivory, even at the transcriptional level, whereas other soil treatments show robust upregulation of defense-related genes (Zhu et al. 2018). We posit that plants grown in cover crop inoculums experienced priming that allowed for a stronger activation of defense pathways controlling VOC emissions than plants grown in fallow inoculums.

Similar to our observation that larval feeding did not influence VOCs emissions by plants grown in fallow inoculum, previous studies with WSS-infested wheat plants found a lack of herbivore-induced VOC emissions when plant VOCs were measured two (Piesik et al. 2009) and three weeks (Peck, 2004) post-oviposition. Further, at ten days post-infestation, analysis of tissue from four different wheat cultivars showed that Reeder exhibited the fewest changes in stem proteins and metabolites (Lavergne et al. 2020). While volatile responses to infestation will likely vary by cultivar, the results we observed in the Reeder variety may, in part, be due to feeding habits of WSS larvae and the fact that our research focused on feeding by smaller larvae. Plants infested with stem boring insects emit far less VOCs than when they are attacked by leaf chewing insects (Turlings et al. 1998), which may be due to highly localized induction of jasmonic acid (JA) (Lee et al. 2017). Jasmonic acid is an immediate product of herbivory via the octadecanoid pathway that acts as a defense hormone, upregulating induced-plant defenses, including the production of herbivore-induced VOCs (Ozawa et al. 2000). Thus, it is likely that the localized induction of JA caused by stem boring insects results in a highly localized induction of VOCs, which may be undetectable outside of the stem and at the scale on which foraging insects are be able to detect. There is evidence that stem boring insects may even suppress a plant's JA-defenses through hormonal secretions (Tooker et al. 2008; Tooker and De Moraes 2007), further diminishing the plant's ability to alter whole-plant VOCs in response to herbivory. It is possible that upon herbivory, plants grown in fallow inoculations experienced highly localized induction of JA inside the stem and sparse production of induced-VOCs, whereas plants grown in cover crop inoculum experienced stronger and faster activation of JA associated with enhanced production of VOCs due to priming by beneficial microbes present in the cover crop inoculum that were not present in the fallow inoculum. Thus, plants grown with soils from cover crop rotations may benefit from increased pest resistance through microbial priming of plant defenses.

Biological control of pests offers an effective strategy for managing destructive insects without the use of pesticides (Rand et al. 2016). Given that natural enemies of herbivores rely on induced volatile cues for effective host-foraging (Arimura et al. 2009; Bruce et al. 2005; De Moraes et al. 1998; Heil 2014), biological control is often dependent on effective volatile signaling by herbivore-attacked crops. While plants commonly alter VOCs in response to herbivory (Heil 2014), lack of induction or modification to VOC blends has been shown to reduce recruitment of natural enemies

(Megali et al. 2015; Pineda et al. 2013), which may lead to increased pest abundance. The absence of herbivore-induced plant volatiles observed in our study in wheat grown in fallow inoculum suggests that these crops may be less effective at recruiting natural enemies for biological control of pests through reduced host-searching efficacy. As such, biological control efforts may be less effective when used in tandem with agricultural practices and crop rotations that diminish the crops' ability to induce indirect VOC defenses.

In addition to increasing emissions of total VOCs, we found that wheat grown in soils from cover crops emitted more nonanal and 2-pentdadecanone when infested by larvae. Nonanal is known to serve as an attractant to natural enemy insects (Yu et al. 2008) and can also have synergistic properties for insect herbivores (Dickens 2006). Nonanal has also been shown be important in plant-plant communication, triggering resistance expression against a bacterial pathogen in uninfected lima bean plants (Yi and Heil 2009). 2-pentadecanone has been shown to repel insect herbivores, including a major pest of wheat, the grain aphid (*Sitobion avenae*) (Drakulic et al. 2015; Sun et al. 2016). Clearly, altered emissions of nonanal and 2-pentadecanone has the potential to alter multitrophic plant-insect interactions, and these should be further studied to understand the how cropping systems can influence pest resistance in agroecosystems.

Finally, it is worth noting that herbivore-induced emissions by wheat grown in cover crop inoculum consisted of significantly more alkane compounds, especially the lower alkane species (C_{11}-C_{13}), than when wheat was grown in fallow inoculum. Higher alkanes (C_{21}-C_{35}) found in cuticular waxes (Lavergne et al. 2018) are often used by phytophagous insects to determine whether plants are suitable for feeding and laying eggs (Morris et al. 2000; Eigenbrode et al. 1995; Müller and Riederer, 2005); however, less is known about how lower alkanes are metabolically regulated in plants and their role in mediating plant-insect interactions. Lower alkanes are known to be produced by insects and help to mediate intraspecific insect interactions (Blum et al. 1960; Lenz et al. 2013). For example, tridecane, which was emitted in higher quantities from infested wheat grown in cover crop inoculum, is a major component of the stink bugs scent glands and signals alarm to others when the stink bug is threatened (Blum et al. 1960). Many common insect VOC signals, including a number of lower alkanes, are also produced by plants, suggesting that plants and insects converge in patterns of volatile production, both for attraction and defense (Schiestl et al. 2014). As such, plants producing higher amounts of lower alkanes may do so to mimic signals displayed by insects, however, while a number of studies report plant emissions of lower alkanes (Kigathi et al. 2009; Pierre et al. 2011; Rembold et al. 1989), they are not reported to influence insect behavior (Finidori-Logli et al. 1995; Weissteiner and Schütz, 2006; Pierre et al. 2011). For insects such as the WSS and its braconid parasitoids that employ GLVs, terpenes, and ketones to locate appropriate plant (Piesik et al. 2008; Weaver et al. 2009) and insect hosts (Pérez, 2009), respectively, altered alkane emissions could serve as synergists or antagonists, but this needs to be confirmed with insect bioassays.

The interactions observed in this greenhouse study were made in isolation of a number of other environmental factors known to impact emission profiles but allowed us to test specific hypotheses on the impact of cover crops in soil microbe-induced effects on constitutive and herbivore-induced volatile emissions. Also, while our study observed the effects of microbial communities that are characteristic of agricultural practices at one point in the growing season, we recognize that these communities are likely to experience temporal shifts independent of agricultural practice in response to seasonal and yearly changes in crop development, temperature, and precipitation (Ishaq et al. 2020). Much work is needed to elucidate temporal trends in soil-mediated pest resistance, and future research should also investigate how soil legacy effects of agricultural practices modify crop VOCs over a range of abiotic and biotic conditions including climate, crop variety, and plant development.

Further studies are required, but here we provide evidence that agricultural systems with unique soil microbiomes can be important drivers for insect-plant interactions in agroecosystems via their influence on herbivore-induced VOC emissions of crops. Our findings indicate that cropping system diversification may have substantial effects on multitrophic interactions and differentially influence recruitment of natural enemies of pests through altered herbivore-induced VOC signaling.

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Authors' contributions

D.K.W., T.F.S., F.D.M., and A.M.T., conceived the project; S.C.M. conducted the experiments with assistance from M.L.H.; S.C.M. analyzed the data with assistance from T.F.S.; S.C.M. drafted the initial manuscript, and all co-authors revised, read, and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data availability

The datasets generated and analyzed during the current study are available in the Dryad repository, https://doi.org/10.5061/dryad.2jm63xskm.

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Figures

Fig. 1

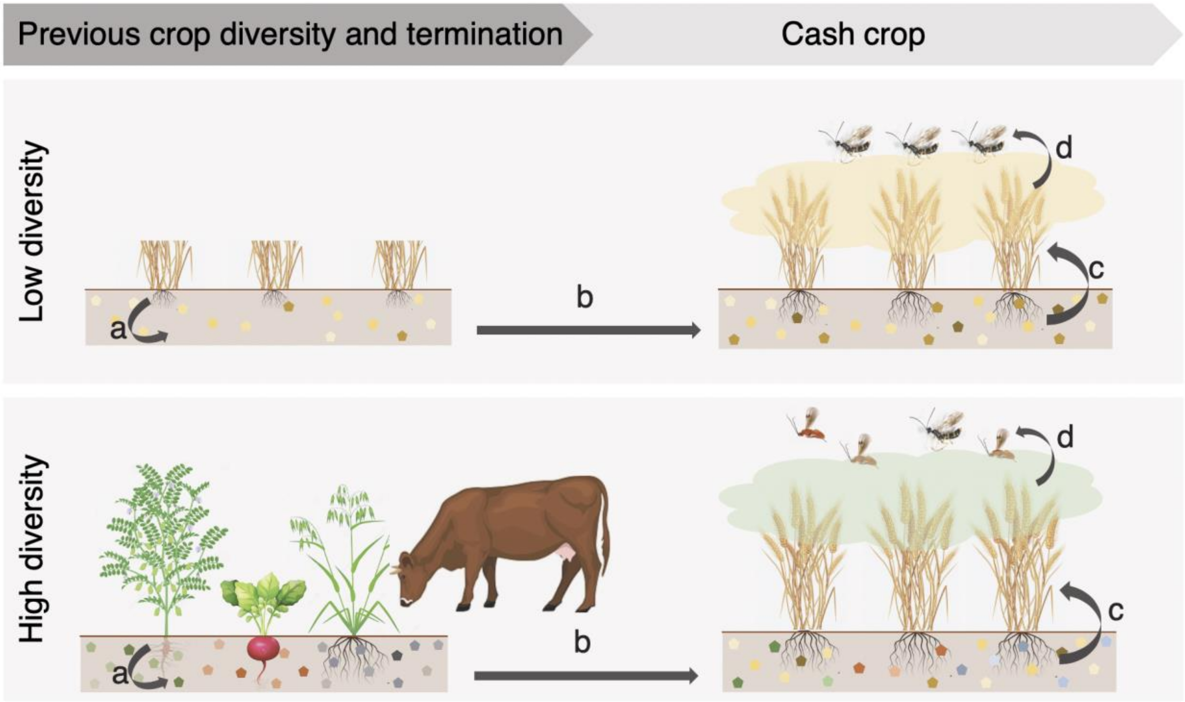

Fig. 2

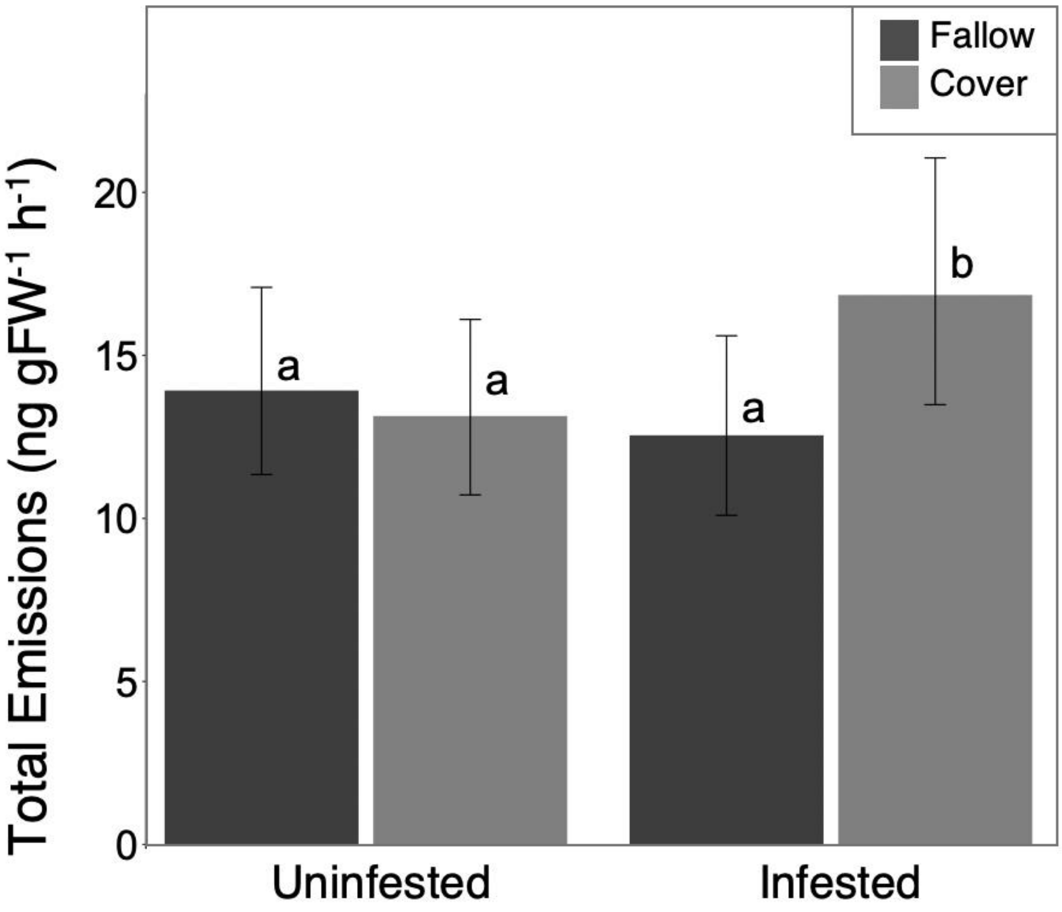
Fallow
Cover
Total Emissions (ng gFW-1 h-1)
0
5
10
15
20
a
a
a
b
Uninfested
Infested

Fig. 3

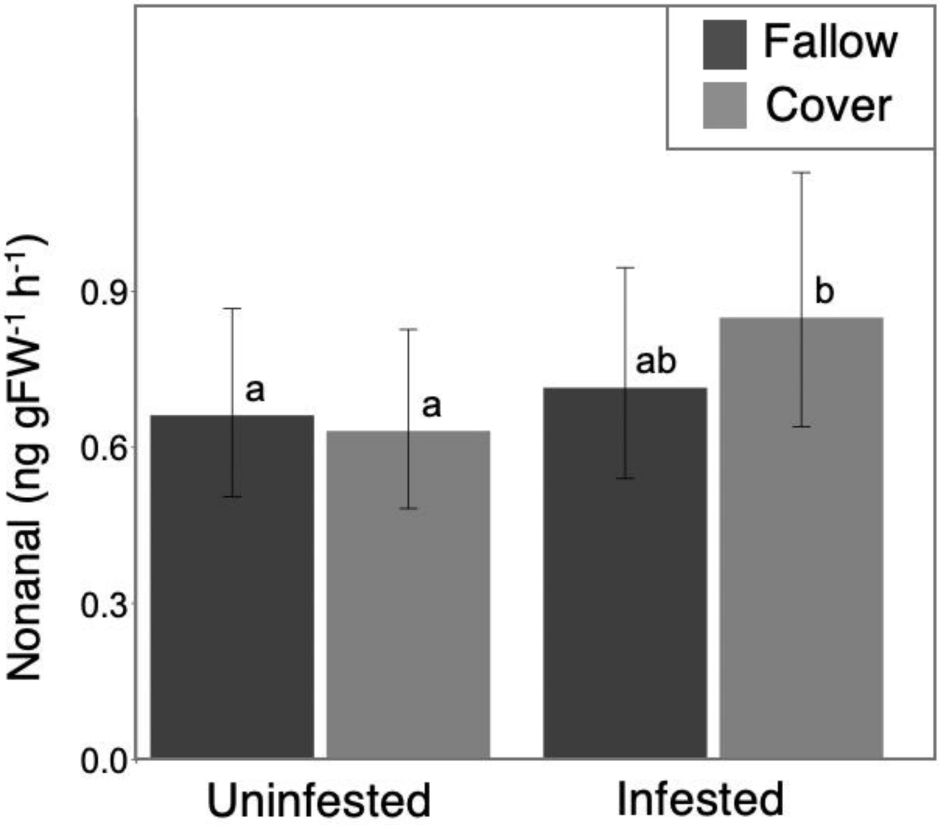
Fallow
Cover
Nonanal (ng gFW^{-1} h^{-1})
0.9
0.6
0.3
0.0
a
a
ab
b
Uninfested
Infested

Fig. 4

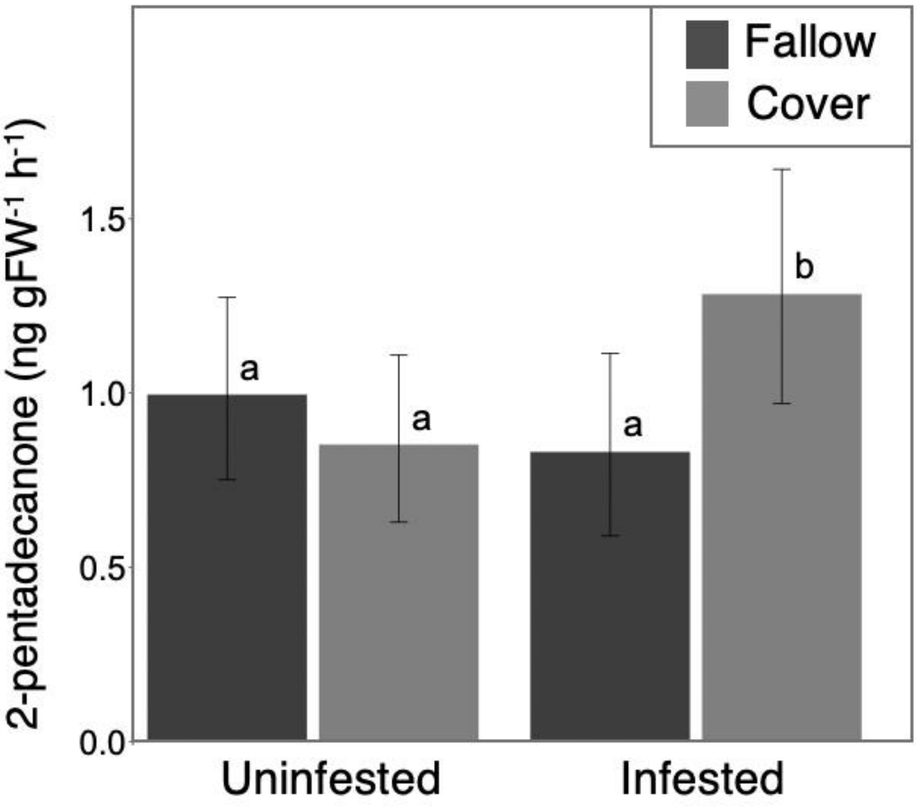
Fallow
Cover
2-pentadecanone (ng gFW^{-1} h^{-1})
1.5
1.0
0.5
0.0
a
a
a
b
Uninfested
Infested

Fig. 5

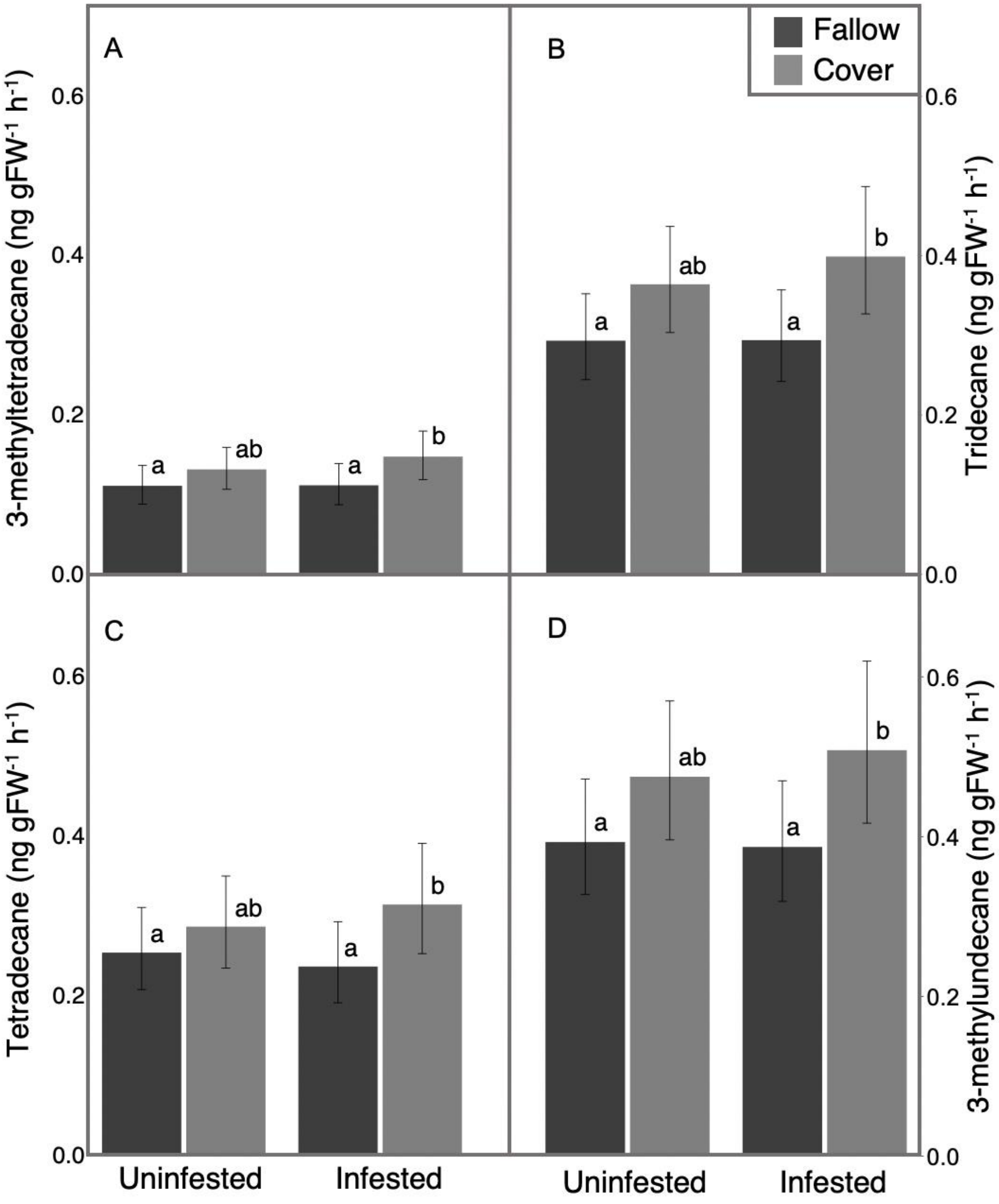
A
B
C
D
Fallow
Cover
3-methyltetradecane (ng gFW^{-1} h^{-1})
Tridecane (ng gFW^{-1} h^{-1})
Tetradecane (ng gFW^{-1} h^{-1})
3-methylundecane (ng gFW^{-1} h^{-1})
0.0
0.2
0.4
0.6
a
ab
a
b
Uninfested
Infested

Figure Captions

Fig. 1 *Experimental framework and overarching hypothesis*. Crop rotations have the potential to modify crop-pest interactions through microbe driven soil-legacies. a) High diversity rotations influence soil microbial communities differentially than low diversity rotations through plant-specific root exudates and manure inputs. b) Soil microbial communities persist into the next growing season where they c) interact with subsequent crops, potentially modifying VOC emissions. d) Altered VOCs may differentially attract pests and their natural enemies, conferring different levels of pest resistance of crops.

Fig. 2 Total VOC (ng gFW^{-1} h^{-1}) as a function of soil inoculum and larval feeding. Means ± SEM are shown and significance between treatments is expressed using different lower-case letters ($\alpha <0.1$)

Fig. 3 Emissions of nonanal as a function of soil and larval feeding. Means ± SEM are shown and significance between treatments is expressed using different lower-case letters ($\alpha <0.05$)

Fig. 4 Emissions of 2-pentadecanone as a function of soil and larval feeding. Means ± SEM are shown and significance between treatments is expressed using different lower-case letters ($\alpha <0.1$)

Fig. 5 Emissions of 3-methyltetradecane (panel a), tridecane (panel b), tetradecane (panel c), and 3-methylundecane (panel d) as a function of soil and larval feeding. Means ± SEM are shown and significance between treatments is expressed using different lower-case letters ($\alpha <0.05$)

Tables

Table 1. Constitutive and induced mean emission rates (ng nonyl acetate equivalents gFW^{-1} h^{-1}) of total compounds, families of compounds, and individual compounds, in response to larval feeding by soil treatment.

Compound	**ID**[1]	**Fallow**		**Cover**	
		− *WSS*	**+ *WSS***	**− *WSS***	**+ *WSS***
Total		13.92^{a} (11.35, 17.09)	12.55^{a} (10.09, 15.60)	13.14^{a} (10.72, 16.10)	16.85^{b} (13.49, 21.06)
Monoterpenes		1.02^{a} (0.83, 1.26)	1.02^{a} (0.82, 1.28)	1.11^{a} (0.90, 1.37)	1.22^{a} (0.98, 1.53)
β-pinene	SS	0.15^{a} (0.12, 0.19)	0.16^{a} (0.12, 0.19)	0.17^{a} (0.14, 0.20)	0.19^{a} (0.15, 0.23)
Limonene	SS	0.18^{a} (0.14, 0.22)	0.15^{a} (0.11, 0.19)	0.15^{a} (0.11, 0.19)	0.18^{a} (0.14, 0.23)
β-ocimene	SS	0.31^{a} (0.23, 0.40)	0.34^{a} (0.25, 0.45)	0.41^{a} (0.31, 0.52)	0.37^{a} (0.27, 0.48)
linalool	SS	0.21^{a} (0.17, 0.26)	0.19^{a} (0.16, 0.24)	0.22^{a} (0.18, 0.28)	0.25^{a} (0.20, 0.31)
α-terpineol	NIST$_{>50}$	0.15^{a} (0.10, 0.20)	0.16^{a} (0.11, 0.22)	0.17^{a} (0.12, 0.23)	0.21^{a} (0.15, 0.28)
Sesquiterpenes		1.62^{a} (1.18, 2.22)	1.46^{a} (1.05, 2.02)	1.70^{a} (1.24, 2.32)	1.90^{a} (1.36, 2.65)
α-farnesene	NIST$_{>50}$	0.40^{a} (0.24, 0.62)	0.37^{a} (0.21, 0.59)	0.33^{a} (0.18, 0.53)	0.43^{a} (0.24, 0.66)
Unknown sesquiterpene	NIST	0.10^{a} (0.07, 0.14)	0.09^{a} (0.06, 0.13)	0.09^{a} (0.06, 0.13)	0.13^{a} (0.09, 0.18)
Alkanes		1.72^{a} (1.43, 2.08)	1.63^{a} (1.33, 1.98)	1.98^{ab} (1.64, 2.39)	2.20^{b} (1.79, 2.69)
Dodecane	NIST	0.10^{a} (0.08, 0.12)	0.11^{a} (0.08, 0.13)	0.12^{a} (0.10, 0.15)	0.12^{a} (0.10, 0.15)
3-methylundecane	NIST	0.39^{a} (0.33, 0.47)	0.39^{a} (0.32, 0.47)	0.47^{ab} (0.39, 0.57)	0.51^{b} (0.42, 0.62)
4-methyldodecane	NIST	0.12^{a} (0.10, 0.16)	0.13^{a} (0.10, 0.17)	0.15^{a} (0.12, 0.19)	0.16^{a} (0.13, 0.20)
2-methyldodecane	NIST	0.12^{a} (0.08, 0.16)	0.11^{a} (0.08, 0.16)	0.14^{a} (0.10, 0.18)	0.14^{a} (0.10, 0.19)
Tridecane	NIST$_{>50}$	0.29^{a} (0.24, 0.35)	0.29^{a} (0.24, 0.35)	0.36^{ab} (0.30, 0.43)	0.40^{b} (0.33, 0.49)
4-methyltetradecane	NIST	0.50^{a} (0.41, 0.60)	0.47^{a} (0.38, 0.57)	0.58^{a} (0.48, 0.68)	0.58^{a} (0.47, 0.69)
Tetradecane	NIST	0.25^{a} (0.21, 0.31)	0.24^{a} (0.19, 0.29)	0.29^{ab} (0.23, 0.35)	0.31^{b} (0.25, 0.39)
3-methyltetradecane	NIST	0.11^{a} (0.09, 0.14)	0.11^{a} (0.09, 0.14)	0.13^{ab} (0.10, 0.16)	0.15^{b} (0.12, 0.18)
2-acetoxytridecane	NIST	0.27^{a} (0.20, 0.36)	0.25^{a} (0.17, 0.34)	0.29^{a} (0.21, 0.37)	0.38^{a} (0.28, 0.50)

Ketones		6.69^{a} (5.18, 8.63)	6.20^{a} (4.69, 8.21)	6.07^{a} (4.80, 7.67)	8.30^{a} (6.29, 10.95)
6-methyl-5-heptene-2-one	SS	0.13^{a} (0.10, 0.16)	0.12^{a} (0.09, 0.16)	0.12^{a} (0.09, 0.16)	0.14^{a} (0.10, 0.18)
2-undecanone	NIST$_{>50}$	0.52^{a} (0.38, 0.67)	0.43^{a} (0.30, 0.58)	0.41^{a} (0.29, 0.55)	0.59^{a} (0.43, 0.78)
2-tridecanone	NIST$_{>50}$	2.58^{a} (1.95, 3.41)	2.32^{a} (1.72, 3.13)	2.16^{a} (1.64, 2.86)	3.13^{a} (2.30, 4.26)
2-pentadecanone	NIST$_{>50}$	1.00^{a} (0.75, 1.27)	0.83^{a} (0.59, 1.11)	0.85^{a} (0.63, 1.11)	1.28^{b} (0.97, 1.64)
Hexahydrofarnesyl acetone	SS	2.63^{a} (1.83, 3.58)	2.50^{a} (1.67, 3.50)	2.37^{a} (1.61, 3.26)	3.21^{a} (2.23, 4.35)
Aldehydes		1.11^{a} (0.85, 1.44)	1.12^{a} (0.84, 1.47)	1.06^{a} (0.81, 1.38)	1.34^{a} (1.01, 1.77)
Nonanal	SS	0.66^{a} (0.51, 0.87)	0.71^{ab} (0.54, 0.95)	0.63^{a} (0.48, 0.83)	0.85^{b} (0.64, 1.13)
Decanal	SS	0.44^{a} (0.34, 0.57)	0.40^{a} (0.30, 0.52)	0.42^{a} (0.33, 0.54)	0.48^{a} (0.37, 0.63)
Green leaf volatiles (GLV)		0.21^{a} (0.14, 0.31)	0.21^{a} (0.14, 0.32)	0.21^{a} (0.14, 0.31)	0.23^{a} (0.15, 0.35)
Z-3-hexenyl acetate	SS	0.21^{a} (0.14, 0.31)	0.21^{a} (0.14, 0.32)	0.21^{a} (0.14, 0.31)	0.23^{a} (0.15, 0.35)
Irregular terpenes		0.10^{a} (0.07, 0.14)	0.09^{a} (0.06, 0.13)	0.09^{a} (0.06, 0.13)	0.13^{a} (0.09, 0.18)
Z-geranyl acetone	NIST	0.10^{a} (0.07, 0.14)	0.09^{a} (0.06, 0.13)	0.09^{a} (0.06, 0.13)	0.13^{a} (0.09, 0.18)

Differences in lower case letters represent significant differences in emissions between treatments ($\alpha < 0.1$).

[1] Identification (ID) of compounds based upon comparison of retention time and mass spectra with synthetic standards (SS) or comparison of mass spectra using NIST Mass Spectral Search Program (NIST); NIST$_{>50}$ = Denotes NIST match probability greater than 50%

CHAPTER FOUR

SYNTHESIS

The objective of this conclusion chapter is to highlight the novelty of my research and discuss how my experimental findings can advance our understanding of plant-soil feedbacks in terms herbivore resistance.

It is well established that belowground plant-microbe associations can alter whole plant defenses against herbivores (Grunseich et al. 2020; Jung et al. 2012; Sharifi et al. 2018; Shikano et al. 2017). While several studies have suggested that cover crops and management practices might enhance pest resistance through their impact on soil microbiomes (Huang et al. 2013; Kaplan et al. 2018; Pineda et al. 2017), none have specifically shown their ability to alter the emissions of plant volatile organic compounds (VOCs), secondary metabolites important in the mediation of crop-pest interactions. In this thesis, I demonstrate: i) the potential of management practices to condition soils that alter VOC phenotypes of crops, ii) the importance of considering abiotic stressors when assessing the impacts of soil microbes on VOC emission; and iii) the interactive effects of herbivory and soil microbes on VOC emissions. These findings can be combined to develop future experiments to better understand the ways in which soil legacy effects influence the production of plant secondary metabolites, and the subsequent effects on the ecosystem interactions.

In Chapter II, coupling a greenhouse and a field study, we revealed that plants grown in the presence of soil microbes from agricultural fields emit altered VOCs compared to plants grown in sterile soil, highlighting the potential of agricultural soil microbe communities to influence volatile emissions. We revealed that more diverse crop rotations produce altered VOC blends, including key shifts in volatiles known to be important in pest resistance against the wheat stem sawfly (WSS). While we failed to observe a difference in VOC emissions during our first field season, wheat plants grown in rotation with cover crops in our second field season emitted fewer compounds known to be attractive to the WSS. This result suggests that these plants may be less easily detected by ovipositing females when compared to plants grown in rotation with fallow.

While these studies provide support for microbe-mediated priming and induction of plant defenses, we observed extreme differences in plant biomass and emissions of aldehyde compounds—both signs of physiological stress— by treatment, causing us to hypothesize that changes in VOC emissions were not due to plant-microbe interactions alone. In the greenhouse, our conditioning methods may have caused plants grown in soil inoculum to experience stress through negative plant-soil feedbacks that plants grown in sterile soil did not experience. In the second season of our field study, soil moisture data suggest that wheat plants grown in rotation with cover crops experienced limited water availability relative to plants grown following fallow. In the first field season, when we had no evidence of water stress among treatments, we observed no differences in VOC emissions by rotation. Taken together, these results suggest that soil microbes may elicit changes to plant physiology that influence VOC emissions, but their effects manifest only after plants experience some level of environmental stress.

To further support this idea, in Chapter III, we evaluated the interactive effect of herbivory and soil microbe community on VOC emissions by growing wheat in aliquots of soil from fallow or cover crop rotations (taken from the same field from Chapter II). We found no difference in VOC emissions by soil inoculum alone. However, when plants were fed upon by WSS larvae, we observed altered emissions of VOCs, but only in plants grown in cover crop inoculum. Given these observations, it is possible that soil microbes from the cover crop rotation were able prime plant defenses, and their effects became apparent only after plants faced biotic challenge from herbivores. Taken together, our findings from Chapter II and III suggest that the extent to which soil microbes influence plant VOC phenotypes is dependent on the degree of stress the plant is under. More work will be necessary to better understand the role of the soil microbiome on pest resistance across a spectrum of environmental conditions.

Results from this thesis have important implications for research on pest management, plant-soil feedbacks and plant-microbe-insect interactions. Most obvious is the role of farming management practices on plant VOCs. Cover crops and management practices have traditionally been recognized for their ability to influence pest prevalence directly by disrupting pest lifecycles. For example, planting cover crops reduces pest pressure from insects that rely on specific plant hosts for the completion of their lifecycle. This thesis demonstrates that farming practices may have additional, indirect effects on pests through soil legacy effects that influence plant VOC emissions. While soil health has been long recognized as an important component of productive agriculture, this work highlights the soil's additional role in pest suppression and emphasizes the reverberating, unintentional temporal effects that management may have on agroecosystem function through soil legacies.

Our study focused on established crop rotations (i.e. seven- and eight-year-old rotations). However, evidence suggests that many functions of the soil microbiome are resilient to changes caused by land management practices up to, and possibly beyond, ten years (Mackelprang et al. 2018). Thus, altered land management practices may exhibit aboveground changes, while microbial communities may remain relatively static. It is uncertain how this dichotomy between above- and below-ground communities may ultimately influence aboveground interactions and is especially worth exploring in agricultural systems where the introduction of new farming management practices may require several years for soil microbial communities to carry out key functions on plant phenotypes.

This is the first study, to our knowledge, to provide evidence that whole soil microbiomes can alter plant VOCs, not just single strains. This is important not only for agricultural systems, but also natural systems where VOCs mediate important plant-insect ecosystem interactions including herbivory, pollination, and biological control. Shifts in plant-microbe interactions over community succession has previously been shown to influence plant resistance to herbivores (Howard et al. 2018), and our work provides additional mechanisms through which soil microbes may influence aboveground community dynamics. Clearly, the bottom-up effects of the soil microbiome on plant physiology and subsequent community interactions is an important consideration for future ecological work.

This thesis lays the groundwork for future studies aimed at elucidating the potential of different cover crops and management practices to condition soil microbe communities that enhance crop protection. Traditionally, cover crop rotations are chosen to avoid pathogenic microbes; however, future research should focus on how cover crop rotations can be optimized to encourage the accumulation of beneficial soil microbes that enhance crop production through increased growth and resistance. Surprisingly, phylogenetic relatedness of crop pairings in rotation seems to be a poor predictor for their success (Ingerslew & Kaplan 2018; Miller & Menalled 2015) despite being widely accepted in agronomic practice. Better understanding the species-specific effects of soil conditioning will lend insight to designing better crop rotations that optimize plant growth and protection.

This work provides strong evidence that abiotic and biotic environmental conditions interact with plant-microbe associations to influence the degree of change within the VOC aspect of the plant phenotype. Previous work has demonstrated the effect of abiotic environmental factors such as drought stress on plant-microbe associations, causing symbioses to range from beneficial to pathogenic. However, more research is necessary to understand how these factors together influence secondary chemistry in plants. Herbivory has also been shown to influence the strength of plant-microbe associations on plant phenotypes. One study exploring the effects of aboveground herbivory on plant-soil feedback effects for explaining plant species abundance found that plant-soil feedbacks alone have limited power in explaining species abundances and that herbivory had stronger effects on plant biomass and growth in the landscape (Heinze et al. 2020). While this study generally focuses on plant-soil feedbacks, it is well-established that plant-soil feedbacks are partially defined by soil microbial communities.

The degree to which microbe-mediate plant-soil feedbacks exert influence over secondary metabolism likely changes in response to factors such as drought, nutrient availability, and pests. It is important to better understand these interactions in order to determine whether plant-soil feedbacks can truly reduce susceptibility to insect pests in agricultural and natural systems, or whether their effects are overpowered by other environmental conditions. Specifically, more research is necessary to understand the role of the microbiome in determining plant volatile blends versus other factors that are also known to influence plant volatiles.

Finally, this work lends itself to understanding the important implications of microbe x environmental shifts in plant VOCs in determining insect behavior of pests and their natural enemies. While this thesis does not specifically test insect behavior, this is a critical next step for understanding the role of farming management practices on pest resistance in crops. While many plant-insect systems have identified bioactive compounds that influence behavior and host-location, there remain many unknowns in how compound blends are perceived and influence insect behavior through synergistic or antagonist effects (Junker et al. 2018). Future work should test how the altered VOC emissions observed here influence behavior of the wheat stem sawfly and/or braconid parasitoids through the use of insect bioassays. Further, field work should be conducted to determine whether differences observed in laboratory experiments play out similarly on a landscape scale in the field. For example, future studies could compare the effect of farming management practices on parasitoid recruitment by comparing wheat fields that are lightly or heavily infested by sawfly larvae and quantify adult parasitoid presence and the rate of sawfly parasitism. While there is a rich potential for the application of the plant-soil feedback framework for reducing insect pests in agriculture, there are many nuanced variables in the system that must be better understood in order to better understand the mechanisms of action and the parameters necessary for effective pest management.

This dissertation sets forth potential mechanisms by which farming management practices influence VOC emissions by crops through plant-soil feedbacks. Overall, this research contributes to the body of knowledge on the role of soil microbes in the mediation of plant-insect interactions with important implications for the integrated management of agricultural pests.

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