MICROARTHROPOD-MICROBE INTERACTIONS ON SOIL CARBON DYNAMICS IN BIOENERGY CROPPING SYSTEMS

By

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ABSTRACT

Soils, together with the vegetation they support, constitute the largest terrestrial carbon reservoir. However, human-driven land use change has caused significant carbon depletions in soils worldwide, particularly in managed ecosystems. As rising emissions threaten to exacerbate an already worsening climate crisis, strategies to replenish soil carbon stocks are imperative to mitigate further global warming. This is the main motivation for expanding second-generation bioenergy crop production in North America, with potentially substantial carbon gains possible in bioenergy cropping systems established on degraded soils. Yet in the face of uncertainties regarding the factors regulating carbon turnover and stabilization across diverse bioenergy cropping systems, the true potential for these systems to accrue carbon at meaningful rates remains unresolved. As the soil carbon cycle is directly controlled by microbial and plant processes, research has largely focused on investigating the physiological, climatic, and physiochemical factors regulating them in bioenergy cropping systems. However, very little is known about the microarthropods, small yet highly abundant and diverse soil fauna ubiquitous across ecosystems, these systems harbor. Microarthropod activity has long been known to have an important role in organic matter decomposition, yet they directly contribute relatively little to net soil carbon gains and losses due to their low biomass and metabolism. However, microarthropods can strongly influence microbes via a variety of mechanisms, including via direct microbivory, promoting nutrient availability, and altering organic matter quantity and chemistry. Thus, microarthropods can indirectly affect soil carbon dynamics by regulating microbial community structure, activity, and access to organic matter. The strength and direction of these microarthropod-microbe effects on carbon accrual, while presently unclear, will largely depend on the microarthropods and microbial communities involved, both of which are strongly dependent upon aboveground land use. In this dissertation, I approached this broad yet important knowledge by addressing the following key uncertainties: **1)** what microarthropods are currently present in bioenergy cropping systems, **2)** how do cropping system attributes affect their community structure, and **3)** given the multitude of interactions and mechanisms operating simultaneously, what is the net impact of microarthropod-microbe interactions on soil carbon dynamics in these systems? In Chapter 1, I reviewed the literature

on soil fauna effects on soil carbon cycling, narrowing my focus on these dynamics in context of perennial grass bioenergy cropping systems, and identifying where research is most needed to someday incorporate faunal activity in soil carbon models. In Chapter 2, I addressed the first and second major uncertainties by surveying microarthropod communities from bioenergy cropping systems ranging from an annual monoculture to a perennial polyculture. Over the span of two years, I found that perennial cropping systems consistently supported greater total microarthropod and mite abundances compared to the annual system. Having characterized the microarthropod communities in these systems, I addressed the third major uncertainty by conducting two greenhouse mesocosm experiments to evaluate the potential effects of these communities on key predictors of soil carbon accrual and stability. In Chapter 3, I investigated the effects of microarthropods from either a perennial or annual monoculture on microbial carbon use efficiency using a ^{18}O-water tracer method. In Chapter 4, I narrowed my focus to perennial monoculture (switchgrass) communities to assess the relative effects of microarthropods and nematodes, alone and in combination, on nitrogen mineralization and switchgrass productivity. While I did not find conclusive evidence to suggest an effect on carbon use efficiency, I did find that microarthropods in combination with nematodes stimulated nitrogen mineralization from litter and subsequent assimilation into switchgrass roots, though only nematodes individually retained this positive effect. I conclude in Chapter 5 by reviewing the key takeaways from this research, discussing the broader implications of my findings as well as some of the methodological challenges associated soil fauna research I encountered, and suggesting future studies to address remaining questions. Despite the daunting uncertainties that remain, continued research into the complex interactions between microarthropods, microbes, and bioenergy crops will doubtlessly be important to better understand their potential contributions to soil carbon accrual and storage in bioenergy cropping systems.

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LIST OF ABBREVIATIONS

AMF	arbuscular mycorrhizal fungi
ANOVA	analysis of variance
BCSE	Biofuel Cropping System Experiment
C	carbon
CUE	carbon use efficiency
GLBRC	Great Lakes Bioenergy Research Center
GLMM	generalized linear mixed model
KBS	W. K. Kellogg Biological Station
MBC	microbial biomass carbon
MBN	microbial biomass nitrogen
N	nitrogen
NMDS	non-metric multidimensional scaling
O	oxygen
PERMANOVA	permutational multivariate analysis of variance
PGCS	perennial grass bioenergy cropping system
SOC	soil organic carbon
SOM	soil organic matter
WHC	water holding capacity

CHAPTER 1: PERENNIAL GRASS BIOENERGY CROPPING SYSTEMS: IMPACTS ON SOIL FAUNA AND IMPLICATIONS FOR SOIL CARBON ACCRUAL[1]

ABSTRACT

Perennial grass energy crop production is necessary for the successful and sustainable expansion of bioenergy in North America. Numerous environmental advantages are associated with perennial grass cropping systems, including their potential to promote soil carbon accrual. Despite growing research interest in the abiotic and biotic factors driving soil carbon cycling within perennial grass cropping systems, soil fauna remain a critical yet largely unexplored component of these ecosystems. By regulating microbial activity and organic matter decomposition dynamics, soil fauna influence soil carbon stability with potentially significant implications for soil carbon accrual. I begin by reviewing the diverse, predominantly indirect effects of soil fauna on soil carbon dynamics in the context of perennial grass cropping systems. Since the impacts of perennial grass energy crop production on soil fauna will mediate their potential contributions to soil carbon accrual, I then discuss how perennial grass energy crop traits, diversity, and management influence soil fauna community structure and activity. I assert that continued research into the interactions of soil fauna, microbes, and organic matter will be important for advancing our understanding of soil carbon dynamics in perennial grass cropping systems. Further, explicit consideration of faunal effects on soil carbon can improve our ability to predict changes in soil carbon following perennial grass cropping system establishment. I conclude by addressing the major knowledge gaps that should be prioritized to better understand and model the complex connections between perennial grass bioenergy systems, soil fauna, and carbon accrual.

INTRODUCTION

Bioenergy production will likely be a key element for reaching renewable energy and carbon (C) emission mitigation targets to limit further climate change. Stimulated by global food insecurity and environmental concerns, focus has shifted away from first-generation biofuels

[1] Zahorec, A., Reid, M. L., Tiemann, L. K., & Landis, D. A. (2022). Perennial grass bioenergy cropping systems: Impacts on soil fauna and implications for soil carbon accrual. *GCB Bioenergy, 14*(1), 4-23.

produced from food crops to second-generation biofuels produced from non-food sources (Nanda et al., 2015; Valentine et al., 2012). Following extensive research initiated by the US Department of Energy into diverse biomass sources, perennial grasses such as switchgrass (*Panicum virgatum*) are now widely believed to be the future of bioenergy in North America (US DOE, 2011; Wright & Turhollow, 2008; McLaughlin & Kszos, 2005) (**Table 1.1**). Multiple key advantages of perennial grass production over that of first-generation and annual crops have been identified, suggesting that dedicated perennial grass energy crops have the greatest potential for sustainable biomass production. Perennial grasses can successfully grow on degraded, marginal soils that are unsuitable for annual crop production, minimizing competition between bioenergy and food crops for land even as biomass feedstock demands increase (Tilman et al., 2009). Additionally, perennial grasses require less intensive management and fewer chemical inputs than their first-generation counterparts to achieve economically viable yields, providing numerous environmental benefits such as reduced nitrogen (N) leaching and greenhouse gas emissions (Robertson et al., 2017). There is also growing evidence that perennial grass bioenergy cropping systems (PGCS) promote greater biodiversity and ecosystem services, such as increased pollinator abundance and biological pest suppression, than annual, more intensively managed systems (Núñez-Regueiro et al., 2021; Landis et al., 2018; Werling et al., 2014; Meehan et al., 2012; Robertson et al., 2012; Bellamy et al., 2009). A final prominent advantage is the expectation that PGCS have greater potential to accrue and store soil organic C (SOC) (McGowan et al., 2019; Anderson-Teixeira et al., 2009; Fargione et al., 2008). However, uncertainties over the potential for land-use changes associated with PGCS establishment to influence SOC stability and storage remain critical concerns (Agostini et al., 2015). As the viability of bioenergy as a C mitigation strategy hinges on the ability of bioenergy cropping systems to accrue SOC, further knowledge of SOC dynamics under PGCS is crucial for understanding the long-term sustainability of these systems.

Given the critical importance of SOC accrual for the successful expansion of bioenergy crop production (Lemus & Lal, 2005), research into SOC dynamics under PGCS has greatly expanded in recent decades. Such efforts have reaffirmed the fundamental roles of plants and soil microbes in SOC cycling. Primary production largely determines the amount of fresh SOC

entering soils both belowground via the root system and aboveground as litter. High SOC inputs to PGCS result from the substantial belowground productivity characteristic of perennial grasses as well as surface litter retention in the absence of tillage (Carvalho et al., 2017; Anderson-Teixeira et al., 2013). Once in soils, the fate of these inputs largely depends on soil microbes, with their community dynamics and activity driving both SOC decomposition and stabilization (Kallenbach et al., 2016). Compared to annual cropping systems, the increased activity and densities of microbes, especially for fungi, in PGCS suggest greater potential SOC stabilization and storage within these systems (Jesus et al., 2016; Liang et al., 2012). Furthermore, insights into the interactions between soil microbes and soil characteristics emphasize the importance of microbe-soil dynamics for understanding SOC stabilization within PGCS (Kravchenko et al., 2019; Tiemann & Grandy, 2015). Taken together, SOC accrual in PGCS is thought to occur as a consequence of complex interactions between microbes, soil organic matter (SOM), and mineral soil, with climate and soil characteristics serving as bottom-up regulators of microbial activity and SOC access. However, this view of SOC accrual ignores the contributions of soil fauna, a vital component of soil ecosystems with the potential to exert both bottom-up and top-down control over microbes.

Soil fauna (henceforth "fauna") comprise much of the biodiversity belowground and perform wide ranging functions essential for crop production and overall ecosystem stability (**Table 1.2**). All of the basic processes governing SOC — addition, loss, transformation, and translocation — are influenced directly or indirectly by fauna to some degree (Osler & Sommerkorn, 2007; Fox et al., 2006; Seastedt, 1984). Despite recognition that faunal activity can substantially affect SOC fluxes, the extent to which fauna influence SOC stocks, and hence the ability of soils to accrue SOC, remains uncertain (Schmitz et al., 2014). Greater research into the potential for fauna to regulate SOC stocks in addition to fluxes is needed to improve our understanding of SOC dynamics overall. Indeed, wide discrepancies between major ecosystem models and limited predictive power remain major obstacles for simulating SOC cycling, even with the explicit inclusion of microbial dynamics. This suggests that current models fail to completely incorporate the key controls and mechanisms governing SOC dynamics, further emphasizing the need to consider faunal effects on SOC (Filser et al., 2016; Grandy et al., 2016;

de Vries et al., 2013). However, major uncertainties surrounding fauna community structure, function, and ecological interactions must be addressed before their explicit incorporation into SOC models will be possible. While investigations into these knowledge gaps are necessary across diverse ecosystems, I argue that they are especially needed in managed systems such as PGCS, due to the opportunity for new knowledge to inform management practices to promote SOC accrual.

From the limited research specific to bioenergy cropping systems, evidence suggests that PGCS better support native fauna than annual, more intensively managed systems. However, questions remain regarding faunal responses to different attributes of PGCS, such as crop type, diversity, and management requirements. Less clear is the potential for such cropping system effects to alter the strength or direction of faunal effects on SOC accrual in PGCS. To address these uncertainties, my objectives are to **1)** review the effects of fauna on SOC dynamics, reporting findings from North American PGCS when available and drawing inferences from studies in other managed and natural ecosystems when not, **2)** discuss how plant traits and management practices typical of PGCS influence or are likely to influence fauna community structure and function in ways likely to strongly impact their effects on SOC, **3)** and identify the critical knowledge gaps hindering our ability to parameterize faunal effects on SOC dynamics, using data from a switchgrass bioenergy cropping system to illustrate where future research in this topic is most needed. I synthesize findings from studies conducted specifically in the context of bioenergy as they exist, though limited research into fauna and their effects on SOC within PGCS necessitates drawing inferences from studies conducted in other, comparable arable and natural lands. While I narrow my focus in this review to North American PGCS, further investigations into the interconnections between belowground biodiversity and SOC dynamics will doubtlessly be an important aspect in better understanding the sustainability and efficacy of bioenergy cropping systems dominant in other parts of the world and climate regions.

SOIL FAUNA EFFECTS ON SOC DYNAMICS

Direct effects: SOC inputs and losses from fauna

Fauna-derived SOC inputs include living biomass, necromass, exuviae, fecal pellets, and

other biosynthesized materials. Temperate grasslands support high faunal densities compared to other biomes (Petersen & Luxton, 1982). Similarly, greater densities under PGCS relative to annual systems have been reported for diverse faunal groups: detritivorous invertebrates (Hedde et al., 2013) including collembola (Bellamy et al., 2009), mites (Chapter 2), and earthworms (Emmerling, 2014; Felten & Emmerling, 2011; but see Briones et al., 2019) as well as carabid beetles (Ward & Ward, 2001). Additionally, fecal pellets can be found in abundance in the surface layers of grassland soils (Davidson et al., 2002). While this could indicate a greater quantity of fauna-derived SOC under PGCS, these inputs likely comprise only a small fraction of total SOC stocks. At the Kjettslinge field experiment, fauna only contributed between 1.63-5.48% to total soil biomass, even less when root biomass was considered (Andrén et al.,1990). Similarly, faunal biomass C was estimated to be less than 4% that of soil microbes in temperate grasslands (Fierer et al., 2009). The low relative biomass of fauna suggests that the vast majority of SOC in PGCS will be of plant or microbial origin, with only minor faunal inputs to total SOC stocks.

Direct losses of SOC from fauna result from SOC consumption, most prevalently through detritivory and microbivory, and subsequent respiration of unassimilated C. How much SOC is lost from PGCS by faunal respiration is unknown, though it can be assumed that fauna make lesser contributions to total soil respiration than microbes. Studies from other systems indicate that faunal respiration accounts for as much as ~10% of total soil respiration (Andrén et al., 1990; Schaefer, 1990; Reichle, 1977), though lower faunal contributions are also possible (Persson et al., 1980). While microbes should have the greatest direct influence on SOC losses from PGCS, it remains unclear if fauna will have significant, albeit secondary, direct effects on SOC loss.

The balance between direct faunal SOC inputs, including biomass and biosynthesized products, and respiratory losses determines the net contribution of fauna on SOC stocks. Historically, this was estimated by combining faunal community structure, life history, and energetics data to calculate mean annual SOC flow through fauna. This requires a great deal of taxa-specific information which is largely unexplored except for a relatively small number of soil food web studies. Even less understood is the relative stability of faunal SOC inputs, which is

necessary to determine if faunal SOC gains are ultimately accrued. It is now known that molecular recalcitrance is a poor predictor of SOC stability (Schmidt et al., 2011), challenging traditional assumptions that faunal input C:N ratios should reliably correlate with their turnover times. Faunal fecal production is predicted to stimulate aggregate formation (Maaß et al., 2015) and can enhance dissolved organic C leaching (Joly et al., 2020), both of which could promote long-term fecal-SOC storage. Further research into partitioning of faunal-SOC inputs in PGCS into active, slow, and passive SOC pools will be necessary to understand the true direct contributions of fauna to SOC accrual.

Indirect effects: Interactions with microbes and SOM

While fauna have weak direct effects on SOC gains and losses, their numerous indirect effects on SOC may be significant regulators of SOC dynamics (Wolters, 2000; Seastedt, 1984). These indirect effects can be categorized as direct or indirect microbial interactions or SOM decomposition effects. While this attempts to make broad distinctions between the predominant mechanisms driving these interactions for ease of discussion, these categories are often overlapping. I focus my attention primarily on fauna interactions with microbes and SOM as they have received the greatest research attention to date, and point readers to Gan and Wickings (2020) and Bonkowski et al. (2009) for greater detail on fauna-plant interactions and their potential effects on SOC dynamics.

Effects on microbes: Microbivory can strongly affect SOC decomposition and stability by exerting top-down control over microbial activity and community dynamics. Detritivorous and microbivorous fauna have been found to alter microbial activity (Wickings & Grandy, 2011; Crowther et al., 2012), biomass (Trap et al., 2016; Bradford et al., 2002), and community composition (Janoušková et al., 2018), with the strength and direction of faunal effects depending upon microbivore identity and grazing intensity. A major way in which fauna grazing can stimulate microbial activity is by enhancing microbial turnover and subsequent N mineralization (Bardgett & Chan, 1999). Fauna have significant influence over N availability in soils, with ~30% of total N mineralization attributed to their activity (Verhoef & Brussaard, 1990). This can have important implications on microbial and plant growth, especially when N is limiting as is the case for many marginal soils. Faunal grazing can stimulate microbes via other

mechanisms as well, such as grazing triggering compensatory growth (Hedlund & Augustsson, 1995; Bengtsson et al., 1993; Hanlon & Anderson, 1979), though further research is needed to determine the extent to which such mechanisms observed in simplified microcosm studies occur in natural settings. Taken together, microbivory can significantly influence microbial activity, growth, and composition. Microbivory can thus indirectly affect SOC by altering the quantity of microbial SOC inputs or losses as well as how microbes access and utilize SOM. The strength and direction of the effect on SOC will ultimately depend on the balance between stimulatory and inhibitory effects of microbivory as well as the identities of the microbes preyed upon.

Fauna also have important bottom-up effects on microbes by influencing the physical and chemical properties of soils. These bottom-up effects can arise due to changing microclimatic conditions in response to faunal activity. Tunneling and burrowing by macrofaunal "ecosystem engineers" can profoundly alter soil porosity, water and gas movement, temperature, and chemistry, all of which are important abiotic controls over microbial activity. Ant activity has been shown to have a range of impacts on microbial diversity and activity in north temperate grasslands (Wills & Landis, 2018), with potentially important implications for how these microbes process SOC. Additionally, fauna alter the molecular, chemical, and physical structure of SOM via numerous mechanisms including bioturbation, ingestion/excretion, and litter fragmentation, subsequently impacting its accessibility to microbes (Filser et al., 2016; Wickings & Grandy, 2011). Macrofauna can also enhance the incorporation of C in microaggregates within larger macroaggregates, indicating their potential for macrofaunal activity to enhance SOC stabilization by increasing the amount of C physically protected from microbial decomposition (Franco et al., 2020; Fonte et al., 2007). Thus, faunal activity can influence microbial activity by regulating its access to SOC which in turn affects SOC stabilization. Lastly, belowground herbivory by fauna can have important consequences for rhizosphere microbes, though more research is necessary to understand the implications of these multi-trophic interactions on SOC dynamics (Gan & Wickings, 2020).

SOM decomposition: Detritivorous fauna, particularly meso- and some macrofauna, play a significant role in SOM decomposition with important consequences for plant residue

stabilization and turnover. There is general consensus from litter bag studies that fauna accelerate decomposition, with the strongest positive faunal effects found in grasslands (García-Palacios et al., 2013). Further, Seastedt (1984) reported a ~22% average increase in grass litter decomposition rate with faunal access to litter bags. On the contrary, faunal suppression had no noticeable effect on litter mass loss in miscanthus (*Miscanthus x giganteus*), switchgrass, or prairie bioenergy cropping systems, though fauna had largely recolonized insecticide treated units by the end of the study (Zangerl et al., 2013). As faunal effects on litter mass loss vary with climate and litter quality (Sauvadet et al., 2017; González & Seastedt, 2001), more research within PGCS is needed to predict the impact of fauna on litter turnover in these systems.

Detritivorous fauna strongly influence the physical and chemical properties of litter which can significantly affect how litter-C interacts with decomposer microbes and and soil surfaces. These effects, which are not always captured in litter bag studies, are expected to have important implications for SOC fate and stability. Litter-C becomes more accessible to microbes following faunal fragmentation, gut processing, and excretion (Edwards, 2000; Petersen & Luxton, 1982), such that fauna help to facilitate C flow from litter to microbial pools. Faunal processing of litter is also expected to influence the production and leaching of dissolved organic C (Joly et al., 2020; Osler & Sommerkorn, 2007; Cragg & Bardgett, 2001), a critical component of stabilized SOC for its ability to associate with mineral surfaces (Cotrufo et al., 2019). Both mechanisms can have substantial impacts on overall SOC stability, as was found in a three year decomposition study conducted in a tallgrass prairie. Positive effects of fauna on big bluestem (*Andropogon gerardii*) decomposition during the first 18 months increased microbial uptake of litter-C during the early stages of decomposition (Soong et al., 2016). Another early-stage effect of fauna was increased incorporation of litter-C and N into silt- and clay-sized SOC pools, suggesting enhanced dissolved organic C leaching. When simulated in DayCent, these faunal effects on litter-C transfers into microbial and slow SOC pools increased total SOC by 11% over two centuries. This study puts forth compelling evidence that by altering the properties of SOM, fauna can indirectly promote SOC accrual by affecting when and how microbes, as well as mineral surfaces, associate with SOC inputs.

CROPPING SYSTEM EFFECTS ON SOIL FAUNA

In the previous section, I reviewed the diverse ways fauna directly influence SOC dynamics. I find evidence from the current literature that fauna make relatively minor direct contributions to SOC gains and losses. In contrast, indirect faunal effects on SOC, as mediated by their impacts on microbes and SOM decomposition, can strongly influence the fate and stabilization of SOC. It can be surmised that such indirect effects of fauna should therefore have the greatest potential to impact SOC accrual potential, with direction of their effects on SOC accrual depending upon how fauna alter the interactions between SOC, microbes, and the mineral soil. Theoretically, faunal effects that enhance food web C efficiency and SOC stabilization can promote SOC accrual, whereas the opposite can result in SOC depletion as respiratory losses outpace SOC gains (**Fig. 1.1**). The strength of faunal effects on SOC accrual should depend in part upon the activity and structure of faunal communities, which is largely dependent upon aboveground land-use. For this reason, further research into land-use impacts on fauna and their ability to indirectly affect SOC will be essential to understanding their potential contributions to SOC accrual. This will be especially important in PGCS, wherein management practices can be readily implemented to promote positive faunal effects on SOC accrual.

While studies exploring the impacts of bioenergy cropping system establishment on native biodiversity continue to emerge, few have evaluated the responses of soil fauna (Lask et al., 2020; Immerzeel et al., 2013; Dauber et al., 2010). As stated earlier, current research indicates that PGCS can better support native fauna compared to annual cropping systems. However, the potential variability in faunal community structure, diversity, and activity across diverse PGCS remains largely uninvestigated. Physiological and morphological trait variation across different candidate perennial grass energy crops is expected to have differing effects on fauna. Indeed, Emery et al. (2017) reported significant differences in nematode community composition between switchgrass and miscanthus, with miscanthus communities dominated by plant-parasitic nematodes. Plant diversity and management differences across PGCS may also impact faunal communities with potential implications for their effects on SOC. Therefore, to understand how SOC dynamics in PGCS are influenced by fauna, it will be important to consider

how faunal communities and activities are affected by different PGCS characteristics. I begin by discussing the potential bottom-up effects of perennial grass energy crops on fauna by serving as sources of food and habitat, followed by cropping system characteristics, specifically crop diversity and N additions, as they pertain to faunal communities.

Perennial grasses as the base of soil food webs

Perennial grass energy crops represent a diversity of physiological, morphological, and phenological traits. These traits largely determine the quality and quantity of SOC inputs in soils and thus serve as important bottom-up regulators of consumers in soil food webs. Unlike for aboveground food webs, however, the influence of plant-specific traits in shaping soil fauna communities are expected to be greatest at large spatial and temporal scales, whereas interspecific interactions between soil biota are assumed to be the primary drivers of soil community dynamics at local levels (Wardle, 2006).

The quality of perennial grass residues has important implications for soil food web structure. Bacteria and their consumers dominate the rhizosphere where labile, high-quality substrates (e.g., root exudates) are most heavily concentrated, whereas more C-rich litter selects for increased fungi and fungivore activity (**Fig. 1.2**). Grass litter is relatively low-quality, with warm-season C4 grasses generally producing higher C:N, more recalcitrant residues than cool-season C3 grasses (Vivanco & Austin, 2006; Baer et al., 2002). Across five PGCS at the W. K. Kellogg Biological Research Station (KBS), average surface litter C:N over seven years ranged from 36.2 in native C3 and C4 polycultures to 64.4 for miscanthus monocultures (Robertson, 2021). Residue quality may impact fauna by altering soil food web structure, as C:N is known to influence microbial community composition (Liang et al., 2017; Waldrop & Firestone, 2004). Further, the production of high C:N energy crops on bacterial-dominated soils typical of agroecosystems may lead to N competition between fauna and microbes (Ernst et al., 2009), which may be especially important in marginal soils. However, the limited responses of diverse faunal groups to residues of differing quality may indicate that residue quality impacts on fauna may only become appreciable when C:N differences are very large (Sauvadet et al., 2016; Porazinska et al., 2003). For instance, earthworm densities were similar under miscanthus and SRC willow systems despite miscanthus, a C4 grass, producing higher C:N litter, but densities in

these PGCS were strongly reduced compared to those in an annual arable system (Briones et al., 2019). Soil food webs also exhibit a relatively high degree of omnivory and generalism, and hence moderate-to-minor differences in residue quality between perennial grass energy crops may be largely negligible.

Primary productivity influences soil food web structure by controlling the quantity and relative partitioning of above- and belowground plant-C entering soils. How much plant productivity ultimately enters soil food webs as belowground production or litter depends on interactions between species- or variety-specific traits and external factors. Across three PGCS established in Illinois, US, belowground biomass and surface litter inputs were highest in miscanthus, intermediate in switchgrass, and lowest in native prairie cropping systems (Anderson-Teixeira et al., 2013). The quantity of above- or belowground inputs perennial grasses provision is also regulated by climatic variables such as mean annual temperature and precipitation as well as soil characteristics. Indeed, belowground biomass and litter production differed greatly for both switchgrass and native prairie systems depending on PGCS site geography (von Haden et al., 2019). As soil food webs are thought to be largely donor-controlled, productivity helps determine the faunal densities PGCS can sustain. Thus, perennial grass energy crops that provision greater residue quantities to soil can theoretically support more abundant belowground communities. However, few empirical studies have specifically investigated the effects of residue quantity on fauna. While positive effects of fauna on above- and belowground productivity have been observed, it remains uncertain what, if any, feedbacks these changes in residue quantity have on faunal communities (Bais et al., 2006). Fauna consumers are widely believed to be less constrained by resource competition compared to those aboveground, and many possess physiological adaptations to persist during periods of resource scarcity or environmental unfavourability. Indeed, litter quantity was found to have only transient effects on fauna in a cultivated soil, with faunal densities similarly high across soils with differing amounts of litter after 11 mo, even in treatments with no litter (Sauvadet et al., 2016). Thus, the influence of primary productivity in PGCS, and thus residue quantity, may have only minor effects on fauna.

Perennial grasses as habitat provisioners and modifiers

Plants exert significant influence over the physical and climatic conditions of surface soils which has important implications for soil microclimate and habitat conditions. Many traits characteristic of perennial grasses linked to their enhanced environmental sustainability can also benefit fauna. The greater root biomass associated with perennial grasses enhances soil porosity, facilitating water drainage and gas exchange as well as creating channels for faunal migration (Marshall et al., 2016). This improved soil structure increases the volume of soil accessible to fauna, connectivity between resource patches, and spatial heterogeneity within the soil matrix. This may become increasingly important in subsurface soils as SOM becomes scarcer and bulk density increases with depth. Meso- and microfauna should be especially sensitive to root effects on soil structure, as they rely on pre-existing pore spaces to move throughout the soil. Tallgrass species such as big bluestem tend to have greater fine and coarse root production at depth compared to shortgrass species (e.g., blue grama, *Bouteloua gracilis*, also a C4 grass) with shallower root systems (Craine et al., 2002). Switchgrass and miscanthus root systems can extend several meters below the soil surface, though environmental conditions, soil characteristics, and management can significantly influence the depth and distribution of their roots (Mann et al., 2013; Ma et al., 2000). Other traits affecting root architecture may also influence fauna in PGCS, especially microfauna and root-associate groups. For instance, under blue grama dominated fields, increased specific root length and branching intensity corresponded to elevated and depressed densities of root feeding nematodes, respectively (Otfinowski & Coffey, 2020). While variation in perennial grass root architecture has been shown to have important implications for SOC decomposition (de Graaff et al., 2013), the extent to which fauna effects on SOC may be impacted by root architecture differences between species remains unknown.

Furthermore, the lack of tillage in PGCS allows the accumulation of a stable litter layer at the soil surface. The litter layer provides habitat for surface-dwelling species and regulates soil microclimate conditions as it buffers the underlying soil from diurnal temperature fluctuations and moisture loss (Andrade et al., 2010), both of which can have important impacts on fauna. Though the effects of litter on fauna in the context of PGCS specifically remains to be

investigated, litter has been linked to greater densities for earthworms (Melman et al., 2019), isopods (Souty-Grosset & Faberi, 2018), microarthropods (Santos et al., 1978), and other surface-dwelling species (Facelli, 1994) in a range of habitat types. As fauna are highly sensitive to soil temperature and moisture, PGCS management practices that influence litter layer thickness and stability are predicted to have substantial effects on fauna communities. For instance, baling after harvest can strongly depress the amount of aboveground residues that become incorporated into the litter layer (Kantola et al., 2017; Anderson-Teixeira et al., 2013), which may negatively impact fauna.

Cropping system diversity effects

While plant diversity is a driver of aboveground diversity and abundance in PGCS (Webster et al., 2010), the effects on belowground communities are expected to be much more complex. Faunal responses to increasing plant richness are largely idiosyncratic across systems (Wardle, 2006; de Deyn et al., 2004, Hooper et al., 2000). As faunal communities exhibit high functional redundancy and resiliency, plant composition, particularly plant functional trait composition, is predicted to influence fauna more strongly than plant diversity *per se* (Beugnon et al., 2019). To date, the potential relationships between plant richness, plant functional diversity, and faunal diversity remain poorly understood within PGCS. Restored prairie cropping systems containing a mix of native grasses, legumes, and forbs contained more diverse ant communities than switchgrass (Helms IV et al., 2020). In contrast, nematode diversity under switchgrass stands was similar regardless of whether switchgrass was grown in monoculture or within a diverse prairie polyculture (Bliss et al., 2010). While nematodes have smaller spatial distributions than meso- and macrofauna and thus the potential effects of diversity may not have been captured, it is also likely that different size classes, and thus different functional groups, may exhibit variable responses to plant diversity.

While plant and faunal diversity appear to be relatively uncoupled, plant diversity has been found to influence other aspects of belowground communities that may become important when considering faunal effects on SOC. Greater plant richness can increase the quality range for residues entering soil food webs, potentially allowing more trophic levels to coexist than when plant richness, and thus residue quality diversity, is low. Indeed, there is

evidence that plant diversity aboveground is associated with increased soil food web complexity. Eisenhauer et al. (2011) reported functional shifts in nematode communities as a function of grassland plant richness, with high-diversity grasslands supporting increased fungivorous and predatory nematodes and relatively lower herbivorous nematodes compared to species-poor grasslands. Furthermore, Helms IV et al. (2020) analyzed arthropod food webs across diverse bioenergy cropping systems and found restored prairie polycultures supported longer food chains than switchgrass monocultures. Plant diversity may also influence fauna community structure indirectly. For instance, perennial grass energy crop monocultures produce significantly less fine roots than perennial polycultures (Sprunger et al., 2017), which can influence the quality and quantity of root-derived SOC entering soil food webs as well as soil microhabitat or microclimate conditions.

A final aspect of plant diversity to consider is the potential effect of unplanned non-crop diversity (e.g., weeds). No-till, low-input PGCS can contain relatively high weed diversity and biomass, especially after initial planting (Werling et al., 2014; Holguin et al., 2010). Weeds, which inflate plant diversity and heterogeneity within PGCS, can serve as additional habitat and food sources for fauna. Weed biomass and diversity can promote soil arthropod abundance and diversity with potentially significant impacts on soil food web structure. Following herbicide application and subsequent herbicide-resistant weed invasion, Wardle et al. (1999) observed increased faunal abundances in agricultural fields, indicating that fauna were more influenced by plant community changes than the herbicide itself. Additionally, Semere and Slater (2007) greater carabid beetle diversity and abundance associated with miscanthus bioenergy cropping systems compared to reed canary grass, but that these differences resulted from the greater abundance of weeds in miscanthus rather than crop type. As PGCS generally receive no or low weed control, weeds may provide beneficial diversity effects for belowground communities, the extent to which warrants further investigation.

Impacts of nitrogen addition

Land-use intensification has consistently strong adverse effects on soil fauna (de Vries et al., 2013). Reduced management intensity and land conversion (e.g., conventional annual cropping systems to grassland) have a range of potential benefits on soil communities (Tsiafouli

et al., 2015; Felten & Emmerling, 2011; Postma-Blaauw et al., 2010) which are expected to favor fauna in minimally managed PGCS. While management practices known to be detrimental to fauna (e.g., annual tillage) are largely absent, many PGCS still receive some degree of N fertilization. Within the United States, a range of 67-110 kg N ha^{-1} yr^{-1} is recommended for native warm-season grasses, while cool-season grasses have greater N demands (Brejda, 2000). Though this is a reduction compared to the amount of N required for annual systems, the strong effects of N additions on soil chemistry have the potential to impact fauna even at relatively low fertilization rates. For this reason, the potential for N additions in PGCS to affect fauna and their effects SOC dynamics warrant investigation.

N additions can indirectly affect fauna via their substantial impacts on soil microbial communities (Geisseler & Scow, 2014; Fierer et al., 2012). Increased bacterial dominance (de Vries & Bardgett, 2012; de Vries et al., 2006) as well as reductions in sensitive functional groups such as arbuscular mycorrhizal fungi (AMF) and gram-negative bacteria (Zhang et al., 2018; Oates et al., 2016; Leff et al., 2015) have been associated with increased N fertilization. These changes in microbial community structure may have cascading effects on higher trophic levels. The lower fungi-to-bacteria ratio in N fertilized soils should favor bacterivores and disfavor fungivores, as has been reported with nematodes (Emery et al., 2017; Gruzdeva et al., 2007; Murray et al., 2006; but see Ikoyi et al., 2020). However, faunal responses to N addition are difficult to generalize, perhaps in part due to the types of N additions applied varying across studies (**Table 3**). A recent meta-analysis reported generally negative effects of fertilization on faunal diversity, with high variability in faunal response depending upon the type, amount, and duration of N additions (de Graaff et al., 2019).

Despite this variability in faunal responses across studies, evidence suggests that organic, ecologically based N additions are generally better for fauna (de Graaff et al., 2019). Incorporation of legumes as cover crops enhanced invertebrate richness as well as macrofauna abundance and diversity (Sileshi et al., 2008; Blanchart et al., 2006; Sileshi & Mafongoya, 2006). Organic N fertilizers may also benefit soil fauna, as they have been found to promote higher protist (Forge et al., 2005), nematode (Hu & Qi, 2010), prostigamtid mite (suborder Prostigmata), and collembola (Wang et al., 2016) abundances compared to inorganic

alternatives. Additionally, combining inorganic and organic N fertilizer may support higher faunal abundances than inorganic fertilizers alone (Zhang et al., 2016; Zhu & Zhu, 2015). However, of these studies, only two (Wang et al., 2016; Forge et al., 2005) were conducted within perennial cropping systems, with the others conducted in more conventional annual, largely maize (*Zea mays*), cropping systems. As N tends to cycle more efficiently and be less limiting under PGCS, it is possible that the soil communities they support will respond less strongly than fauna in annual, more-intensely managed systems.

Furthermore, the effect of N additions alone cannot be decoupled from other potential effects from the use of legumes or organic fertilizers, such as increased plant diversity. Additionally, both inorganic and organic N additions can significantly impact the quality and quantity of plant residues, resulting in additional indirect effects on fauna. For instance, the concentration of C compounds exuded by switchgrass roots doubled at high compared to low N availability (Smercina et al., 2021), which may further promote bacteria-based food webs over fungal-base webs. To date, no standardized investigations into N addition effects on diverse faunal groups across large geographic scales, as have been done for microbes (Zhang et al., 2018), have been attempted to my knowledge. Thus, potential patterns in faunal responses to N additions in grassland ecosystems remain obscure, as are any implications they may subsequently have on SOC accrual. The potential for N additions to impact soil food web structure, which can alter the balance between C and N mineralization and immobilization as microbes and fauna compete for N and subsequently affect SOC accrual, warrants further investigation in PGCS.

IDENTIFYING THE UNKNOWNS

Substantial work had gone into identifying the factors driving SOC accrual in PGCS, with the ultimate goal of predicting SOC changes following cropping system establishment. Selected as the most promising herbaceous energy crop for biomass production within the continental United States (Wright & Turhollow, 2010), switchgrass production has received considerable research focus to better understand potential impacts on SOC stocks. Current evidence suggests that switchgrass cropping systems establishment is generally associated with SOC gains exceeding the 0.25 Mg C ha^{-1} yr^{-1} minimum necessary for cropping system C neutrality

(Martinez-Feria & Basso, 2020; Agostini et al., 2015; Liebig et al., 2008; Frank et al., 2004). However, variability in SOC accrual potential across sites is high, with switchgrass cropping system establishment resulting in SOC losses in some circumstances. Understanding the sources of this variability will be necessary to better model SOC dynamics in switchgrass and other PGCS. To date, research into potential sources of variation in SOC changes have focused on climate, soil characteristics, management practices, and, increasingly, microbial dynamics (Tiemann & Grandy, 2015; Garten & Wullschuleger, 2000). As was detailed in previous sections, fauna have diverse influences over SOC, largely through their effects on microbes and decomposition dynamics, with potentially significant implications for the SOC accrual ability of PGCS soils. Thus, I argue that the explicit incorporation of fauna community structure and activity can improve our ability to accurately model SOC dynamics, predict SOC changes with PGCS establishment, and account for site-specific variability. However, there remain critical knowledge gaps relating to which, how, and to what degree do soil fauna influence SOC that must first be addressed before this can be achieved. Here, I describe these major knowledge gaps, with particular focus on how they can be addressed within switchgrass cropping systems.

While much data exists and continues to be generated on abiotic, plant, and microbial dynamics from diverse switchgrass ecosystems, soil fauna communities remain one of the last major unexplored ecosystem components of switchgrass cropping systems. Little data exists on the faunal biodiversity associated with switchgrass, and even less is known of the spatial or temporal variability of these communities (**Table 1.4**). Understanding faunal biodiversity within switchgrass cropping systems will be important for identifying dominant or potential "keystone" groups which may have outsized influence on SOC. In an example from a Sitka spruce forest, the dominant litter-dwelling arthropod *Onychiurus latus*, a fungivorous collembola, altered that natural distribution of saprotrophic fungi by preferentially grazing upon the competitively superior *Marasmius androsaceus* (Newell, 1984a; Newell, 1984b). Since *M. androsaceus* was also found to enhance the decomposition rate of litter, *O. latus* may also be indirectly slowing litter decomposition in this forest by allowing the competitive-inferior fungi to strive where it is abundant. Beyond species richness and identification, knowledge on faunal functional richness and food web dynamics can be useful to predict which faunal effects

are likely to have the greatest impact on SOC. For instance, the expansive, deep rooting systems of switchgrass may favor bacterial-based food webs and promote high abundances of microfauna, thus enhancing N mineralization by stimulating microbial turnover. This in turn could alleviate switchgrass-microbe competition for N, which can promote SOC gains by preventing SOC mining by N-limited microbes or improving switchgrass biomass production.

Uncovering the taxonomic and functional composition of faunal communities will also be useful for identifying key microbe-fauna interactions influencing SOC formation, decomposition, or stabilization in switchgrass cropping systems. While the prospect of identifying such interactions influential for SOC dynamics remains a daunting task with so many unknowns surrounding belowground biodiversity in switchgrass cropping systems, insights into the identities of key microbes associated with switchgrass can serve as a starting point. Numerous microbial taxa and functional groups (e.g., AMF) comprising the switchgrass microbiome have been found to increase switchgrass biomass production and stress tolerance (Hestrin et al., 2021). By regulating the abundance or activity of these microbes, fauna have the potential to alter the quantity of switchgrass productivity that ultimately enters SOC pools. Documented effects of soil fauna on AMF, as well as the responses of plants to such interactions, range from positive to negative (Paudel et al., 2016; Dauber et al., 2008; Hol & Cook, 2005; Gange, 2000). In switchgrass cropping systems, the combined reduction of AMF and nematodes altered the lignin composition of aboveground switchgrass tissues (Basyal et al., 2021), which may influence its subsequent decomposition.

Another major knowledge gap is that, of the diverse ways soil fauna can influence SOC (see **Fig. 1.1**), it remains essentially unknown how these effects operate within switchgrass cropping systems. Furthermore, the mechanisms driving many of these effects are not clearly understood, especially regarding how they influence SOC stability. Litter bag studies, long and widely used to study the role of fauna in residue decomposition by tracking residue mass loss over time, provide no information on how residues were lost. Hence, despite the overall positive trend of fauna on litter mass loss from these studies, how much mass loss can be directly attributed to faunal ingestion as indirect faunal effects (e.g., enhanced microbial decomposition or dissolved organic C leaching in the presence of fauna) remains unclear. A

similar issue arises from studies measuring soil respiration in the presence or absence of fauna; increased respiration in the presence of fauna may arise from compensatory growth of grazed microbes, microbial utilization of labile C compounds in fecal pellets, or more suitable microclimate conditions for microbes. As each of these potential mechanisms have differing implications on SOC stability and fate, such investigations provide incomplete information into the true nature of faunal effects on SOC. Investigations conducted within switchgrass cropping systems into the role of fauna on SOC should therefore be designed such that the fate of SOC as it is transferred between pools with different turnover times is measured, rather than simply short-term SOC gains or losses. This was effectively done by Soong et al. (2016), who in addition to measuring the effect of fauna on litter mass loss examined the incorporation of litter-C into SOM fractions as well as microbes.

Once the mechanisms behind faunal effects on SOC are better understood, the direction, strengths, and variability of these effects must be quantified and validated in order to be parameterized and incorporated into SOC models as is currently being done for soil microbes (Wieder et al., 2014). It has already been stated that faunal activity, particularly that of detritivorous groups, generally increases decomposition rates. Thus, reported decomposition rates for switchgrass residues measured in the absence of fauna may be underestimated. However, the degree to which fauna influence residue decomposition will depend on the quality of the residue, which strongly varies between switchgrass tissue types (Johnson et al., 2007). While knowledge on the quantity and quality of switchgrass net primary productivity that becomes incorporated as SOC continues to expand across climate gradients and soil types (von Haden et al., 2019, Agostini et al., 2015), to my knowledge only one study (Zangerl et al., 2013) has attempted to quantify the contribution of fauna to switchgrass residue decomposition. The degree to which switchgrass residues of different quality are fed upon and by which fauna in switchgrass cropping systems can have significant implications for the fate of these inputs as they are made more or less available for subsequent microbial degradation. Furthermore, it will be important to investigate the variability of fauna effects across different climate conditions and soil types as well as under different levels of N fertilization as each of these factors can strongly influence soil fauna community structure, activity, and interactions

with microbes. Temporal variability must also be considered, as seasonal variation in soil fauna communities will influence both the how and how strongly soil fauna communities affect SOC.

CONCLUSIONS

Substantial research has been devoted in recent decades to evaluating the environmental impacts of perennial grass energy crop production in North America. There is general consensus that PGCS are a more sustainable alternative to annual, first-generation energy crops, owing to their higher potential to offset C emissions with less risk for indirect land use effects and lower management requirements (Robertson et al., 2017). Still, there remain critical gaps that challenge assumptions of the long-term success and sustainability of perennial grass energy crop production. Critically, high variability in SOC stock responses to PGCS establishment, coupled with SOC modeling limitations, remain significant barriers to bioenergy expansion. SOC accrual, a prerequisite for bioenergy, can be conceptualized as the consequence of interconnected physical, chemical, and biological processes and interactions. Research developments continue to elucidate the critical roles of plants, particularly their residues, and microbes in SOC formation, decomposition, and stabilization. However, comparatively little research has focused on the potential contributions of fauna, such that their contributions to SOC accrual in PGCS can currently only be speculated at. Despite this, I have shown that soil fauna have diverse effects on soil microbial community structure and activity and SOM decomposition dynamics, the results of which can influence the interactions between microbes, plant residues, and mineral soils. Thus, faunal activity has the potential to indirectly regulate SOC stability. Much research is needed to elucidate the direction, strength, and primary mechanisms driving these faunal effects in PGCS, yet it will be important to gain a more comprehensive understanding of the biotic factors regulating SOC accrual.

While the influence of fauna on SOC accrual ability remains obscure, the strong influence of aboveground land-use on fauna is well-documented. In order to understand the role of fauna on SOC dynamics in PGCS, it will be necessary to consider how PGCS characteristics moderate faunal community structure and function. While current evidence suggests that PGCS favor fauna relative to annual cropping systems, little is known about how faunal communities differ across the diverse range of potential PGCS. Perennial grass traits

expected to have the greatest potential impact on fauna are those that influence the quality (e.g., C:N) and/or quantity (e.g., belowground biomass) of residue inputs to soil food webs or influence the faunal habitat conditions. PGCS design, such as monoculture or polyculture cultivation, and N additions are also expected to have potentially important impacts on fauna. Greater understanding into how faunal communities, as well as their interactions with microbes and SOM, are affected by these characteristics can help to predict faunal effects on SOC across diverse PGCS.

Greater investigation of the primary faunal effects on SOC and the extent to which they can influence SOC accrual potential within varying PGCS could eventually lead to the incorporation of fauna in SOC models. At present, however, basic knowledge of the structure and functioning of native faunal communities within PGCS is incredibly scarce. Even for switchgrass, the "model" perennial grass energy crop in North America, the taxonomic and functional richness of faunal communities it is associated with is practically unknown. Further, which of the numerous diverse faunal effects could be having the greatest impact on SOC in PGCS, and to what extent, can only be speculated at in the absence of empirical study. It will also be necessary to consider how both faunal community structure and their effects on SOC vary both spatially and temporally. While this list of knowledge gaps is far from exhaustive, prioritizing research in these areas is a crucial next step to improve our understanding of fauna, an obscure yet ubiquitous component of PGCS, and their role in SOC dynamics.

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REFERENCES

Agostini, F., Gregory, A. S., & Richter, G. M. (2015). Carbon sequestration by perennial energy crops: Is the jury still out?. *BioEnergy Research*, *8*(3), 1057-1080.

Anderson-Teixeira, K. J., Davis, S. C., Masters, M. D., & Delucia, E. H. (2009). Changes in soil organic carbon under biofuel crops. *GCB Bioenergy*, *1*(1), 75-96.

Anderson-Teixeira, K. J., Masters, M. D., Black, C. K., Zeri, M., Hussain, M. Z., Bernacchi, C. J., & DeLucia, E. H. (2013). Altered belowground carbon cycling following land-use change to perennial bioenergy crops. *Ecosystems*, *16*(3), 508-520.

Andrade, J. A. V., Abreu, F. M. G. D., & Madeira, M. A. V. (2010). Influence of litter layer removal on the soil thermal regime of a pine forest in a Mediterranean climate. *Revista Brasileira de Ciência do Solo*, *34*(5), 1481-1490.

Andrén, O., Lindberg, T., Boström, U., Clarholm, M., Hansson, A. C., Johansson, G., Lagerlöf, J., Paustian, K., Persson, J., Pettersson, R., Schnürer, J., Sohlenius, B., & Wivstad, M. (1990). Organic carbon and nitrogen flows. *Ecological Bulletins*, *40*, 85-126.

Baer, S. G., Kitchen, D. J., Blair, J. M., & Rice, C. W. (2002). Changes in ecosystem structure and function along a chronosequence of restored grasslands. *Ecological Applications*, *12*(6), 1688-1701.

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, *57*(1), 233-266.

Bardgett, R. D., & Chan, K. F. (1999). Experimental evidence that soil fauna enhance nutrient mineralization and plant nutrient uptake in montane grassland ecosystems. *Soil Biology & Biochemistry*, *31*(7), 1007-1014.

Basyal, B., Foster, C., Gross, K. L., & Emery, S. M. (2022). Nitrogen fertilizer, arbuscular mycorrhizal fungi, and soil nematodes affect lignin quality and quantity in switchgrass (*Panicum virgatum* L.). *BioEnergy Research*, *15*, 1033–1041.

Bellamy, P. E., Croxton, P. J., Heard, M. S., Hinsley, S. A., Hulmes, L., Hulmes, S., Nuttall, P, Pywell, R.F., & Rothery, P. (2009). The impact of growing miscanthus for biomass on farmland bird populations. *Biomass and Bioenergy*, *33*(2), 191-199.

Bengtsson, G., Hedlund, K., & Rundgren, S. (1993). Patchiness and compensatory growth in a fungus-Collembola system. *Oecologia*, *93*(2), 296-302.

Beugnon, R., Steinauer, K., Barnes, A. D., Ebeling, A., Roscher, C., & Eisenhauer, N. (2019). Plant functional trait identity and diversity effects on soil meso- and macrofauna in an experimental grassland. *Advances in Ecological Research*, *61*, 163-184.

Blanchart, E., Villenave, C., Viallatoux, A., Barthès, B., Girardin, C., Azontonde, A., & Feller, C. (2006). Long-term effect of a legume cover crop (*Mucuna pruriens* var. *utilis*) on the communities of soil macrofauna and nematofauna, under maize cultivation, in southern

Benin. *European Journal of Soil Biology*, *42*, S136-S144.

Bliss, T., Powers, T. O., & Brassil, C. E. (2010). The spatial influence of aboveground diversity on belowground communities. *Ecosphere*, *1*(2-3), 1-12.

Bonkowski, M., Villenave, C., & Griffiths, B. (2009). Rhizosphere fauna: The functional and structural diversity of intimate interactions of soil fauna with plant roots. *Plant and Soil*, *321*(1), 213-233.

Bradford, M. A., Jones, T. H., Bardgett, R. D., Black, H. I., Boag, B., Bonkowski, M., Cook, R., Eggers, T., Gange, A. C., Grayston, S. J., Kandeler, E., McCaig, A. E., Newington, J. E., Prosser, J. I., Setälä, H., Staddon, P. L., Tordoff, G. M., Tscherko, D., & Lawton, J. H. (2002). Impacts of soil faunal community composition on model grassland ecosystems. *Science*, *298*(5593), 615-618.

Brejda, J. J. (2000). Fertilization of native warm-season grasses. *Native Warm-Season Grasses: Research Trends and Issues*, *30*, 177-200.

Briones, M. J. I., Elias, D. M. O., Grant, H. K., & McNamara, N. P. (2019). Plant identity control on soil food web structure and C transfers under perennial bioenergy plantations. *Soil Biology & Biochemistry*, *138*, 107603.

Carvalho, J. L., Hudiburg, T. W., Franco, H. C., & DeLucia, E. H. (2017). Contribution of above- and belowground bioenergy crop residues to soil carbon. *GCB Bioenergy*, *9*(8), 1333-1343.

Cassida, K. A., Kirkpatrick, T. L., Robbins, R. T., Muir, J. P., Venuto, B. C., & Hussey, M. A. R. K. (2005). Plant-parasitic nematodes associated with switchgrass (*Panicum virgatum* L.) grown for biofuel in the South Central United States. *Nematropica*, *35*(1), 1-10.

Ceja-Navarro, J. A., Wang, Y., Ning, D., Arellano, A., Ramanculova, L., Yuan, M. M., Byer, A., Craven, K. D., Saha, M. C., Brodie, E. L., Pett-Ridge, J., & Firestone, M. K. (2021). Protist diversity and community complexity in the rhizosphere of switchgrass are dynamic as plants develop. *Microbiome*, *9*(1), 1-18.

Chauvat, M., Perez, G., Hedde, M., & Lamy, I. (2014). Establishment of bioenergy crops on metal contaminated soils stimulates belowground fauna. *Biomass & Bioenergy*, *62*, 207-211.

Cluzeau, D., Guernion, M., Chaussod, R., Martin-Laurent, F., Villenave, C., Cortet, J., Ruiz-Camacho, N., Pernin, C., Mateille, T., Philippot, L., Bellido, A., Rougé, L., Arrouays, D., Bispo, A., & Pérès, G. (2012). Integration of biodiversity in soil quality monitoring: Baselines for microbial and soil fauna parameters for different land-use types. *European Journal of Soil Biology*, *49*, 63-72.

Cragg, R. G., & Bardgett, R. D. (2001). How changes in soil faunal diversity and composition within a trophic group influence decomposition processes. *Soil Biology & Biochemistry*, *33*(15), 2073-2081.

Craine, J. M., Tilman, D., Wedin, D., Reich, P., Tjoelker, M., & Knops, J. (2002). Functional traits,

productivity and effects on nitrogen cycling of 33 grassland species. *Functional Ecology*, *16*(5), 563-574.

Crossley Jr, D. A., Mueller, B. R., & Perdue, J. C. (1992). Biodiversity of microarthropods in agricultural soils: Relations to processes. *Agriculture, Ecosystems & Environment*, *40*(1-4), 37-46.

Crowther, T. W., Boddy, L., & Jones, T. H. (2012). Functional and ecological consequences of saprotrophic fungus–grazer interactions. *ISME Journal*, *6*(11), 1992-2001.

Dauber, J., Jones, M. B., & Stout, J. C. (2010). The impact of biomass crop cultivation on temperate biodiversity. *GCB Bioenergy*, *2*(6), 289-309.

Dauber, J., Niechoj, R., Baltruschat, H., & Wolters, V. (2008). Soil engineering ants increase grass root arbuscular mycorrhizal colonization. *Biology and Fertility of Soils*, *44*(5), 791-796.

Davidson, D. A., Grieve, I. C., & Young, I. M. (2002). Impacts of fauna on an upland grassland soil as determined by micromorphological analysis. *Applied Soil Ecology*, *20*(2), 133-143.

de Deyn, G. B., Raaijmakers, C. E., van Ruijven, J., Berendse, F., & van der Putten, W. H. (2004). Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. *Oikos*, *106*(3), 576-586.

Edwards, C. A. (2000). Soil invertebrate controls and microbial interactions in nutrient and organic matter dynamics in natural and agroecosystems. In D. C., Coleman & P. F. Hendrix (Eds.), *Invertebrates as webmasters in ecosystems*, (pp. 141-159). CAB International.

Eisenhauer, N., Migunova, V. D., Ackermann, M., Ruess, L., & Scheu, S. (2011). Changes in plant species richness induce functional shifts in soil nematode communities in experimental grassland. *PLoS ONE*, *6*(9), e24087.

Emery, S. M., Reid, M. L., Bell-Dereske, L., & Gross, K. L. (2017). Soil mycorrhizal and nematode diversity vary in response to bioenergy crop identity and fertilization. *GCB Bioenergy*, *9*(11), 1644-1656.

Emmerling, C. (2014). Impact of land-use change towards perennial energy crops on earthworm population. *Applied Soil Ecology*, *84*, 12-15.

Ernst, G., Henseler, I., Felten, D., & Emmerling, C. (2009). Decomposition and mineralization of energy crop residues governed by earthworms. *Soil Biology & Biochemistry*, *41*(7), 1548-1554.

Facelli, J. M. (1994). Multiple indirect effects of plant litter affect the establishment of woody seedlings in old fields. *Ecology*, *75*(6), 1727-1735.

Fargione, J., Hill, J., Tilman, D., Polasky, S., & Hawthorne, P. (2008). Land clearing and the biofuel carbon debt. *Science*, *319*(5867), 1235-1238.

Felten, D., & Emmerling, C. (2011). Effects of bioenergy crop cultivation on earthworm

communities—A comparative study of perennial (*Miscanthus*) and annual crops with consideration of graded land-use intensity. *Applied Soil Ecology*, *49*, 167-177.

Fierer, N., Strickland, M. S., Liptzin, D., Bradford, M. A., & Cleveland, C. C. (2009). Global patterns in belowground communities. *Ecology Letters*, *12*(11), 1238-1249.

Fierer, N., Lauber, C. L., Ramirez, K. S., Zaneveld, J., Bradford, M. A., & Knight, R. (2012). Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME Journal*, *6*(5), 1007-1017.

Filser, J., Faber, J. H., Tiunov, A. V., Brussaard, L., Frouz, J., de Deyn, G. D., Uvarov, A. V., Berg, M. P., Lavelle, P., Loreau, M., Wall, D. H., Querner, P., Eijsackers, H., & Jiménez, J. J. (2016). Soil fauna: Key to new carbon models. *Soil*, *2*(4), 565-582.

Fonte, S. J., Kong, A. Y., van Kessel, C., Hendrix, P. F., & Six, J. (2007). Influence of earthworm activity on aggregate-associated carbon and nitrogen dynamics differs with agroecosystem management. *Soil Biology & Biochemistry*, *39*(5), 1014-1022.

Forge, T. A., Bittman, S., & Kowalenko, C. G. (2005). Responses of grassland soil nematodes and protozoa to multi-year and single-year applications of dairy manure slurry and fertilizer. *Soil Biology & Biochemistry*, *37*(10), 1751-1762.

Fox, O., Vetter, S., Ekschmitt, K., & Wolters, V. (2006). Soil fauna modifies the recalcitrance-persistence relationship of soil carbon pools. *Soil Biology & Biochemistry*, *38*(6), 1353-1363.

Franco, A. L., Cherubin, M. R., Cerri, C. E., Six, J., Wall, D. H., & Cerri, C. C. (2020). Linking soil engineers, structural stability, and organic matter allocation to unravel soil carbon responses to land-use change. *Soil Biology & Biochemistry*, *150*, 107998.

Frank, A. B., Berdahl, J. D., Hanson, J. D., Liebig, M. A., & Johnson, H. A. (2004). Biomass and carbon partitioning in switchgrass. *Crop Science*, *44*(4), 1391-1396.

Gan, H., & Wickings, K. (2020). Root herbivory and soil carbon cycling: Shedding “green” light onto a “brown” world. *Soil Biology & Biochemistry*, *150*, 107972.

Gange, A. (2000). Arbuscular mycorrhizal fungi, collembola and plant growth. *Trends in Ecology & Evolution*, *15*(9), 369-372.

García-Palacios, P., Maestre, F. T., Kattge, J., & Wall, D. H. (2013). Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. *Ecology Letters*, *16*(8), 1045-1053.

Garten Jr, C. T., & Wullschleger, S. D. (2000). Soil carbon dynamics beneath switchgrass as indicated by stable isotope analysis. *Journal of Environmental Quality*, *29*, 645-653.

Geisseler, D., & Scow, K. M. (2014). Long-term effects of mineral fertilizers on soil microorganisms–A review. *Soil Biology & Biochemistry*, *75*, 54-63.

González, G., & Seastedt, T. R. (2001). Soil fauna and plant litter decomposition in tropical and

subalpine forests. *Ecology*, *82*(4), 955-964.

de Graaff, M. A., Six, J., Jastrow, J. D., Schadt, C. W., & Wullschleger, S. D. (2013). Variation in root architecture among switchgrass cultivars impacts root decomposition rates. *Soil Biology & Biochemistry*, *58*, 198-206.

de Graaff, M. A., Hornslein, N., Throop, H. L., Kardol, P., & van Diepen, L. T. (2019). Effects of agricultural intensification on soil biodiversity and implications for ecosystem functioning: A meta-analysis. *Advances in Agronomy*, *155*, 1-44.

Grandy, A. S., Wieder, W. R., Wickings, K., & Kyker-Snowman, E. (2016). Beyond microbes: Are fauna the next frontier in soil biogeochemical models?. *Soil Biology & Biochemistry*, *102*, 40-44.

Gruzdeva, L. I., Matveeva, E. M., & Kovalenko, T. E. (2007). Changes in soil nematode communities under the impact of fertilizers. *Eurasian Soil Science*, *40*(6), 681-693.

von Haden, A. C., Kucharik, C. J., Jackson, R. D., & Marín-Spiotta, E. (2019). Litter quantity, litter chemistry, and soil texture control changes in soil organic carbon fractions under bioenergy cropping systems of the North Central US. *Biogeochemistry*, *143*(3), 313-326.

Hanlon, R. D. G., & Anderson, J. M. (1979). The effects of Collembola grazing on microbial activity in decomposing leaf litter. *Oecologia*, *38*(1), 93-99.

Hedde, M., van Oort, F., Renouf, E., Thénard, J., & Lamy, I. (2013). Dynamics of soil fauna after plantation of perennial energy crops on polluted soils. *Applied Soil Ecology*, *66*, 29-39.

Hedlund, K., & Augustsson, A. (1995). Effects of enchytraeid grazing on fungal growth and respiration. *Soil Biology & Biochemistry*, *27*(7), 905-909.

Helms IV, J. A., Ijelu, S. E., Wills, B. D., Landis, D. A., & Haddad, N. M. (2020). Ant biodiversity and ecosystem services in bioenergy landscapes. *Agriculture, Ecosystems & Environment*, *290*, 106780.

Hestrin, R., Lee, M. R., Whitaker, B. K., & Pett-Ridge, J. (2021). The switchgrass microbiome: A review of structure, function, and taxonomic distribution. *Phytobiomes Journal*, *5*(1), 14-28.

Hol, W. G., & Cook, R. (2005). An overview of arbuscular mycorrhizal fungi–nematode interactions. *Basic and Applied Ecology*, *6*(6), 489-503.

Holguin, C. M., Reay-Jones, F. P. F., Frederick, J. R., Adler, P. H., Chong, J. H., & Savereno, A. (2010). Insect diversity in switchgrass grown for biofuel in South Carolina. *Journal of Agricultural and Urban Entomology*, *27*(1), 1-19.

Hooper, D. U., Bignell, D. E., Brown, V. K., Brussard, L., Dangerfield, J. M., Wall, D. H., Wardle, D. A., Coleman, C. A., Giller, K. E., Lavelle, P., van der Putten, W. H., de Ruiter, P. C., Rusek, J., Silver, W. L., Tiedje, J. M., & Wolters, V. (2000). Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. *Bioscience*, *50*(12), 1049-1061.

Hu, C., & Qi, Y. (2010). Effect of compost and chemical fertilizer on soil nematode community in a Chinese maize field. *European Journal of Soil Biology*, *46*(3-4), 230-236.

Ikoyi, I., Fowler, A., Storey, S., Doyle, E., & Schmalenberger, A. (2020). Sulfate fertilization supports growth of ryegrass in soil columns but changes microbial community structures and reduces abundances of nematodes and arbuscular mycorrhiza. *Science of the Total Environment*, *704*, 135315.

Immerzeel, D. J., Verweij, P. A., Van Der Hilst, F., & Faaij, A. P. (2014). Biodiversity impacts of bioenergy crop production: A state-of-the-art review. *GCB Bioenergy*, *6*(3), 183-209.

Janoušková, M., Kohout, P., Moradi, J., Doubková, P., Frouz, J., Vosolsobě, S., & Rydlová, J. (2018). Microarthropods influence the composition of rhizospheric fungal communities by stimulating specific taxa. *Soil Biology & Biochemistry*, *122*, 120-130.

Jesus, E. D. C., Liang, C., Quensen, J. F., Susilawati, E., Jackson, R. D., Balser, T. C., & Tiedje, J. M. (2016). Influence of corn, switchgrass, and prairie cropping systems on soil microbial communities in the upper Midwest of the United States. *GCB Bioenergy*, *8*(2), 481-494.

Jiang, Y., Wang, B., Niu, X., Dong, Z., & Wang, P. (2016). Contribution of soil fauna respiration to CO_2 flux in subtropical Moso bamboo (*Phyllostachys pubescens*) forests: A comparison of different soil treatment methods. *Environmental Earth Sciences*, *75*(13), 1-11.

Johnson, J. M. F., Barbour, N. W., & Weyers, S. L. (2007). Chemical composition of crop biomass impacts its decomposition. *Soil Science Society of America Journal*, *71*(1), 155-162.

Joly, F. X., Coq, S., Coulis, M., David, J. F., Hättenschwiler, S., Mueller, C. W., Prater, I., & Subke, J. A. (2020). Detritivore conversion of litter into faeces accelerates organic matter turnover. *Communications Biology*, *3*(1), 1-9.

Kallenbach, C. M., Frey, S. D., & Grandy, A. S. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications*, *7*(1), 1-10.

Kantola, I. B., Masters, M. D., & DeLucia, E. H. (2017). Soil particulate organic matter increases under perennial bioenergy crop agriculture. *Soil Biology & Biochemistry*, *113*, 184-191.

Kravchenko, A. N., Guber, A. K., Razavi, B. S., Koestel, J., Quigley, M. Y., Robertson, G. P., & Kuzyakov, Y. (2019). Microbial spatial footprint as a driver of soil carbon stabilization. *Nature Communications, 10*(1), 1-10.

Landis, D. A., Gratton, C., Jackson, R. D., Gross, K. L., Duncan, D. S., Liang, C., Meehan, T. D., Robertson, B. A., Schmidt, T. M., Stahlheber, K. A., Tiedje, J. M., & Werling, B. P. (2018). Biomass and biofuel crop effects on biodiversity and ecosystem services in the North Central US. *Biomass & Bioenergy*, *114*, 18-29.

Lask, J., Magenau, E., Ferrarini, A., Kiesel, A., Wagner, M., & Lewandowski, I. (2020). Perennial rhizomatous grasses: Can they really increase species richness and abundance in arable land?—A meta-analysis. *GCB Bioenergy*, *12*(11), 968-978.

Leff, J. W., Jones, S. E., Prober, S. M., Barberán, A., Borer, E. T., Firn, J. L., Harpole, S. W., Hobbie, S. E., Hofmockel, K. S., Knops, J. M. H., McCulley, R. L., La Pierre, K., Risch, A. C., Seabloom, E. W., Schütz, M., Steenbock, C., Stevens, C. J., & Fierer, N. (2015). Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences*, *112*(35), 10967-10972.

Lemus, R., & Lal, R. (2005). Bioenergy crops and carbon sequestration. *Critical Reviews in Plant Sciences*, *24*(1), 1-21.

Lentendu, G., Wubet, T., Chatzinotas, A., Wilhelm, C., Buscot, F., & Schlegel, M. (2014). Effects of long-term differential fertilization on eukaryotic microbial communities in an arable soil: A multiple barcoding approach. *Molecular Ecology*, *23*(13), 3341-3355.

Lewandowski, I., Scurlock, J. M., Lindvall, E., & Christou, M. (2003). The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass & Bioenergy*, *25*(4), 335-361.

Liang, C., Jesus, E. D. C., Duncan, D. S., Jackson, R. D., Tiedje, J. M., & Balser, T. C. (2012). Soil microbial communities under model biofuel cropping systems in southern Wisconsin, USA: Impact of crop species and soil properties. *Applied Soil Ecology*, *54*, 24-31.

Liang, C., Schimel, J. P., & Jastrow, J. D. (2017). The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology*, *2*(8), 1-6.

Liebig, M. A., Schmer, M. R., Vogel, K. P., & Mitchell, R. B. (2008). Soil carbon storage by switchgrass grown for bioenergy. *BioEnergy Research*, *1*(3), 215-222.

Ma, W. C., Brussaard, L., & De Ridder, J. A. (1990). Long-term effects of nitrogenous fertilizers on grassland earthworms (Oligochaeta: Lumbricidae): Their relation to soil acidification. *Agriculture, Ecosystems & Environment*, *30*(1-2), 71-80.

Ma, Z., Wood, C. W., & Bransby, D. I. (2000). Soil management impacts on soil carbon sequestration by switchgrass. *Biomass & Bioenergy*, *18*(6), 469-477.

Maaß, S., Caruso, T., & Rillig, M. C. (2015). Functional role of microarthropods in soil aggregation. *Pedobiologia*, *58*(2-3), 59-63.

Mann, J. J., Barney, J. N., Kyser, G. B., & DiTomaso, J. M. (2013). Root system dynamics of *Miscanthus × giganteus* and *Panicum virgatum* in response to rainfed and irrigated conditions in California. *BioEnergy Research*, *6*(2), 678-687.

Mao, Y., Li, X., Smyth, E. M., Yannarell, A. C., & Mackie, R. I. (2014). Enrichment of specific bacterial and eukaryotic microbes in the rhizosphere of switchgrass (*Panicum virgatum* L.) through root exudates. *Environmental Microbiology Reports*, *6*(3), 293-306.

Marshall, A. H., Collins, R. P., Humphreys, M. W., & Scullion, J. (2016). A new emphasis on root traits for perennial grass and legume varieties with environmental and ecological benefits. *Food and Energy Security*, *5*(1), 26-39.

Martinez-Feria, R., & Basso, B. (2020). Predicting soil carbon changes in switchgrass grown on

marginal lands under climate change and adaptation strategies. *GCB Bioenergy*, *12*(9), 742-755.

McGowan, A. R., Nicoloso, R. S., Diop, H. E., Roozeboom, K. L., & Rice, C. W. (2019). Soil organic carbon, aggregation, and microbial community structure in annual and perennial biofuel crops. *Agronomy Journal*, *111*(1), 128-142.

McLaughlin, S. B., & Kszos, L. A. (2005). Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. *Biomass & Bioenergy*, *28*(6), 515-535.

Meehan, T. D., Werling, B. P., Landis, D. A., & Gratton, C. (2012). Pest-suppression potential of midwestern landscapes under contrasting bioenergy scenarios. *PLoS One*, *7*(7), e41728.

Mekete, T., Reynolds, K., Lopez-Nicora, H. D., Gray, M. E., & Niblack, T. L. (2011a). Plant-parasitic nematodes are potential pathogens of *Miscanthus × giganteus* and *Panicum virgatum* used for biofuels. *Plant Disease*, *95*(4), 413-418.

Mekete, T., Reynolds, K., Lopez-Nicora, H. D., Gray, M. E., & Niblack, T. L. (2011b). Distribution and diversity of root-lession nematode (*Pratylenchus* spp.) associated with *Miscanthus × giganteus* and *Panicum virgatum* used for biofuels, and species identification in a multiplex polymerase chain reaction. *Nematology*, *13*(6), 673-686.

Murray, P. J., Cook, R., Currie, A. F., Dawson, L. A., Gange, A. C., Grayston, S. J., & Treonis, A. M. (2006). Interactions between fertilizer addition, plants and the soil environment: Implications for soil faunal structure and diversity. *Applied Soil Ecology*, *33*(2), 199-207.

Nanda, S., Azargohar, R., Dalai, A. K., & Kozinski, J. A. (2015). An assessment on the sustainability of lignocellulosic biomass for biorefining. *Renewable & Sustainable Energy Reviews*, *50*, 925-941.

Newell, K. (1984a). Interaction between two decomposer basidiomycetes and a collembolan under Sitka spruce: Grazing and its potential effects on fungal distribution and litter decomposition. *Soil Biology & Biochemistry*, *16*(3), 235-239.

Newell, K. (1984b). Interaction between two decomposer basidiomycetes and a collembolan under Sitka spruce: Distribution, abundance and selective grazing. *Soil Biology & Biochemistry*, *16*(3), 227-233.

Núñez-Regueiro, M. M., Siddiqui, S. F., & Fletcher Jr, R. J. (2021). Effects of bioenergy on biodiversity arising from land-use change and crop type. *Conservation Biology*, *35*(1), 77-87.

Oates, L. G., Duncan, D. S., Sanford, G. R., Liang, C., & Jackson, R. D. (2016). Bioenergy cropping systems that incorporate native grasses stimulate growth of plant-associated soil microbes in the absence of nitrogen fertilization. *Agriculture, Ecosystems & Environment*, *233*, 396-403.

Osler, G. H., & Sommerkorn, M. (2007). Toward a complete soil C and N cycle: Incorporating the soil fauna. *Ecology*, *88*(7), 1611-1621.

Otfinowski, R., & Coffey, V. (2020). Can root traits predict communities of soil nematodes in restored northern prairies?. *Plant and Soil*, *453*(1), 459-471.

Paudel, S., Longcore, T., MacDonald, B., McCormick, M. K., Szlavecz, K., Wilson, G. W., & Loss, S. R. (2016). Belowground interactions with aboveground consequences: Invasive earthworms and arbuscular mycorrhizal fungi. *Ecology*, *97*(3), 605-614.

Petersen, H., & Luxton, M. (1982). A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos*, *39*, 288-388.

Porazinska, D. L., Bardgett, R. D., Blaauw, M. B., Hunt, H. W., Parsons, A. N., Seastedt, T. R., & Wall, D. H. (2003). Relationships at the aboveground–belowground interface: Plants, soil biota, and soil processes. *Ecological Monographs*, *73*(3), 377-395.

Postma-Blaauw, M. B., de Goede, R. G. M., Bloem, J., Faber, J. H., & Brussaard, L. (2010). Soil biota community structure and abundance under agricultural intensification and extensification. *Ecology*, *91*(2), 460-473.

Raworth, D. A., Robertson, M. C., & Bittman, S. (2004). Effects of dairy slurry application on carabid beetles in tall fescue, British Columbia, Canada. *Agriculture, Ecosystems & Environment*, *103*(3), 527-534.

Reichle, D. E. (1977). The role of soil invertebrates in nutrient cycling. *Ecological Bulletins*, *25*, 145-156.

Robertson, B. A., Porter, C., Landis, D. A., & Schemske, D. W. (2012). Agroenergy crops influence the diversity, biomass, and guild structure of terrestrial arthropod communities. *BioEnergy Research*, *5*(1), 179-188.

Robertson, G. P., Hamilton, S. K., Barham, B. L., Dale, B. E., Izaurralde, R. C., Jackson, R. D., Landis, D. A., Swinton, S. M., Thelen, K. D., & Tiedje, J. M. (2017). Cellulosic biofuel contributions to a sustainable energy future: Choices and outcomes. *Science*, *356*(6345), eaal2324.

Robertson, G. P. (2021) GLBRC plant carbon and nitrogen content (KBS087-003.1) [Unpublished data set]. Kellogg Biological Station Long-Term Ecological Research (LTER) Program.

Sądej, W., Kosewska, A., Sądej, W., & Nietupski, M. (2012). Effects of fertilizer and land-use type on soil properties and ground beetle communities. *Bulletin of Insectology*, *65*(2), 239-246.

Sanderson, M. A., & Adler, P. R. (2008). Perennial forages as second generation bioenergy crops. *International Journal of Molecular Sciences*, *9*(5), 768-788.

Santos, P. F., DePree, E., & Whitford, W. G. (1978). Spatial distribution of litter and microarthropods in a Chihuahuan desert ecosystem. *Journal of Arid Environments*, *1*(1), 41-48.

Sauvadet, M., Chauvat, M., Cluzeau, D., Maron, P. A., Villenave, C., & Bertrand, I. (2016). The dynamics of soil micro-food web structure and functions vary according to litter quality.

Soil Biology & Biochemistry, *95*, 262-274.

Sauvadet, M., Chauvat, M., Brunet, N., & Bertrand, I. (2017). Can changes in litter quality drive soil fauna structure and functions?. Soil *Biology & Biochemistry*, 107, 94-103.

Schaefer, M. (1990). The soil fauna of a beech forest on limestone: Trophic structure and energy budget. *Oecologia*, *82*(1), 128-136.

Schmidt, M. W., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. A. C., Nannipieri, P., Rasse, D. P., Weiner, S., & Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, *478*(7367), 49-56.

Schmitz, O. J., Raymond, P. A., Estes, J. A., Kurz, W. A., Holtgrieve, G. W., Ritchie, M. E., Schindler, D. E., Spivak, A. C., Wilson, R. W., Bradford, M. A., Christensen, V., Deegan, L., Smetacek, V., Vanni, M. J., & Wilmers, C. C. (2014). Animating the carbon cycle. *Ecosystems*, *17*(2), 344-359.

Searle, S. Y., & Malins, C. J. (2014). Will energy crop yields meet expectations?. *Biomass & Bioenergy*, *65*, 3-12.

Seastedt, T. R. (1984). The role of microarthropods in decomposition and mineralization processes. *Annual Review of Entomology*, *29*(1), 25-46.

Semere, T., & Slater, F. M. (2007). Invertebrate populations in miscanthus (*Miscanthus × giganteus*) and reed canary-grass (*Phalaris arundinacea*) fields. *Biomass & Bioenergy*, *31*(1), 30-39.

Sileshi, G., & Mafongoya, P. L. (2006). Long-term effects of improved legume fallows on soil invertebrate macrofauna and maize yield in eastern Zambia. *Agriculture, Ecosystems & Environment*, *115*(1-4), 69-78.

Sileshi, G., Mafongoya, P. L., Chintu, R., & Akinnifesi, F. K. (2008). Mixed-species legume fallows affect faunal abundance and richness and N cycling compared to single species in maize-fallow rotations. *Soil Biology & Biochemistry*, *40*(12), 3065-3075.

Smercina, D. N., Bowsher, A. W., Evans, S. E., Friesen, M. L., Eder, E. K., Hoyt, D. W., & Tiemann, L. K. (2021). Switchgrass rhizosphere metabolite chemistry driven by nitrogen availability. *Phytobiomes Journal*, *5*(1), 88-96.

Soong, J. L., Vandegehuchte, M. L., Horton, A. J., Nielsen, U. N., Denef, K., Shaw, E. A., de Tomasel, C. M., Parton, W., Wall, D. H., & Cotrufo, M. F. (2016). Soil microarthropods support ecosystem productivity and soil C accrual: Evidence from a litter decomposition study in the tallgrass prairie. *Soil Biology & Biochemistry*, *92*, 230-238.

Souty-Grosset, C., & Faberi, A. (2018). Effect of agricultural practices on terrestrial isopods: A review. *ZooKeys*, *801*, 63-96.

Sprunger, C. D., Oates, L. G., Jackson, R. D., & Robertson, G. P. (2017). Plant community composition influences fine root production and biomass allocation in perennial

bioenergy cropping systems of the upper Midwest, USA. *Biomass & Bioenergy*, *105*, 248-258.

Tiemann, L. K., & Grandy, A. S. (2015). Mechanisms of soil carbon accrual and storage in bioenergy cropping systems. *GCB Bioenergy*, *7*(2), 161-174.

Tilman, D., Socolow, R., Foley, J. A., Hill, J., Larson, E., Lynd, L., Pacala, S., Reilly, J., Searchinger, T., Somerville, C., & Williams, R. (2009). Beneficial biofuels — The food, energy, and environment trilemma. *Science*, *325*(5938), 270-271.

Trap, J., Bonkowski, M., Plassard, C., Villenave, C., & Blanchart, E. (2016). Ecological importance of soil bacterivores for ecosystem functions. *Plant and Soil*, *398*(1-2), 1-24.

Tsiafouli, M. A., Thébault, E., Sgardelis, S. P., de Ruiter, P. C., van der Putten, W. H., Birkhofer, K., Hemerik, L., de Vries, F. T., Bardgett, R. D., Brady, M. V., Bjornlund, L., Jørgensen, H. B., Christensen, S., d'Hertefeldt, T., Hotes, S., Gera Hol, W. H., Frouz, J., Liiri, M., Mortimer, … Hedlund, K. (2015). Intensive agriculture reduces soil biodiversity across Europe. *Global Change Biology*, *21*(2), 973-985.

U.S. Department of Energy. (2011). U.S. Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry. R.D. Perlack and B.J. Stokes (Leads), ORNL/TM-2011/224. Oak Ridge National Laboratory, Oak Ridge, TN. 227p.

Valentine, J., Clifton-Brown, J., Hastings, A., Robson, P., Allison, G., & Smith, P. (2012). Food vs. fuel: The use of land for lignocellulosic 'next generation' energy crops that minimize competition with primary food production. *GCB Bioenergy*, *4*(1), 1-19.

Verhoef, H. A., & Brussaard, L. (1990). Decomposition and nitrogen mineralization in natural and agroecosystems: The contribution of soil animals. *Biogeochemistry*, *11*(3), 175-211.

Vivanco, L., & Austin, A. T. (2006). Intrinsic effects of species on leaf litter and root decomposition: A comparison of temperate grasses from North and South America. *Oecologia*, *150*(1), 97-107.

de Vries, F. T., Hoffland, E., van Eekeren, N., Brussaard, L., & Bloem, J. (2006). Fungal/bacterial ratios in grasslands with contrasting nitrogen management. *Soil Biology & Biochemistry*, *38*(8), 2092-2103.

de Vries, F. T., & Bardgett, R. D. (2012). Plant–microbial linkages and ecosystem nitrogen retention: Lessons for sustainable agriculture. *Frontiers in Ecology and the Environment*, *10*(8), 425-432.

de Vries, F. T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M. A., Bjørnlund, L., Jørgensen, H. B., Brady, M. V., Christensen, S., de Ruiter, P. C., d'Hertefeldt, T., Frouz, J., Hedlund, K., Hemerik, L., Gera Hol, W. H., Hotes, S., Mortimer, S. R., Setälä, H., Sgardelis, S. P., … Bardgett, R. D. (2013). Soil food web properties explain ecosystem services across European land use systems. *Proceedings of the National Academy of Sciences*, *110*(35), 14296-14301.

Waldrop, M. P., & Firestone, M. K. (2004). Microbial community utilization of recalcitrant and simple carbon compounds: Impact of oak-woodland plant communities. *Oecologia*, *138*(2), 275-284.

Wang, S., Chen, H. Y., Tan, Y., Fan, H., & Ruan, H. (2016). Fertilizer regime impacts on abundance and diversity of soil fauna across a poplar plantation chronosequence in coastal Eastern China. *Scientific Reports*, *6*(1), 1-10.

Ward, K. E., & Ward, R. N. (2001). Diversity and abundance of carabid beetles in short-rotation plantings of sweetgum, maize and switchgrass in Alabama. *Agroforestry Systems*, *53*(3), 261.

Wardle, D. A., Nicholson, K. S., Bonner, K. I., & Yeates, G. W. (1999). Effects of agricultural intensification on soil-associated arthropod population dynamics, community structure, diversity and temporal variability over a seven-year period. *Soil Biology & Biochemistry*, *31*(12), 1691-1706.

Wardle, D. A. (2006). The influence of biotic interactions on soil biodiversity. *Ecology Letters*, *9*(7), 870-886.

Webster, C. R., Flaspohler, D. J., Jackson, R. D., Meehan, T. D., & Gratton, C. (2010). Diversity, productivity and landscape-level effects in North American grasslands managed for biomass production. *Biofuels*, *1*(3), 451-461.

Werling, B. P., Dickson, T. L., Isaacs, R., Gaines, H., Gratton, C., Gross, K. L., Liere, H., Malmstrom, C. M., Meehan, T. D., Ruan, L., Robertson, B. A., Robertson, G. P., Schmidt, T. M., Schrotenboer, A. C., Teal, T. K., Wilson, J. K., & Landis, D. A. (2014). Perennial grasslands enhance biodiversity and multiple ecosystem services in bioenergy landscapes. *Proceedings of the National Academy of Sciences*, *111*(4), 1652-1657.

Wickings, K., & Grandy, A. S. (2011). The oribatid mite *Scheloribates moestus* (Acari: Oribatida) alters litter chemistry and nutrient cycling during decomposition. *Soil Biology & Biochemistry*, *43*(2), 351-358.

Wieder, W. R., Grandy, A. S., Kallenbach, C. M., & Bonan, G. B. (2014). Integrating microbial physiology and physio-chemical principles in soils with the MIcrobial-MIneral Carbon Stabilization (MIMICS) model. *Biogeosciences*, *11*(14), 3899-3917.

Wills, B. D., & Landis, D. A. (2018). The role of ants in north temperate grasslands: A review. *Oecologia*, *186*(2), 323-338.

Wolters, V. (2000). Invertebrate control of soil organic matter stability. *Biology and fertility of Soils*, *31*(1), 1-19.

Wright, L., & Turhollow, A. (2010). Switchgrass selection as a “model” bioenergy crop: A history of the process. *Biomass & Bioenergy*, *34*(6), 851-868.

Zangerl, A. R., Miresmailli, S., Nabity, P., Lawrance, A., Yanahan, A., Mitchell, C. A., Anderson-Teixeira, K. J., David, M. B., Berenbaum, M. R., & DeLucia, E. H. (2013). Role of arthropod

communities in bioenergy crop litter decomposition. *Insect Science*, *20*(5), 671-678.

Zhang, Z., Zhang, X., Mahamood, M., Zhang, S., Huang, S., & Liang, W. (2016). Effect of long-term combined application of organic and inorganic fertilizers on soil nematode communities within aggregates. *Scientific Reports*, *6*(1), 1-12.

Zhang, T. A., Chen, H. Y., & Ruan, H. (2018). Global negative effects of nitrogen deposition on soil microbes. *ISME Journal*, *12*(7), 1817-1825.

Zhang, K., Johnson, L., Prasad, P. V., Pei, Z., & Wang, D. (2015). Big bluestem as a bioenergy crop: A review. *Renewable & Sustainable Energy Reviews*, *52*, 740-756.

Zhao, Z. B., He, J. Z., Geisen, S., Han, L. L., Wang, J. T., Shen, J. P., Wei, W. X., Fang, Y. T., Li, P. P., & Zhang, L. M. (2019). Protist communities are more sensitive to nitrogen fertilization than other microorganisms in diverse agricultural soils. *Microbiome*, *7*(1), 33.

Zhu, X., & Zhu, B. (2015). Diversity and abundance of soil fauna as influenced by long-term fertilization in cropland of purple soil, China. *Soil & Tillage Research*, *146*, 39-46.

APPENDIX

CHAPTER 1 FIGURES & TABLES

Table 1.1. Select perennial grasses studied for their utilization as dedicated energy crops in the temperate Northern Hemisphere.

Species	Description	Yield range (Mg ha^{-1} yr^{-1})	Advantages	Limitations
Big bluestem (*Andropogon gerardii*)	warm-season (C4) grass	6.8 - 11.9[1] 3.2 - 11.4[2]	Dominant native grassland species, productive across a wide geographic range	Limited research into energy crop potential
Miscanthus (*Miscanthus x giganteus*)	warm-season (C4) grass	4 - 44[1] 5 - 38[3] 1.4 - 40.9[4]	Vigorous growth with high yield potential under suitable conditions	Narrow genetic base, sterile, non-native to N. America, poor overwintering at northern latitudes
Reed canary grass (*Phalaris arundinacea*)	cool-season (C3) grass	1.6 - 12.2[1] 5.5 - 10.2[4]	Broad genetic variability, productive at low temperatures	Lower N and water efficiencies than C4 grasses, potential to become invasive
Switchgrass (*Panicum virgatum*)	warm-season C4 grass	0.9 - 34.6[1] 1 - 35[3] 5.2 - 11.1[4]	Dominant native grassland species, broad genetic variability, productive across a wide geographic range	Weed competition can hinder crop establishment

[1]Lewandowski et al. (2002), [2]Zhang et al. (2015), [3]Searle & Malins (2014), [4]Sanderson & Adler (2008)

Table 1.2. Functions and ecological importance of the major soil fauna groups.

	Functional Roles	Ecological Importance	Examples
Microfauna	Microbivores	Regulate microbial prey populations; mineralize nutrients	*Rhabditis spp.*
	Herbivores/ plant parasites	Cause plant damage, disease, or death	*Meloidogyne incognita*
	Predators/ omnivores	Regulate prey populations; mineralize nutrients	*Dorylaimus spp.*
	Entomopathogens	Infect and kill insects, including some important pest species	*Heterorhabditis bacteriophora*
Mesofauna	Decomposers/ microbivores	Enhance SOM decomposition rates by fragmenting plant litter; mineralize nutrients; regulate microbial prey	Oribatid mites, collembola
	Predators/ omnivores	Regulate prey populations; mineralize nutrients	Mesostigmatid mites
Macrofauna	Ecosystem engineers	Alter the physical and chemical conditions of the soil	Earthworms, ants, moles
	Decomposers	Degrade SOM; mineralize nutrients	Isopods, millipedes, earthworms
	Predators	Regulate prey populations; mineralize nutrients	Carabid beetles, centipedes

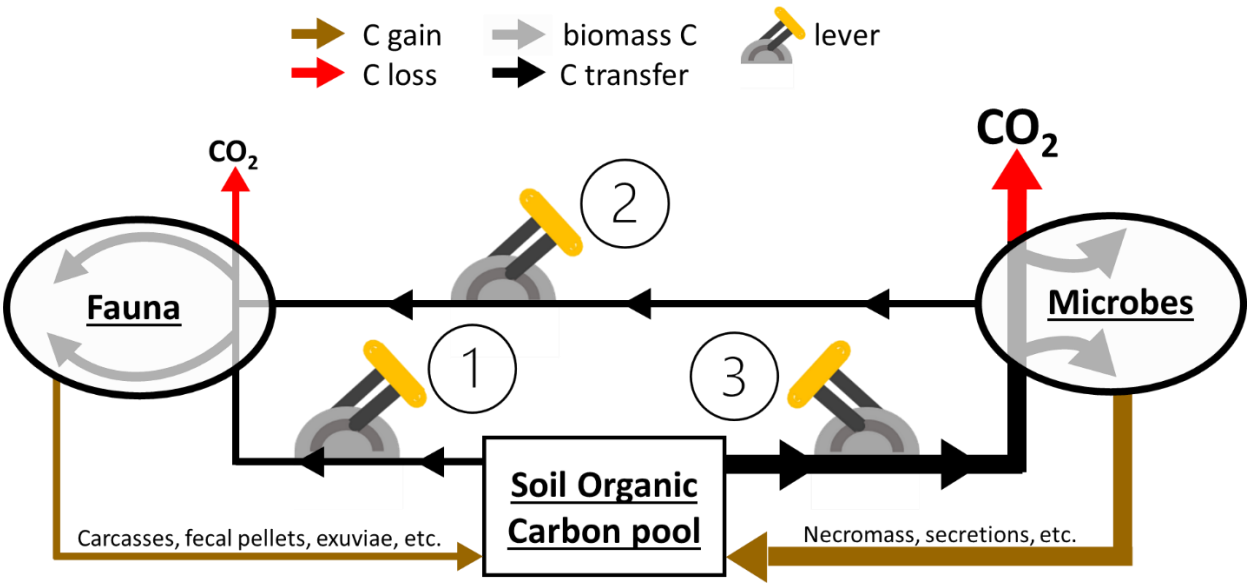

Figure 1.1. Conceptual diagram showing C transfers between living (circles) and non-living (square) SOC pools with levers representing the interactions where fauna theoretically have the greatest potential to influence SOC accrual. These interactions include **1)** effects on SOM properties (i.e., size, distribution, quality) which alter SOM availability and accessibility to decomposer microbes, **2)** trophic interactions with microbes which alter microbial community dynamics, biomass turnover, and/or functioning, and **3)** indirect effects on microbes (i.e., microhabitat modifications via bioturbation, N availability, etc.) that subsequently affect how microbes and SOM interact. All of these interactions are primarily indirect, with direct faunal contributions to SOC gains and losses expected to have only minor influence relative to that of microbes. Interactions with living plants have been omitted for simplicity and substantial uncertainties regarding the mechanisms and relative significance of fauna–plant and fauna–microbe–plant interactions on SOC accrual.

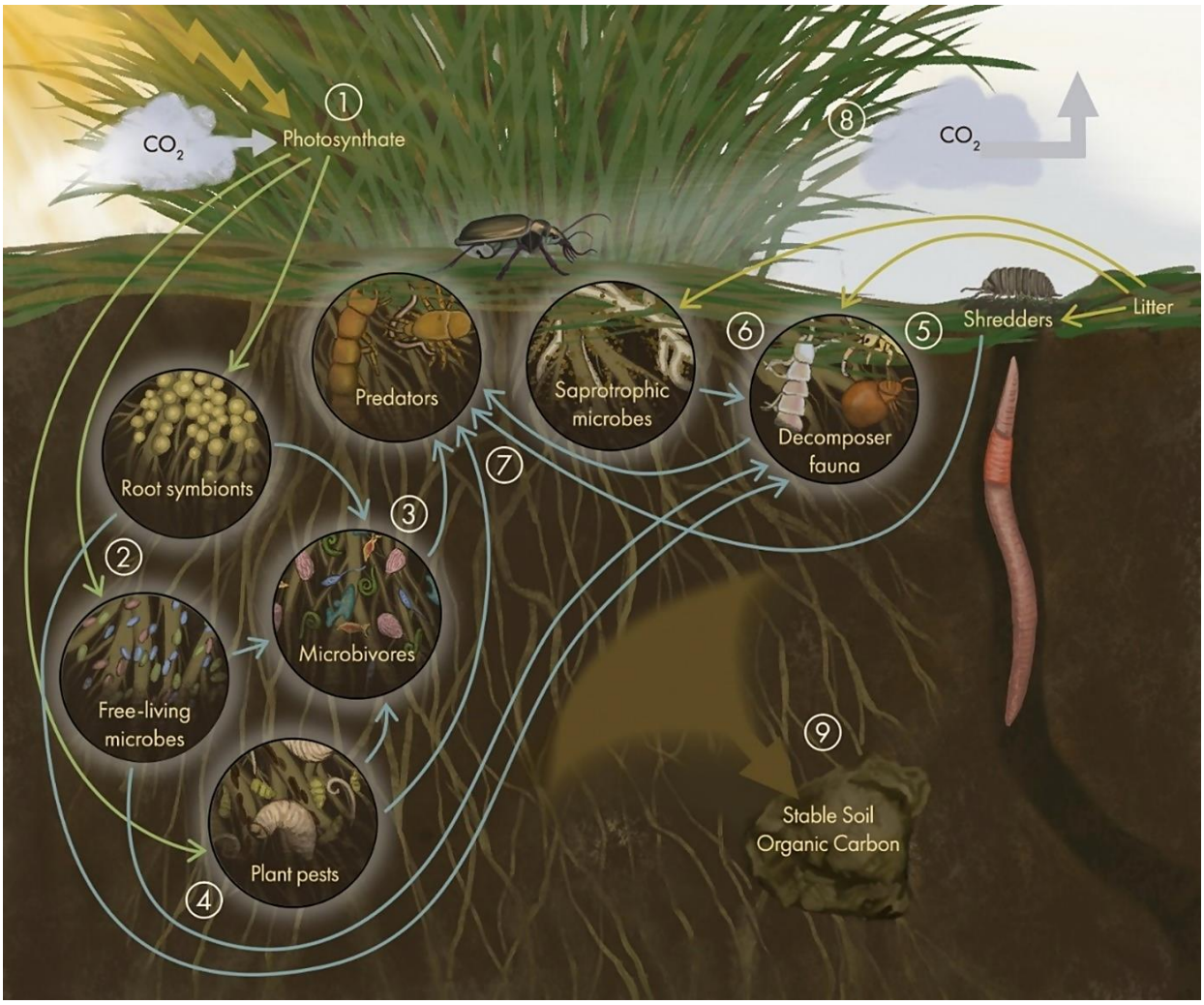

Figure 1.2. Potential pathways of SOC flows through soil food web channels. Arrows represent C transfers color-coded by C origin: root-derived (green), living heterotroph biomass (blue), and litter-derived (yellow). **1)** Much of the photosynthetically fixed C (photosynthate) produced by grasses is allocated to the rhizosphere, **2)** fueling root symbionts and copiotrophic microbes, especially bacteria. **3)** High microbial growth and activity in the rhizosphere is regulated by correspondingly high rates of microbivory, which subsequently promote N mineralization. **4)** Parasites, pathogens, and other pests obtain C directly, which can impact grass productivity and C allocation. Outside of the rhizosphere, reduced nutrient availability and accessibility in bulk soil favors saprotrophs, especially fungi, which are capable of degrading more recalcitrant SOC sources like grass litter. **5)** Decomposer and shredder fauna enhance SOM decomposition rates, feeding directly on particulate SOM (e.g., litter) **6)** as well as the saprotrophs colonizing it. **7)** The distinction between trophic dynamics in the rhizosphere and bulk soil diminishes at higher trophic levels, with larger predators preying on consumers from both root- and litter-C based energy channels. In addition to cycling between trophic levels, **8)** C can be lost from soil via respiration or **9)** become stabilized where, at least for some time, protected from further decomposition.

Table 1.3. Soil fauna responses to N fertilization. Results from perennial grass systems are green, other perennial systems are blue, and annual systems are orange. Responses: positive (+), negative (–), or no effect (NA). Fertilizer type: organic (O) or inorganic (I).

	Abundance			Diversity		
	–	+	NA	–	+	NA
Macrofauna	1		2, 3 (I)			
Earthworms	4 (I)	5 (O)	1	1		
Carabid beetles		5 (O)	6 (O, I)	5 (O)		5 (I) 6 (O, I)
Mesofauna					7	
Collembola		1, 8 (O, I)				1
Prostigmatid mites		8 (O, I)				
Microfauna						
Nematodes	2 (I)	9, 10 (O) 11 (O, I)	1 12 (I)			1 9 (O)
bacterivore	13 (I)	2, 14 (I) 9, 10 (O) 11 (O, I)	3 (I)		2 (I)	
fungivore	2, 3, 11 (I)	10 (O) 9 (O, I)	13 (I)			
plant parasite		9 (O) 11 (O, I)	12 (I)			
omnivore	10 (O, I)		13 (I)			
predator		2 (I)	3 (I) 9 (O)			
Protists		10 (O)	15 (O, I)	16 (I)		

[1]Cluzeau et al., 2012; [2]Murray et al., 2006, [3]Blanchart et al., 2006; [4]Ma et al., 1990; [5]Raworth et al., 2004; [6]Sądej et al., 2012; [7]Crossley et al., 1992; [8]Wang et al., 2016; [9]Hu & Qi, 2010; [10]Forge et al., 2005; [11]Zhang et al., 2016; [12]Emery et al., 2017; [13]Ikoyi et al., 2020; [14]Gruzdeva et al., 2007; [15]Lentendu et al., 2014; [16]Zhao et al., 2019.

Table 1.4. Faunal groups associated with switchgrass from the literature. For studies accounting for spatial or temporal variation in faunal abundance, richness, or diversity, sampling site number and/or sampling event timing are noted.

Faunal group	Sampling type	Spatial variation	Temporal variation	Identification level
Protists	Soil DNA sequencing[1]	2 sites	5 events over 6 mo	Family to genus, feeding group
Nematodes	Surveyed from soil cores[2]	NA	2 events over 3 mo	Family to genus, feeding group
	Surveyed from soil cores[3]	4 sites	NA	Genus to species (plant parasites)
	Surveyed from soil cores[4]	17 sites*	NA	Genus to species (plant parasites)
	Surveyed from soil cores[5]	At least 7 sites	NA	Species (only *Pratylenchus* spp.)
Collembola	Surveyed from soil cores[6]	NA	NA	Genus to species, morphotype
Earthworms	Surveyed from soil samples[7]	NA	NA	Species
Ants	Surveyed from pitfall traps[8]	3 sites	NA	Species, feeding guild
Carabid beetles	Surveyed from pitfall traps[9]	NA	4 events over 2 mo, 3 events 1 yr later	Genus to species
Rhizospheric eukaryotes	Soil DNA sequencing[10]	NA	NA	Supergroup to genus
Surface-dwelling arthropods	Surveyed from pitfall traps[11]	NA	4 events over 3 mo, repeated for 3 yr	Subclass to species (for select groups)

*Multiple plots from a single site were not counted separately

[1]Ceja-Navarro et al., 2021; [2]Emery et al., 2017; [3]Cassida et al., 2005; [4]Mekete et al., 2011a; [5]Mekete et al., 2011b; [6]Chauvat et al., 2014; [7]Emmerling, 2014; [8]Helms IV et al., 2020; [9]Ward & Ward, 2001; [10]Mao et al., 2014; [11]Holquin et al., 2010.

CHAPTER 2: PERENNIALITY INCREASES MICROARTHROPOD ABUNDANCE AND ALTERS COMMUNITY COMPOSITION IN BIOENERGY CROPPING SYSTEM

ABSTRACT

Agricultural intensification strongly impacts soil ecosystems in numerous ways, many of which are detrimental to soil fauna. Microarthropod activity and community structure are heavily influenced by the soil environment, rendering them highly sensitive to agricultural practices with implications for soil nutrient cycling dynamics. However, microarthropod taxa respond in varying, sometimes contrasting ways to different agricultural practices, making it challenging to predict how microarthropod communities will respond to new agricultural systems. This is particularly true for potential bioenergy cropping systems which can range from intensively managed annual monocultures to low-intensity perennial polycultures and are projected to become increasingly common to help reach carbon mitigation goals. To better understand how bioenergy crop type, diversity, and management intensity influence microarthropod communities, I surveyed microarthropods over two years from soil (both years) and litter (2019 only) across a gradient of annual monoculture, perennial monoculture, and perennial polyculture bioenergy cropping systems. I found consistent evidence that perennial systems, which included planted monocultures of switchgrass and restored prairie polycultures, favored higher overall microarthropod abundances, especially those of mites, compared to an annual monoculture of energy sorghum. Moreover, I found that the perennial cropping systems supported communities of similar composition, suggesting that bioenergy crop perenniality, rather than diversity, was the major driver of microarthropod community structure. These findings add to the growing body of research indicating that perennial bioenergy cropping systems better support above- and belowground biodiversity and ecosystem services than annual cropping systems.

INTRODUCTION

Agroecosystems cover over a third of the planet's terrestrial surface (FAO, 2014), with agricultural intensification impacting a substantial proportion of Earth's soils. Within these systems, factors such as crop species, type (i.e., annual or perennial), and diversity (i.e., monocultures or polycultures) as well as management practices (e.g., fertilization, pesticide

use, tillage, etc.) have varied and often strong effects on both soil physical and chemical characteristics, including soil structure, moisture, spatial heterogeneity, and soil organic matter (SOM) content (Alhameid et al., 2019; McDaniel et al., 2014). Depending on soil type and land use history, these factors can drastically change the soil characteristics which profoundly influence belowground microarthropod communities (Crossley et al., 1992). Moreover, many agricultural systems have undergone intensification with increased production of annual monocultures supported by high inputs of chemical fertilizers and pesticides (Matson et al., 1997). While favorable responses to changes in soil characteristics due to crop production are possible (Hendrix et al., 1990), the impacts of agricultural intensification on soil fauna, including microarthropods, are largely negative (Ponge et al., 2013; Postma-Blaauw et al., 2010; Crossley et al., 1992).

Microarthropods are the numerically dominant arthropod group in most soil ecosystems, including agroecosystems (Bardgett, 2005; Rusek, 1998; Dindal, 1990). Microarthropods are small (0.1 – 2 mm), rendering them physically unable to create their own channels to migrate throughout the soil (Coleman & Wall, 2015). This non-taxonomic group is often largely comprised of collembola and soil mites, though other microarthropod orders can be numerically dominant in some contexts. Coupled with the limited mobility of many species, most microarthropods largely spend their entire lives belowground or at the soil surface and rely heavily on pre-existing soil pores to facilitate dispersal. Due to this, any changes to soil structure resulting from agricultural management, such as increased compaction from tillage or shallow-rooted crop production, can greatly affect the distribution of microarthropods (Eo & Nakamoto, 2008; Cortet et al., 2002). Additionally, microarthropods are strongly influenced by soil temperature and moisture (Butcher et al., 1971), both of which change in response to agricultural management. For instance, soils lacking a stable surface litter layer, as is characteristic in many annual cropping systems, show increased diurnal fluctuations in temperature (Andrade et al., 2010) and decreased moisture retention (Deutsch et al., 2010).

Cropping practices can further impact microarthropod community structure and activity by influencing SOM quality, quantity, and accessibility. Most microarthropods, especially collembola and oribatid mites (suborder Oribatida), are detritivores and/or microbivores

(Potapov et al., 2022) and therefore directly or indirectly rely on plant-derived residues. Crop species vary broadly in the amount of carbon (C) allocated for above- or belowground production, though perennial crops generally exhibit increased belowground productivity and decreased ratio of above- to belowground production compared to annuals (Anderson-Teixeira et al., 2013; Conant et al., 2001). The diversity of crops produced in agroecosystems can also influence SOM properties, though clear patterns relating crop and microarthropod diversity have yet to be described (Hooper et al., 2000).

While microarthropods overall generally respond negatively to agricultural intensification, management practices can have varying impacts on microarthropods, ranging from negative to neutral or even positive effects. Fertilization, a hallmark of agricultural intensification, can lead to an increase in microarthropod diversity (Crossley et al., 1992) and abundance (Wang et al., 2016). Additionally, different microarthropod taxa can have differing or even contrasting responses to cropping systems. For example, while tillage disfavors oribatid mites (Adl et al., 2006), collembola may be unaffected by (Cluzeau et al., 2012) or even benefit (Crossley et al., 1992) from tillage. These divergent responses between microarthropod groups may reflect their differing life histories, with slow-growing, longer-lived groups such as oribatid mites generally less tolerant to soil disturbance from agriculture compared to fast-growing, short-lived groups with high fecundity such as collembola (Behan-Pelletier, 1999; Mallow et al., 1985; Moore et al., 1984).

The varying responses within and among different microarthropod groups to various cropping system traits complicate understanding and predicting how new crops and management will influence microarthropod communities. This is especially true in newly developing agroecosystems such as bioenergy cropping systems, which are projected to account for an increasing proportion of agricultural land use in the coming decades to help meet global climate change mitigation strategies (Reid et al., 2020). In the US Midwest, bioenergy crop production systems ranging from intensively managed annual monocultures to low-intensity perennial polycultures are being developed (Sanford et al., 2016), particularly on low-productivity or other soils unsuited for food crop production (Gelfand et al., 2013). There is growing evidence that low-input, perennial bioenergy cropping systems better support native

arthropod communities and their services aboveground (Landis et al., 2018; Werling et al., 2014). However, there have been no investigations to my knowledge to evaluate microarthropod community structure across diverse bioenergy cropping systems. This knowledge gap is especially problematic as microarthropods are directly and indirectly involved in important soil processes, including the cycling of organic nitrogen (N) via mineralization and soil organic C (SOC) due to their influence on decomposition. As N retention and SOC accrual and storage are necessary for the long-term sustainability of bioenergy (Robertson et al., 2017), it is important to understand how bioenergy cropping system design and management will influence the community structure and activity of microarthropods.

In 2018 and 2019, I conducted microarthropod surveys to investigate how bioenergy crop type, crop diversity, and management affect microarthropod abundance and community structure. Microarthropods were surveyed from three bioenergy cropping systems across a range of agricultural intensity: an annual monoculture receiving full chemical inputs, a perennial monoculture with reduced chemical inputs, and a perennial polyculture with no chemical inputs. As perennial crops have greater belowground productivity, remain active year-round, and support the formation of a stable litter layer at the soil surface, I predicted that the perennial cropping systems would support higher microarthropod abundances than the annual systems. Between the two perennial systems, I predicted that microarthropod abundance would increase with crop diversity aboveground due to the greater diversity of SOM inputs belowground. Finally, I predicted that crop perenniality would have the greatest influence over microarthropod community structure and therefore the communities under the perennial monoculture and polyculture systems would be more similar to each other than either would be toward the annual monoculture.

METHODS

Study Site and Bioenergy Cropping System Treatments

This study was conducted at the Great Lakes Bioenergy Research Center's (GLBRC) Biofuel Cropping System Experiment (BCSE) at the KBS in Hickory Corners, Michigan (42.394948, -85.373103). Established in 2008, the BCSE serves as a long-term field study site to investigate the performance and sustainability of a wide range of potential cellulosic bioenergy

cropping systems (Robertson & Hamilton, 2015). The site consists of ten bioenergy cropping system treatments in 30x40 m plots replicated five times in a randomized complete block design (for more information see ref). The bioenergy cropping systems investigated at the BCSE vary in terms of crop type, life history, and management requirements, ranging from intensively managed annual monocultures to minimally disturbed perennial polycultures. The soil series at the BCSE is a Kalamazoo loam (fine-loamy, mixed, mesic Typic Hapludalfs). The average annual temperature for the region is 10.1 °C with a mean annual precipitation of 1005 mm yr^{-1} (Robertson & Hamilton, 2015).

Microarthropod surveys were conducted within three of the ten bioenergy cropping system treatments: an energy sorghum monoculture, switchgrass monoculture, and restored prairie polyculture. These treatments were chosen to represent a gradient of increasing management intensification and exhibit a variable combination of crop diversity and life history traits. Energy sorghum (*Sorghum bicolor* L. Moench) is an annual C4 grass and important cereal crop native to north-eastern Africa which has been identified as a potentially important dedicated bioenergy crop due to its high yield potential and drought tolerance (Rooney et al., 2007). This treatment was first established in 2018, replacing corn as the model bioenergy crop in the experiment in part due to its high management requirements. Despite this cropping system not requiring tillage, annual replanting of the crop in rows along with chemical pest and weed control resulted in a shallow, transient surface litter layer. Switchgrass (*Panicum virgatum* L. cv. Cave-in-Rock), also a C4 grass, is native to North America and has been developed as a model biomass crop since the early 1990's (McLaughlin & Kszos, 2005). These plots grow as dense monocultures with well-developed surface litter layers. The final treatment was a restored prairie polyculture composed of 18 species of native grasses, legumes, and forbs. Restored prairie plots at the BCSE have not been tilled since their establishment in 2008, receive no fertilizer inputs, and have a well-established surface litter layer. For simplicity, these treatments will subsequently be referred to as annual monoculture (energy sorghum), perennial monoculture (switchgrass), and perennial polyculture (restored prairie). For further details on bioenergy cropping system treatments, see **Table 2.1**.

Microarthropod Sampling and Extraction

Microarthropods were sampled from soil (2018 & 2019) and surface litter (2019) from each bioenergy cropping system treatment. Sampling occurred over three time periods each year. In 2018, sampling was focused on the peak crop growing period with sampling occurring every three weeks between early July and mid-August. In 2019, I broadened the temporal timeframe and sampled once in spring (May), peak growing period (mid-August), and fall (October). Soil-dwelling microarthropods were collected using a cone-shaped soil corer to mitigate soil compaction during sampling (**Fig. 2.1**). Soil core samples were 3 cm in diameter and taken to a depth of 15 cm below the surface. Within each replicate plot (n = 5 per treatment), two soil core subsamples were taken near three designated sampling stations (n = 30 subsamples per treatment). Soil core subsamples were taken on opposite sides of a randomly selected energy sorghum stalk in the intercrop rows in the annual monoculture. As there were no distinct intercrop rows in the two perennial treatments, cores were sampled under the drip line on opposite sides of a randomly selected grass to avoid the bulk root zone. The dominant grass species in the sampling area was chosen in the perennial polyculture replicates and was frequently big bluestem (*Andropogon gerardii* L. Vitman). Soil cores were cut into three 5 cm sections during the first sampling period in 2018, but this practice was discontinued to limit soil core disturbance before extraction and reduce the number of soil samples to be processed. Once collected, soil cores were stored at approximately 6 °C prior to microarthropod extraction, after which subsamples were combined to account for the high spatial heterogeneity of microarthropod distribution in soils (n = 15 per treatment).

Litter-dwelling microarthropods were surveyed by collecting surface litter within 25x25 cm^2 PVC quadrats at each of the three designated sampling locations. Surface residues within the quadrat area were collected using hand rakes after removing living and standing dead residues with hand trimmers, with any litter that fell partially outside of the quadrat cut with scissors. It was impossible to separate surface litter from moss, surface soil, and living vegetation intermixed with these residues, but care was taken to remove these non-litter components when possible. As with soil samples, collected litter was stored at approximately 6 °C prior to microarthropod extraction.

Microarthropod extraction was conducted using Tullgren funnels (**Fig. 2.2**), which use a combination of light and heat to induce the downward migration of microarthropods out of soil and litter samples into collection containers (Tullgren, 1918). Due to a limited number of Tullgren funnels available in 2018, some soil samples were randomly chosen for storage in refrigeration (5-7 d maximum) while other samples were undergoing extraction. This was not necessary during the 2019 surveys with the addition of more funnels, and all samples were placed in funnels between 24-48 hr following field collection. Samples remained in Tullgren funnels until fully dry. Microarthropods in soil samples were extracted for 5 d due to the higher specific heat of soil, while those in litter were extracted over 3 d. Extracted microarthropods were collected in 70% propylene glycol before being transferred into vials with 95% ethanol. Following extraction, microarthropods were first separated into broad taxonomic groups and counted by hand-sorting specimens in petri dishes under a dissecting microscope. Specifically, microarthropods were sorted as: collembola, oribatid mites, other soil mites (i.e., prostigmatid, mesostigmatid, and astigmatid mites), and other fauna, which included other microarthropod groups collected at low abundance (i.e., orders Protura and Diplura). Collembola and oribatid mites were then further categorized by morphospecies to obtain community composition and diversity data without requiring the taxonomic and morphological expertise necessary for species-level identification, though collembola morphospecies could not be distinguished for the 2018 soil samples collected in early July due to extended time submerged in propylene glycol. Morphospecies were distinguished by physical traits which could be visualized under a dissecting microscope at 50-90x. For full description of morphospecies, see Appendix B: Morphospecies List (**Tables 2.7 & 2.8**).

Statistical Analysis

Microarthropod diversity was evaluated at the treatment level for each year by calculating species richness as well as Shannon's and Simpson's diversity indices, which give greater emphasis to rare and common species, respectively, and were calculated using the *vegan* package in R (Oksanen et al., 2022). Juvenile oribatid mite morphospecies were excluded from these calculations so as to not artificially inflate diversity values due to the likelihood of them belonging to the same species as adult morphospecies. Diversity differences across

treatments were assessed via Kruskal-Wallis test as these data lacked normality. Pairwise comparisons were assessed using Dunn's test *post hoc* with the *FSA* package (Ogle et al., 2022). To determine how robust these surveys were in detecting uncommon and rare morphospecies, species accumulation curves were generated for collembola and oribatid mites using the *vegan* package. A single curve was produced for each group with the slope of the curves providing an approximation of the degree to which the total sampling effort over two years was able to approach the true collembola and oribatid mite richness at the BCSE. Differences in community composition were detected using permutational multivariate analysis of variance (PERMANOVA) and visualized with non-metric multidimensional scaling (NMDS) using the *vegan* package. Microarthropod abundance was determined for the levels of total microarthropods, oribatid mites, other mites, and collembola. Generalized linear mixed models (GLMMs) with a Poisson distribution were used to assess differences in microarthropod abundances at each of these levels using the *lme4* package (Bates et al., 2015). Bioenergy cropping system treatment and sampling period treated as interactive fixed effects. Random effects in the models included replicate field plot and sampling location nested within replicate field plot and date. Pairwise comparisons across treatments were assessed *post-hoc* using the package *emmeans* with a Tukey adjustment (Lenth, 2022; Searle et al., 1980). Statistical analyses were performed in R ver. 4. 2. 1. (R Core Team, 2018).

RESULTS

Microarthropod community composition and diversity

We collected over 89,000 total microarthropods across the two years, of which 11% were collembola and 89% were mites. Oribatid mites comprised 22% of the total number of mites collected, with the remaining predominately prostigmatid and astigmatid mites while mesostigmatid mites were the least numerous overall. Other fauna collected were predominantly insects, especially booklice and true bugs, but occasionally included myriapods, earthworms, slugs, and other arachnids. Total abundance in soil was highest in 2018, with 18,416 microarthropods collected compared to 11,811 collected in 2019 (**Fig. 2.3**). However, litter samples collected in 2019 yielded the highest densities of microarthropods across sampling periods and treatments, with 59,669 microarthropods collected in total. In 2019, total

collembola and mite densities in litter were approximately 4.5 to 5.1 times higher than in soil, respectively. Despite the large numerical difference in total abundances, the relative proportions of broad taxonomic groups remained consistent between years with collembola least abundant (~10%), followed by oribatid (~20%) and other mites (~70%). In contrast to their lower overall abundances, collembola accounted for the highest number of morphospecies collected over both years.

In total, 72 morphospecies were collected by the end of the two-year survey. Species accumulation curves indicate that the sampling effort throughout the entirety of the survey was relatively robust in accounting for morphospecies richness at the BCSE, especially for oribatid mites (**Fig. 2.4**). In contrast to their relative contribution to the total number of microarthropods collected, collembola exhibited markedly higher morphospecies richness than oribatid mites, with 62 collembola morphospecies compared to 10 oribatid mite morphospecies. An additional 8 juvenile oribatid mite morphospecies were collected and were assumed to be the nymphal stages for some of the 10 adult morphospecies, hence only the adult mite morphospecies were used in diversity measures to avoid artificially inflating oribatid mite richness. Of the 62 collembola morphospecies, 31 possessed the elongate body form of collembola in the orders Entomobryomorpha and Poduromorpha with the remaining 31 belonging to the order Symphypleona and thus having a globular form. No members of the Neelipleona, also an order of globular collembola, were collected from the BCSE. Over half of these were rare, with 17 elongate and 22 globular morphospecies having relative abundances of less than 1% (**Table 2.2**). In comparison, only 2 adult and 3 juvenile oribatid mite morphospecies were encountered infrequently and at very low abundance (**Table 2.3**). The majority of morphospecies from both groups were collected from all three cropping systems, and those that were present in only one or two cropping systems were always rare. The annual monoculture had the greatest number of unique morphospecies (10 collembola morphospecies), followed by the perennial polyculture (6 collembola and 2 oribatid morphospecies) with the perennial monoculture having only two unique morphospecies, both being collembola.

Microarthropod diversity was generally similar across the three cropping systems across

both years when looking at oribatid and collembola diversity together, though morphospecies richness was highest in the annual monoculture and lowest in the perennial monoculture in 2019 ($p = 0.020$). When examining these two taxonomic groups separately, however, I observed diversity differences across treatments and years (**Table 2.4**). For collembola, all three diversity measures were similar across cropping systems in 2018. This contrasts with 2019, in which collembola richness was highest in the annual monoculture (Kruskal-Wallis; $\chi^2 = 7.546$, $p = 0.023$) and both Shannon's and Simpson's diversity was highest in the perennial polyculture (Shannon's: Kruskal-Wallis; $\chi^2 = 9.100$, $p = 0.011$ & Simpson's: Kruskal-Wallis; $\chi^2 = 6.834$, $p = 0.033$). Collembola richness, Shannon's diversity, and Simpson's diversity were all lowest in the perennial monoculture during this year. Oribatid mites exhibited a different pattern, with morphospecies richness differing across treatments for both years while Shannon's and Simpson's diversity remained consistent across cropping systems. In both years, oribatid richness was greatest in the perennial polyculture (2018: Kruskal-Wallis; $\chi^2 = 7.414$, $p = 0.0246$ & 2019: Kruskal-Wallis; $\chi^2 = 7.548$, $p = 0.023$), with the lowest richness found in the annual monoculture in 2018 and perennial monoculture in 2019. Additionally, community composition differed between cropping system treatments for both soil- and litter-dwelling microarthropods across both years (PERMANOVA; $F_{[2,42]} = 5.138$, $p = 0.001$). In particular, there were strong differences between the communities supported by perennial cropping systems and annual monoculture, whereas there were no substantial differences found between perennial monoculture and polyculture (**Fig. 2.5**).

Microarthropod abundance

In both years, I observed temporal and crop treatment differences in the abundance of microarthropods. In 2018, total microarthropod abundance in all cropping systems increased from early- to late-July before decreasing in mid-August, though these temporal changes were generally slight (**Fig. 2.5**). Assessing abundance patterns by bioenergy cropping system treatment, I found consistently higher average microarthropod abundances in the perennial monoculture and polyculture treatments compared to those in the annual monoculture ($p < 0.001$, **Fig. 2.5A**). These differences were especially pronounced in the first two survey periods while microarthropod abundances between treatments became more similar in mid-August

(**Table 2.5**). When accounting for the contribution of broad taxonomic groups individually, I found that the differences in microarthropod abundance were largely driven by oribatid ($p < 0.001$, **Fig. 2.5B**) and other soil mites ($p < 0.001$, **Fig. 2.5C**), which were more abundant in perennial cropping systems. Conversely, collembola abundances were either similar across treatments or lower in perennial cropping systems compared to the annual monoculture ($p < 0.001$, **Fig. 2.5D**).

There were marked temporal differences in the numbers of microarthropods collected at each sampling period in 2019 (**Fig. 2.6**). Total microarthropod abundances in the perennial mono- and polyculture were lower in May 2019 than at any other point in the survey before increasing slightly in August and greatly into October. In comparison, total abundances in the annual monoculture declined between May and August before increasing into October. As in 2018, microarthropods in 2019 were on average more abundant in the perennial bioenergy cropping system treatments ($p < 0.001$) (**Table 2.6**). However, this was only the case in August and October, with the perennial monoculture having the lowest total microarthropod abundance in May (**Fig. 2.6A**). This pattern in total abundance was broadly mirrored by oribatid and more so other mites, indicating that the increased abundances of microarthropods overall in the perennial treatments was largely driven by mites ($p < 0.001$, **Fig. 2.6B and 2.6C**). In comparison, collembola abundances were similar across sampling periods ($p < 0.001$, **Fig. 2.6D**).

DISCUSSION

Crop species, within-cropping system plant diversity, and management intensity exert substantial impacts on the soils in agroecosystems with consequential influences on belowground communities. These cropping system effects are predicted to be especially important for small, less-mobile organisms with low dispersal capabilities (Hedlund et al., 2004). By surveying microarthropods over two years from three bioenergy cropping systems ranging from an annual monoculture to a perennial polyculture, I found evidence supporting my hypothesis that perennial bioenergy cropping systems support more microarthropods compared to annual cropping systems. Additionally, I found strong differences in microarthropod community composition across cropping systems, with the annual monoculture supporting a distinct community compared to the two perennial systems. These findings were

consistent across sampling years, most individual sampling periods, and between soil- and litter-dwelling microarthropods, despite there being large differences in overall abundance depending on sample type and timing.

There has been increasing effort in recent years to better understand bioenergy cropping system effects on biodiversity, although most studies to date have focused on aboveground or microbial communities. Both perennial switchgrass monocultures and prairie polycultures can support greater abundances and diversity of agriculturally important arthropod groups, including herbivores and predators, as well as methanotrophic microbes and breeding birds (Werling et al., 2014). Studies have previously indicated that the positive effects of perennial systems on biodiversity may also extend to belowground fauna (Emmerling, 2014; Hedde et al., 2013; Ward & Ward, 2001). Compared to annual crops, perennial crops allocate more C and biomass belowground, have deeper and more extensive root systems, and are present on the landscape year-round. In addition to ensuring a continuous flow of SOM to support belowground food webs, perennial root growth increases the porosity of soils, creating channels to facilitate increased transport for mesofauna and other smaller organisms. Furthermore, the lack of tillage and largely unrestrained weed growth characteristic of perennial cropping systems lead to the formation of a stable litter layer at the soil surface. This litter layer provides habitat for surface-dwelling fauna and it insulates the underlying soil from diurnal temperature and moisture fluctuations, increasing the stability of soil microhabitats for mesofauna. While energy sorghum does not receive annual tillage, routine tillage in these field plots in the decade prior to its cultivation had resulted in this system having sparse, shallow patches of litter interspersing bare soil. As sorghum stover and other post-harvest residues accumulate, it is possible that microarthropod abundances in the annual monoculture will increase over time.

Of the major microarthropod taxa examined, I found that soil mites were particularly abundant in perennial cropping systems. In contrast, collembola appeared to be either unaffected by cropping system type or were more abundant in the annual system. This finding was unsurprising, as soil mites, particularly oribatids, typically respond negatively to agricultural intensification while collembola may exhibit negative, neutral, or even positive responses

(Cortet et al., 2002; Maraun & Scheu, 2000; Crossley et al., 1992). This differential, sometimes contrasting, response to agricultural intensification is thought to largely be driven by the differing life history strategies of these taxa. Oribatid mites are generally slow growing with low fecundity and metabolism, limiting their ability to respond to changes (Behan-Pelletier, 1999). Conversely, collembola tend to exhibit higher development rates, fecundity, and metabolism as well as greater mobility, especially species possessing a furcula for jumping (Butcher et al., 1971). While oribatid populations may be at low abundance in annual systems due to greater fluctuation in abiotic conditions and more frequent disturbance, collembola populations are better able to rebound due to their greater reproductive output.

We found that microarthropod diversity differences across cropping systems depended both on survey year and taxa examined. Though only statistically significant in 2019, the annual monoculture had the greatest collembola richness while both Shannon's and Simpson's diversity for collembola remained similar to those found for the two perennial systems. As collembola abundance also tended to be higher in the annual monoculture, this further suggests that collembola can successfully inhabit, and possibly even benefit from, the more disturbance-prone conditions of the annual monoculture system. In comparison, oribatid richness was consistently greatest in the perennial polyculture. Agricultural practices including monocropping and fertilization have a homogenizing effect on soils which can still be detected decades after cultivation has stopped (Li et al., 2010; Robertson et al., 1993). It is likely that the 10 y of production in the annual monoculture prior to the establishment of energy sorghum in 2018, especially the legacy of annual tillage, is influencing microarthropods in this system. Though the effects of land use history cannot be decoupled from that of annual crop production alone in the current study, potentially positive effects on microarthropods with time following the establishment of energy sorghum are not predicted to outweigh the negative effects of annual monocultures (i.e., shallow rooting systems, soil homogenization). There is growing evidence that polycultures are particularly beneficial for restoring soil heterogeneity (Eisenhauer, 2016). As microarthropod richness, especially that of oribatid mites, has been found to respond positively to soil spatial heterogeneity (Nielsen et al., 2010), it is possible that differences in soil heterogeneity between annual and perennial monocultures with the

perennial polyculture could underpin the latter system supporting a richer oribatid community.

Interestingly, I did not find strong differences in microarthropod abundance or community composition between perennial monocultures or perennial polycultures, suggesting that perenniality is a stronger driver of microarthropod community structure than crop diversity. Unlike in aboveground ecosystems, there does not seem to be a clear relationship between plant and belowground faunal species richness (Hooper et al., 2000). Microarthropods are largely dominated by generalist detritivores and/or microbivores and thus should exhibit high functional redundancy (Cole et al., 2006; Liiri et al., 2002; Dindal, 1990). Therefore, microarthropod communities should be largely unaffected by the increased diversity of plant-derived SOM in diverse polycultures. Instead, the functional diversity of the aboveground plant community, especially regarding plant traits that will influence microclimate and/or microhabitat conditions of the underlying soil (i.e., deep, expansive root structures), is likely to have a greater impact on microarthropods than diversity *per se*. Additionally, the lack of weed control in either perennial treatment resulted in the presence of weeds, which could have potential hidden diversity effects on microarthropod communities.

In addition to my comparisons of microarthropod communities across cropping systems, one unexpected finding was the relatively low number of soil-dwelling microarthropods surveyed in 2019 compared to those collected in 2018. Microarthropods are highly sensitive to both temperature and humidity (Butcher et al., 1971) and may also exhibit biannual abundance peaks in spring-early summer and autumn (Vikram Reddy & Venkataiah, 1990; Wallwork, 1970). Despite this, overall microarthropod abundance in May 2019 declined substantially from the summer sampling periods in 2018, all of which remained relatively similar. Abundances were similarly low in August 2019, approximately half of that collected at nearly the same time in the prior year. Microarthropod abundances in 2019 only approached that of in 2018 in October. One probable explanation for this finding was the region experienced above-average rainfall between January and October 2019. There is evidence that elevated soil moisture content can negatively impact microarthropod abundances as well as influence their community composition and vertical distribution throughout the soil profile (O'Lear & Blair, 1999; Price, 1973).

This study supports the growing body of evidence that perennial bioenergy cropping systems better support biodiversity than annual systems, and that these favorable biodiversity impacts extend belowground to soil communities. It remains to be empirically tested, however, whether the benefits of perennial habitat on microarthropods can be linked to enhanced ecosystem service provisioning, as has been seen aboveground with enhanced pest control and pollination services (Werling et al., 2014). Being largely detritivores and microbivores, microarthropods have numerous direct and indirect effects on SOC dynamics (Zahorec et al., 2022, Chapter 1). They alter the physical and chemical qualities of SOM through their feeding, including fragmenting plant residues where they are more easily accessed by decomposer microbes and producing nutrient-rich fecal pellets which can become hotspots for decomposition (Lussenhop, 1992). Microarthropods also feed directly on microbes, which can alter their activity and composition with potentially strong impacts on soil C (Janouškova et al., 2018; Crowther et al., 2012; Wickings & Grandy, 2011). As SOC accrual is necessary for the wide-spread implementation of bioenergy cropping systems at scales necessary to have a meaningful effect on greenhouse gas emissions, it will be important to further understand how cropping system effects impact microarthropod communities and the implications on SOC dynamics in these systems.

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REFERENCES

Adl, S. M., Coleman, D. C., & Read, F. (2006). Slow recovery of soil biodiversity in sandy loam soils of Georgia after 25 years of no-tillage management. *Agriculture, Ecosystems & Environment*, *114*(2-4), 323-334.

Alhameid, A., Singh, J., Sekaran, U., Ozlu, E., Kumar, S., & Singh, S. (2019). Crop rotational diversity impacts soil physical and hydrological properties under long-term no-and conventional-till soils. *Soil Research*, *58*(1), 84-94.

Anderson-Teixeira, K. J., Masters, M. D., Black, C. K., Zeri, M., Hussain, M. Z., Bernacchi, C. J., & DeLucia, E. H. (2013). Altered belowground carbon cycling following land-use change to perennial bioenergy crops. *Ecosystems*, *16*(3), 508-520.

Andrade, J. A. V., Abreu, F. M. G. D., & Madeira, M. A. V. (2010). Influence of litter layer removal on the soil thermal regime of a pine forest in a Mediterranean climate. *Revista Brasileira de Ciência do Solo*, *34*(5), 1481-1490.

Bardgett, R. (2005). *The biology of soil: A community and ecosystem approach*. Oxford University Press.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1-48.

Behan-Pelletier, V. M. (1999). Oribatid mite biodiversity in agroecosystems: Role for bioindication. *Agriculture, Ecosystems & Environment*, *74*(1-3), 411-423.

Butcher, J. W., Snider, R., & Snider, R. J. (1971). Bioecology of edaphic Collembola and Acarina. *Annual Review of Entomology*, *16*(1), 249-288.

Cluzeau, D., Guernion, M., Chaussod, R., Martin-Laurent, F., Villenave, C., Cortet, J., Ruiz-Camacho, N., Pernin, C., Mateille, T., Philippot, L., Bellido, A., Rougé, L., Arrouays, D., Bispo, A., & Pérès, G. (2012). Integration of biodiversity in soil quality monitoring: Baselines for microbial and soil fauna parameters for different land-use types. *European Journal of Soil Biology*, *49*, 63-72.

Cole, L., Bradford, M. A., Shaw, P. J., & Bardgett, R. D. (2006). The abundance, richness and functional role of soil meso-and macrofauna in temperate grassland — A case study. *Applied Soil Ecology*, *33*(2), 186-198.

Coleman, D. C., & Wall, D. H. (2015). Soil fauna: Occurrence, biodiversity, and roles in ecosystem function. In E. A. Paul (Ed.), *Soil microbiology, ecology and biochemistry* (4th ed.), (pp. 111-149). Academic Press.

Conant, R. T., Paustian, K., & Elliott, E. T. (2001). Grassland management and conversion into grassland: Effects on soil carbon. *Ecological Applications*, *11*(2), 343-355.

Cortet, J., Ronce, D., Poinsot-Balaguer, N., Beaufreton, C., Chabert, A., Viaux, P., & de Fonseca, J. P. C. (2002). Impacts of different agricultural practices on the biodiversity of microarthropod communities in arable crop systems. *European Journal of Soil*

Biology, *38*(3-4), 239-244.

Crossley Jr, D. A., Mueller, B. R., & Perdue, J. C. (1992). Biodiversity of microarthropods in agricultural soils: Relations to processes. *Agriculture, Ecosystems & Environment*, *40*(1-4), 37-46.

Crowther, T. W., Boddy, L., & Jones, T. H. (2012). Functional and ecological consequences of saprotrophic fungus–grazer interactions. *ISME Journal*, *6*(11), 1992-2001.

Deutsch, E. S., Bork, E. W., & Willms, W. D. (2010). Separation of grassland litter and ecosite influences on seasonal soil moisture and plant growth dynamics. *Plant Ecology*, *209*, 135-145.

Dindal, D. L. (1990). *Soil biology guide*. Wiley.

Eisenhauer, N. (2016). Plant diversity effects on soil microorganisms: Spatial and temporal heterogeneity of plant inputs increase soil biodiversity. *Pedobiologia*, *59*(4), 175-177.

Emmerling, C. (2014). Impact of land-use change towards perennial energy crops on earthworm population. *Applied Soil Ecology*, *84*, 12-15.

Eo, J., & Nakamoto, T. (2008). Spatial relationships between roots and soil organisms under different tillage systems. *European Journal of Soil Biology*, *44*(3), 277-282.

FAO. (2014). FAOSTAT database collections. Food and Agriculture Organization of the United Nations, Rome. Accessed December 11, 2020.

Gelfand, I., Sahajpal, R., Zhang, X., Izaurralde, R. C., Gross, K. L., & Robertson, G. P. (2013). Sustainable bioenergy production from marginal lands in the US Midwest. *Nature*, *493*(7433), 514-517.

Hedde, M., van Oort, F., Renouf, E., Thénard, J., & Lamy, I. (2013). Dynamics of soil fauna after plantation of perennial energy crops on polluted soils. *Applied Soil Ecology*, *66*, 29-39.

Hedlund, K., Griffiths, B., Christensen, S., Scheu, S., Setälä, H., Tscharntke, T., & Verhoef, H. (2004). Trophic interactions in changing landscapes: Responses of soil food webs. *Basic and Applied Ecology*, *5*(6), 495-503.

Hendrix, P. F., Crossley, D. A., Blair, J. M., & Coleman, D. C. (2020). Soil biota as components of sustainable agroecosystems. In C. A. Edwards, R. Lal, P. Madden, R. H. Miller, & G. House (Eds.). *Sustainable agricultural systems* (pp. 637-654). Soil and Water Conservation Society.

Hooper, D. U., Bignell, D. E., Brown, V. K., Brussard, L., Dangerfield, J. M., Wall, D. H., Wardle, D. A., Coleman, C. A., Giller, K. E., Lavelle, P., van der Putten, W. H., de Ruiter, P. C., Rusek, J., Silver, W. L., Tiedje, J. M., & Wolters, V. (2000). Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. *Bioscience*, *50*(12), 1049-1061.

Janoušková, M., Kohout, P., Moradi, J., Doubková, P., Frouz, J., Vosolsobě, S., & Rydlová, J.

(2018). Microarthropods influence the composition of rhizospheric fungal communities by stimulating specific taxa. *Soil Biology & Biochemistry*, *122*, 120-130.

Landis, D. A., Gratton, C., Jackson, R. D., Gross, K. L., Duncan, D. S., Liang, C., Meehan, T. D., Robertson, B. A., Schmidt, T. M., Stahlheber, K. A., Tiedje, J. M., & Werling, B. P. (2018). Biomass and biofuel crop effects on biodiversity and ecosystem services in the North Central US. *Biomass & Bioenergy*, *114*, 18-29.

Lenth, R. (2022). Emmeans: Estimated marginal means, aka least-squares means. R package version 1.7.5, <https://CRAN.R-project.org/package=emmeans>.

Li, J., Richter, D. D., Mendoza, A., & Heine, P. (2010). Effects of land-use history on soil spatial heterogeneity of macro-and trace elements in the Southern Piedmont USA. *Geoderma*, *156*(1-2), 60-73.

Liiri, M., Setälä, H., Haimi, J., Pennanen, T., & Fritze, H. (2002). Relationship between soil microarthropod species diversity and plant growth does not change when the system is disturbed. *Oikos*, *96*(1), 137-149.

Lussenhop, J. (1992). Mechanisms of microarthropod-microbial interactions in soil. In M. Begon & A. H. Fitter (Eds.). *Advances in ecological research* (Vol. 23, pp. 1-33). Academic Press.

Mallow, D., Snider, R. J., & Robertson, L. S. (1985). Effects of different management practices on Collembola and Acarina in corn production systems. II: The effects of moldboard plowing and atrazine. *Pedobiologia*, *28*(2), 115-131.

Maraun, M., & Scheu, S. (2000). The structure of oribatid mite communities (Acari, Oribatida): Patterns, mechanisms and implications for future research. *Ecography*, *23*(3), 374-382.

Matson, P. A., Parton, W. J., Power, A. G., & Swift, M. J. (1997). Agricultural intensification and ecosystem properties. *Science*, *277*(5325), 504-509

McDaniel, M. D., Tiemann, L. K., & Grandy, A. S. (2014). Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. *Ecological Applications*, *24*(3), 560-570.

McLaughlin, S. B., & Kszos, L. A. (2005). Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. *Biomass & Bioenergy*, *28*(6), 515-535.

Moore, J.C., Snider, R.J., Robertson, L.S., 1984. Effects of different management-practices on Collembola and Acarina in corn production systems. I: The effects of no-tillage and atrazine. *Pedobiologia*, *26*, 143–152.

Nielsen, U. N., Osler, G. H., Campbell, C. D., Neilson, R., Burslem, D. F., & Van der Wal, R. (2010). The enigma of soil animal species diversity revisited: The role of small-scale heterogeneity. *PLoS One*, *5*(7), e11567.

Ogle, D. H., Doll, J. C., Wheeler, P., & Dinno, A. (2022). FSA: Fisheries stock analysis. R package version 0.9.3, <https://github.com/fishR-Core-Team/FSA>.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., de Caceres, M., Durand, S., … Weedon, J. (2022). Vegan: Community ecology package. R package version 2.6-2, <https://CRAN.R-project.org/package=vegan>.

O'Lear, H. A., & Blair, J. M. (1999). Responses of soil microarthropods to changes in soil water availability in tallgrass prairie. *Biology and Fertility of Soils*, *29*, 207-217.

Ponge, J. F., Pérès, G., Guernion, M., Ruiz-Camacho, N., Cortet, J., Pernin, C., Villenave, C., Chaussod, R., Martin-Laurent, F., Bispo, A., & Cluzeau, D. (2013). The impact of agricultural practices on soil biota: A regional study. *Soil Biology & Biochemistry*, *67*, 271-284.

Postma-Blaauw, M. B., de Goede, R. G. M., Bloem, J., Faber, J. H., & Brussaard, L. (2010). Soil biota community structure and abundance under agricultural intensification and extensification. *Ecology*, *91*(2), 460-473.

Potapov, A. M., Beaulieu, F., Birkhofer, K., Bluhm, S. L., Degtyarev, M. I., Devetter, M., Goncharov, A. A., Klarner, B., Korobushkin, D. I., Liebke, D. F., Maraun, M., McDonnell, R. J., Pollierer, M. M., Schaefer, I., Shrubovych, J., Semenyuk, I. I., Sendra, A., Tuma, J., Tůmová, M., … Scheu, S. (2022). Feeding habits and multifunctional classification of soil-associated consumers from protists to vertebrates. *Biological Reviews*, *97*(3), 1057-1117.

Price, D. (1973). Abundance and vertical distribution of microarthropods in the surface layers of a California pine forest soil. *Hilgardia*, *42*(4), 121-147.

Reid, W. V., Ali, M. K., & Field, C. B. (2020). *The future of bioenergy*. *Global Change Biology*, *26*(1), 274-286.

Robertson, G. P., Crum, J. R., & Ellis, B. G. (1993). The spatial variability of soil resources following long-term disturbance. *Oecologia*, *96*, 451-456.

Robertson, G. P., & Hamilton, S. K. (2015). Long-term ecological research at the Kellogg Biological Station LTER site. In S. K. Hamilton, J. E. Doll, & G. P. Robertson (Eds.), *The ecology of agricultural landscapes: Long-term research on the path to sustainability* (pp. 1-32). Oxford University Press.

Robertson, G. P., Hamilton, S. K., Barham, B. L., Dale, B. E., Izaurralde, R. C., Jackson, R. D., Landis, D. A., Swinton, S. M., Thelen, K. D., & Tiedje, J. M. (2017). Cellulosic biofuel contributions to a sustainable energy future: Choices and outcomes. *Science*, *356*(6345).

Rooney, W. L., Blumenthal, J., Bean, B., & Mullet, J. E. (2007). Designing sorghum as a dedicated bioenergy feedstock. *Biofuels, Bioproducts & Biorefining*, *1*(2), 147-157.

Rusek, J. (1998). Biodiversity of Collembola and their functional role in the ecosystem. *Biodiversity and Conservation*, *7*, 1207-1219.

Sanford, G. R., Oates, L. G., Jasrotia, P., Thelen, K. D., Robertson, G. P., & Jackson, R. D. (2016). Comparative productivity of alternative cellulosic bioenergy cropping systems in the North Central USA. *Agriculture, Ecosystems & Environment*, *216*, 344-355.

Searle, S. R., Speed, F. M., & Milliken, G. A. (1980). Population marginal means in the linear model: An alternative to least squares means. *American Statistician*, *34*(4), 216-221.

Tullgren, A. (1918): Ein sehr einfacher Ausleseapparat für territole Tierfaunen. *Zeitschrift für Angewandte Entomologie*, *4*, 149-150.

Vikram Reddy, M., & Venkataiah, B. (1990). Seasonal abundance of soil-surface arthropods in relation to some meteorological and edaphic variables of the grassland and tree-planted areas in a tropical semi-arid savanna. *International Journal of Biometeorology*, *34*, 49-59.

Wallwork, J. A. (1970). *Ecology of soil animals*. McGraw-Hill.

Wang, S., Chen, H. Y., Tan, Y., Fan, H., & Ruan, H. (2016). Fertilizer regime impacts on abundance and diversity of soil fauna across a poplar plantation chronosequence in coastal Eastern China. *Scientific Reports*, *6*(1), 1-10.

Ward, K. E., & Ward, R. N. (2001). Diversity and abundance of carabid beetles in short-rotation plantings of sweetgum, maize and switchgrass in Alabama. *Agroforestry Systems*, *53*(3), 261.

Werling, B. P., Dickson, T. L., Isaacs, R., Gaines, H., Gratton, C., Gross, K. L., Liere, H., Malmstrom, C. M., Meehan, T. D., Ruan, L., Robertson, B. A., Robertson, G. P., Schmidt, T. M., Schrotenboer, A. C., Teal, T. K., Wilson, J. K., & Landis, D. A. (2014). Perennial grasslands enhance biodiversity and multiple ecosystem services in bioenergy landscapes. Proceedings of the National Academy of Sciences, 111(4), 1652-1657.

Wickings, K., & Grandy, A. S. (2011). The oribatid mite *Scheloribates moestus* (Acari: Oribatida) alters litter chemistry and nutrient cycling during decomposition. *Soil Biology & Biochemistry*, *43*(2), 351-358.

Zahorec, A., Reid, M. L., Tiemann, L. K., & Landis, D. A. (2022). Perennial grass bioenergy cropping systems: Impacts on soil fauna and implications for soil carbon accrual. *GCB Bioenergy*, *14*(1), 4-23.

APPENDIX A: CHAPTER 2 FIGURES & TABLES

Table 2.1. Agronomic management details for the three bioenergy cropping system treatments microarthropods were sampled from. Fertilization and pesticide amounts are given on a per hectare basis. For complete list of species planted in the perennial polyculture see ref.

Treatment	Crop details	Fertilization	Pest control
Energy sorghum	Continuous energy sorghum (*Sorghum bicolor*, photoperiod-sensitive hybrid ES5200), est. 2018 with annual replanting. Previously corn-soybean-canola from 2008-2011 and continuous corn with cover crop from 2012-2017.	2018: blend of 28% urea ammonium nitrate (UAN) & 19-17-0 (9.19 kg N, 5.50 kg P), potash (27.52 kg K) 2019: 28% UAN (9.19kg N), super phosphate (11.01 kg P*), potash (27.52 kg K)	2018: Dual II Magnum (1.87 L), Roundup Powermax (1.61 L) 2019: AMS (3.81 kg), Roundup Powermax (2.34 L), Liberty 280SL (2.34 L)
Switchgrass	Switchgrass (*Panicum virgatum*, Cave-in-Rock variety), est. 2008.	28% UAN (9.19 kg N)	NA
Restored prairie	Mixture of six graminoids, three legumes, three early forbs, three mid forbs, and three late forbs, est. 2008.	NA	NA

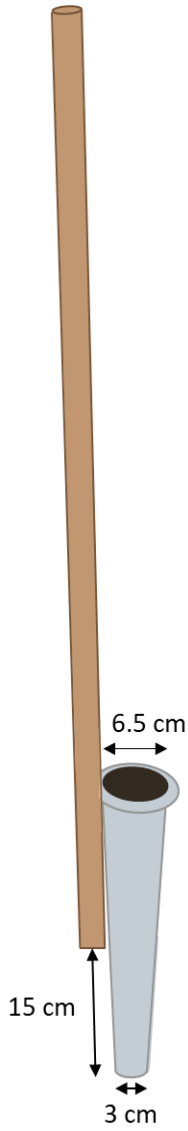

Figure 2.1. Diagram of cone-shaped corer used to collect soil samples for soil-dwelling microarthropod extraction.

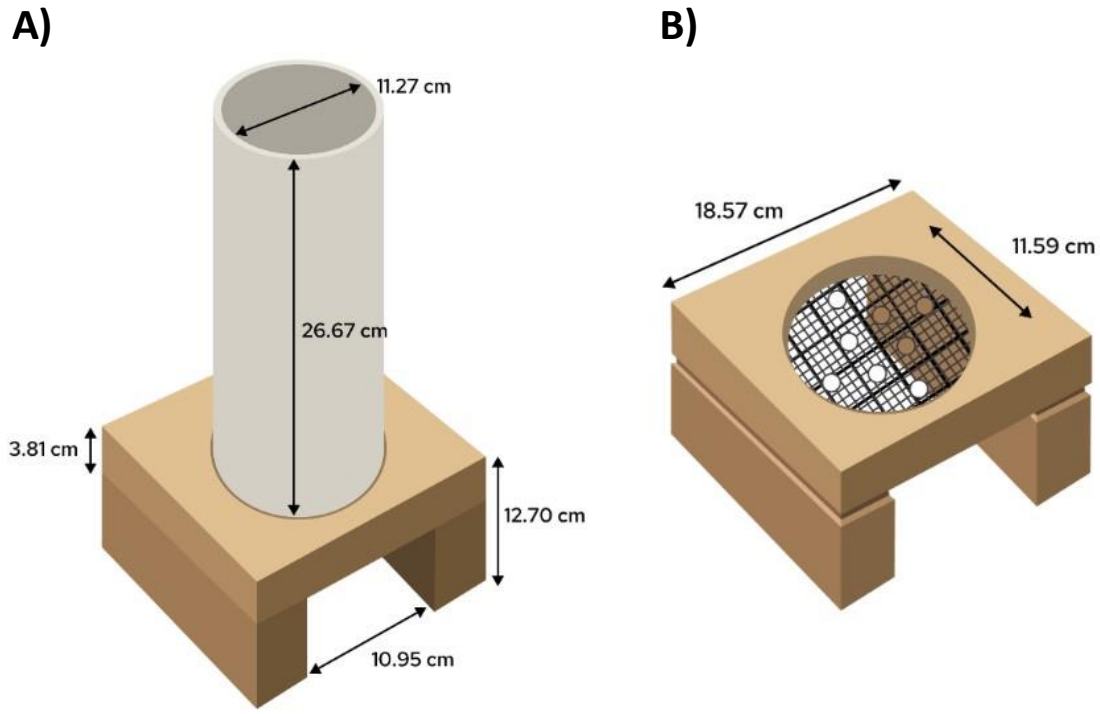

Figure 2.2. Design and dimensions of Tullgren funnels used for microarthropod extraction. **A)** Soil and litter samples were placed into 30 cm tall PVC cylinders over which a lightbulb was hung to subject microarthropods to increasing heat and light intensity. **B)** A circular cut of window screen mesh was held inside the funnel base to prevent debris from falling into collection cups of propylene glycol with additional holes punched in to allow larger fauna to exit samples.

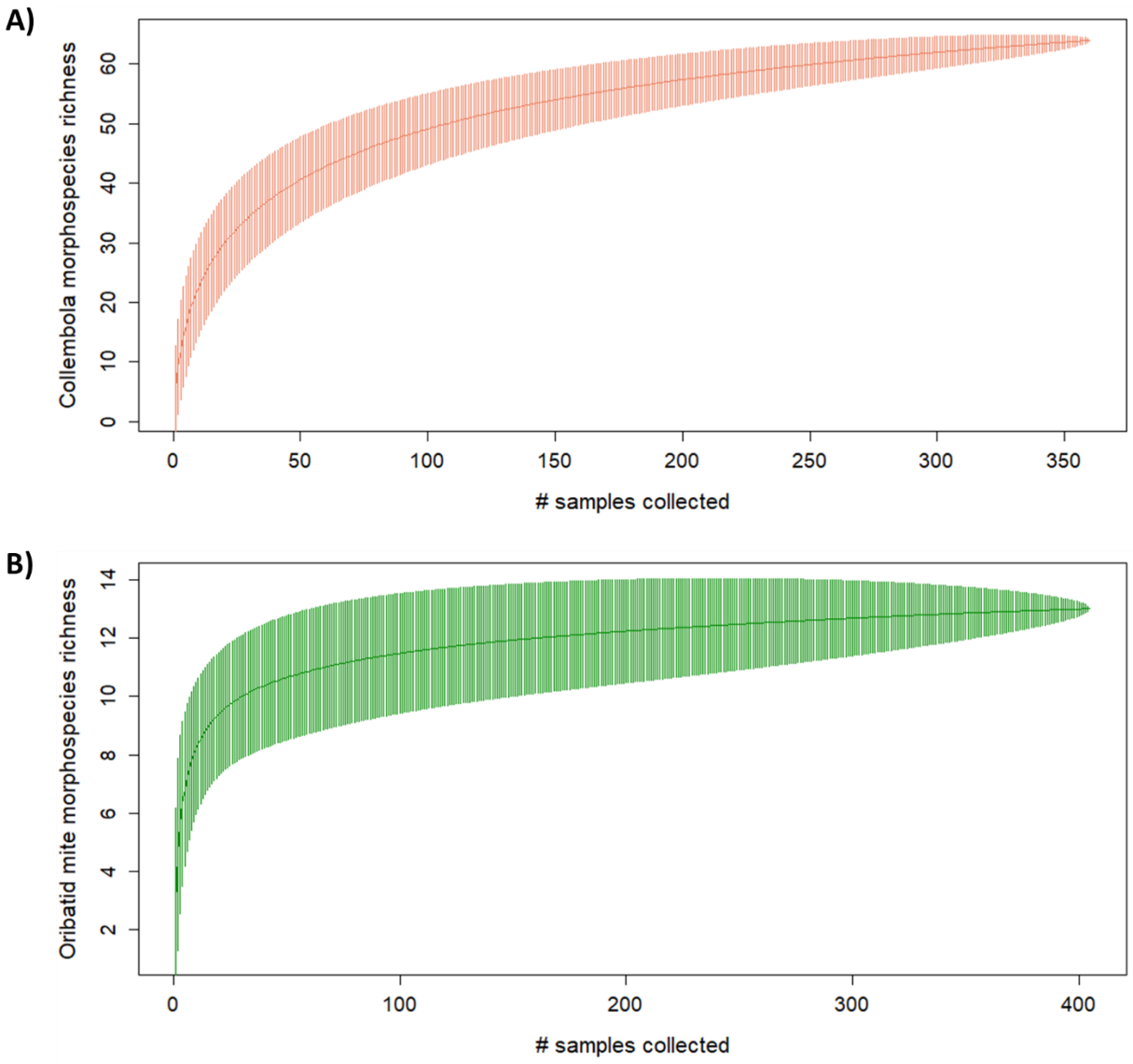

Figure 2.3. Species accumulation curves for **A)** collembola and **B)** oribatid mite morphospecies collected at the BCSE. All samples taken across time, cropping systems, and substrate have been included with the curves representing the accumulation of morphospecies with sampling effort for the BCSE site as a whole.

Table 2.2. Collembola morphospecies collected from the BCSE between 2018 and 2019, including the proportion they contributed to the total collembola abundance, summed across cropping systems and sampling periods, as well as the cropping systems they were collected from. PP: perennial polyculture, PM: perennial monoculture, AM: annual monoculture.

	Proportional abundance (%)			Collected from:		
globular	2018 – soil	2019 – soil	2019 – litter	PP	PM	AM
G1	0	0	<1	X		X
G2	0	<1	<1	X	X	X
G3	5.06	1.23	<1	X	X	X
G5	2.25	3.46	<1	X	X	X
G7	0	<1	1.54	X	X	X
G8	<1	2.30	2.28	X	X	X
G10	0	0	<1	X	X	X
G13	<1	7.76	11.81	X	X	X
G14	0	<1	<1	X	X	
G15	0	<1	<1	X	X	X
G16	0	<1	4.17	X	X	X
G17	0	<1	<1	X	X	X
G18	0	1.77	<1	X		X
G20	0	<1	<1			X
G21	<1	1.38	<1	X	X	X
G23	0	<1	0		X	X
G24	0	<1	<1	X		X
G25	0	<1	<1	X	X	X
G26	0	<1	<1	X	X	X
G27	0	<1	0		X	
G28	<1	<1	1.27	X	X	X
G29	0	<1	0			X
G30	0	<1	0			X
G31	<1	0	<1	X	X	X
G34	0	0	<1			X
G35	0	0	<1			X
G38	0	<1	0			X
G39	0	<1	0			X
G41	<1	<1	0		X	X
G42	0	0	<1	X		
G43	<1	0	0	X		
elongate						
E1	9.74	5.38	1.01	X	X	X
E2	9.93	1.46	6.31	X	X	X
E3	2.31	6.61	1.71	X	X	X
E4	6.18	6.07	<1	X	X	X
E5	17.30	29.65	9.48	X	X	X

Table 2.2 (cont'd)

E6	11.31	3.53	2.97	X	X	X
E7	7.31	10.98	12.88	X	X	X
E10	0	<1	<1	X	X	X
E12	2.44	2.00	<1	X	X	X
E14	2.44	<1	<1	X	X	X
E15	3.25	5.84	6.12	X	X	X
E16	<1	<1	<1	X	X	
E17	0	<1	0	X		
E20	<1	<1	0	X		X
E21	<1	<1	0	X	X	X
E22	0	1.08	0	X	X	X
E24	0	<1	<1			X
E25	<1	<1	<1	X	X	X
E30	1.56	<1	<1	X	X	X
E31	2.75	<1	31.72	X	X	X
E34	0	<1	0	X		
E36	0	0	<1	X	X	
E37	0	0	<1	X		
E40	0	0	<1			X
E41	<1	0	0		X	
E42	<1	0	0	X		
E43	10.56	0	<1	X	X	X
E44	<1	0	<1	X		X
E45	<1	0	0	X	X	X
E46	<1	0	0			X
E47	<1	0	0		X	X

Table 2.3. Oribatid mite morphospecies collected from the BCSE between 2018 and 2019, including the proportion they contributed to the total oribatid mite abundance (% abundance), summed across cropping systems and sampling periods, as well as the cropping systems they were collected from. PP: perennial polyculture, PM: perennial monoculture, AM: annual monoculture.

	Proportional abundance (%)			**Collected from:**		
adults	2018 – soil	2019 – soil	2019 – litter	PP	PM	AM
O1	14.11	5.08	8.15	X	X	X
O2	22.86	34.90	21.47	X	X	X
O3	10.29	15.71	4.61	X	X	X
O4	2.03	3.70	8.00	X	X	X
O5	5.66	6.34	2.21	X	X	X
O6	2.80	7.13	23.08	X	X	X
O10	<1	2.72	<1	X		X
O11	<1	0	<1	X	X	X
O13	9.83	10.91	<1	X	X	X
O22	<1	0	0	X		
juveniles						
O7	3.42	3.82	20.42	X	X	X
O8	17.85	6.10	4.80	X	X	X
O12	<1	<1	3.48	X	X	X
O14	<1	<1	<1	X	X	X
O18	1.86	<1	1.20	X	X	X
O20	7.05	1.18	1.31	X	X	X
O23	<1	0	0	X		
O24	<1	0	<1	X	X	

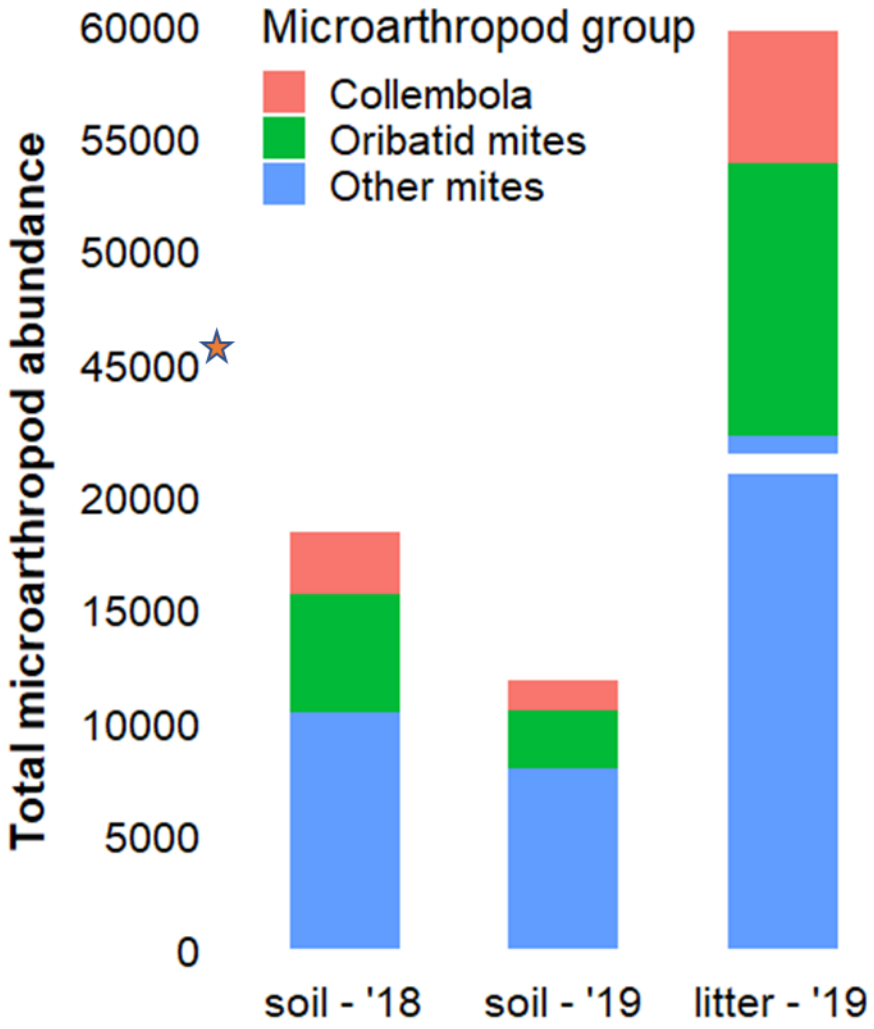

Figure 2.4. Total microarthropods collected from soil (2018 & 2019) and litter (2019), summed across sampling periods and cropping systems. Note the break in the y-axis indicated by a star as well as the gap in the column for litter microarthropods.

Table 2.4. Measures of diversity of microarthropods surveyed in 2018 (soil) and 2019 (soil + litter). Averages are provided for morphospecies richness, Shannon's diversity, and Simpson's diversity. PP: perennial polyculture, PM: perennial monoculture, AM: annual monoculture. Different letters indicate statistically significant results across treatments within a single year ($p < 0.05$, Dunn's test *post hoc*).

		Collembola			Oribatid mites		
		PP	PM	AM	PP	PM	AM
2018	Richness	11.27	12.00	13.07	11.87 **a**	11.20 **ab**	10.80 **b**
	Shannon's diversity	1.680	1.748	1.866	1.640	1.548	1.475
	Simpson's diversity	0.775	0.785	0.814	0.759	0.735	0.714
2019	Richness	16.20 **ab**	15.33 **b**	18.73 **a**	9.07 **a**	8.20 **b**	8.93 **ab**
	Shannon's diversity	2.210 **a**	1.989 **b**	1.978 **ab**	1.618	1.448	1.508
	Simpson's diversity	0.854 **a**	0.807 **b**	0.767 **ab**	0.745	0.695	0.700

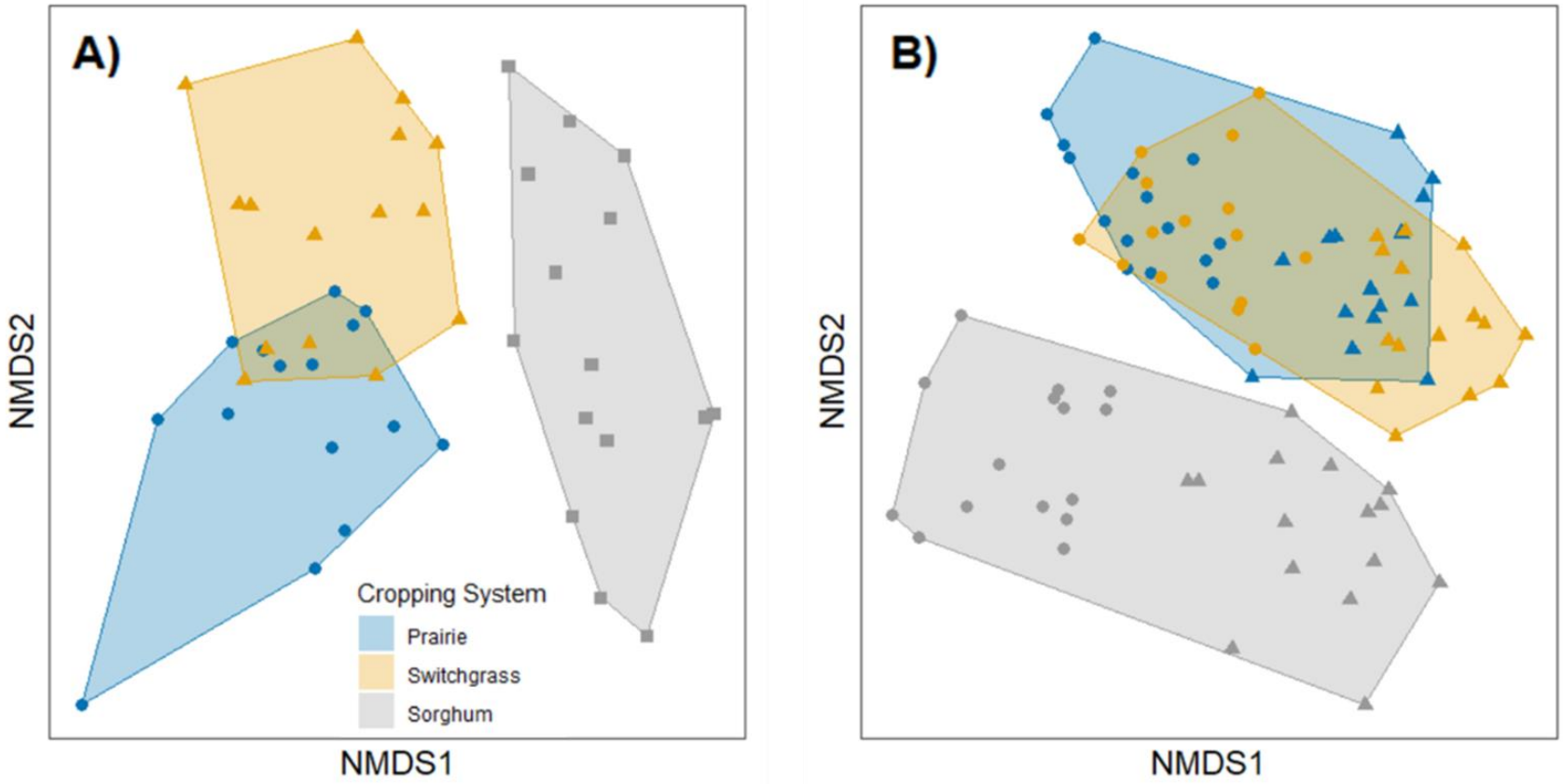

Figure 2.5. NMDS of collembola and oribatid communities surveyed in **A)** 2018 (soil) (k = 3, r^2 = 0.967, stress = 0.183) and **B)** 2019 (soil + litter) (k = 3, r^2 = 0.968, stress = 0.178). Singleton and doubleton morphospecies have been removed. Note that only collembola morphospecies collected from two of the three occasions in 2018 (late-July and mid-August) were used.

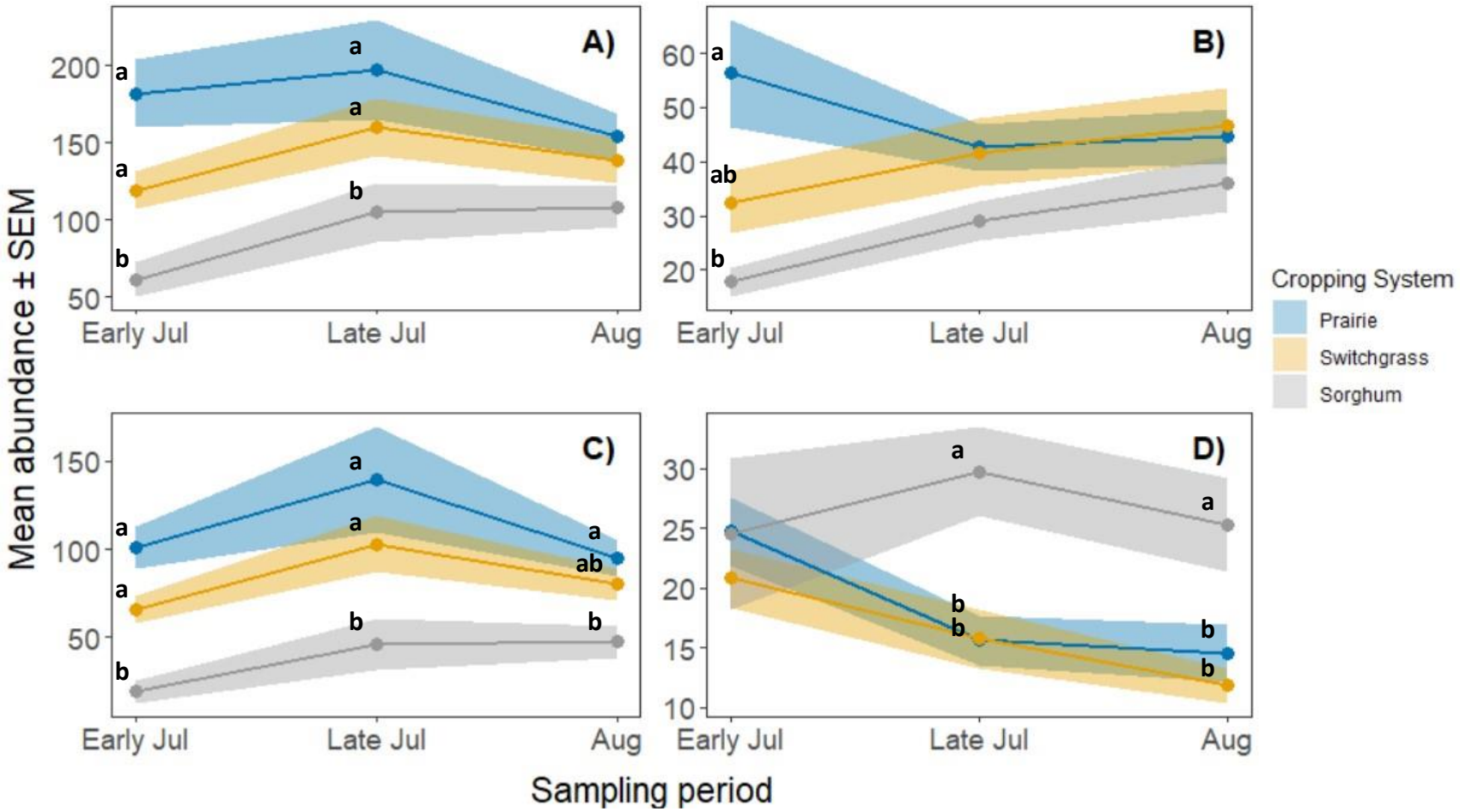

Figure 2.6. Average **A)** overall microarthropod, **B)** oribatid mite, **C)** non-oribatid mite, and **D)** collembola abundances collected from soil in 2018. Shading around the points (sampling round) indicates the standard error of the mean. Different letters next to symbols indicate significant treatment differences ($p < 0.05$, Tukey's *post hoc*).

Table 2.5. Results of GLMMs used to assess bioenergy cropping system treatment effects on the abundance of microarthropod groups surveyed from soil in 2018 (soil only). In the case of significant treatment effects, pairwise comparisons between microarthropod abundances in perennial polyculture (PP), perennial monoculture (PM), and annual monoculture (AM) were assessed via Tukey's test. Statistically significant results ($p < 0.05$, Tukey's *post hoc*) are bolded.

	Pairwise comparisons		
Early July	PP – PM	PP – AM	PM – AM
Total	0.1235	**<0.0001**	**0.0003**
Oribatid mites	0.0933	**<0.0001**	0.0523
Other mites	0.2816	**<0.0001**	**<0.0001**
Collembola	0.6107	0.4977	0.9814
Late July	PP – PM	PP – AM	PM – AM
Total	0.6629	**0.0025**	**0.0360**
Oribatid mites	0.9621	0.2120	0.3317
Other mites	0.5961	**<0.0001**	**0.0001**
Collembola	0.9981	**0.0077**	**0.0064**
August	PP – PM	PP – AM	PM – AM
Total	0.7754	0.1140	0.3879
Oribatid mites	0.9998	0.5988	0.6103
Other mites	0.7307	**0.0062**	0.0544
Collembola	0.7657	**0.0265**	**0.0030**

Table 2.6. Results of GLMMs used to assess bioenergy cropping system treatment effects on the abundance of microarthropod groups surveyed in 2019 (soil + litter). In the case of significant treatment effects, pairwise comparisons between microarthropod abundances in perennial polyculture (PP), perennial monoculture (PM), and annual monoculture (AM) were assessed via Tukey's test. Statistically significant results ($p < 0.05$, Tukey's *post hoc*) are bolded.

	Pairwise comparisons		
May	PP – PM	PP – AM	PM – AM
Total	**0.0306**	0.9778	**0.0175**
Oribatid mites	**0.0242**	0.8157	0.1114
Other mites	**0.0337**	0.5953	0.2829
Collembola	0.3364	**0.0128**	**0.0001**
August	PP – PM	PP – AM	PM – AM
Total	0.2146	**<0.0001**	**<0.0001**
Oribatid mites	0.1040	**<0.0001**	**<0.0001**
Other mites	0.3527	**<0.0001**	**<0.0001**
Collembola	0.5288	0.1797	0.7551
October	PP – PM	PP – AM	PM – AM
Total	0.8171	**0.0012**	**0.0098**
Oribatid mites	0.9316	0.1462	0.0659
Other mites	0.5170	**<0.0001**	**0.0005**
Collembola	0.5496	0.1979	0.7773

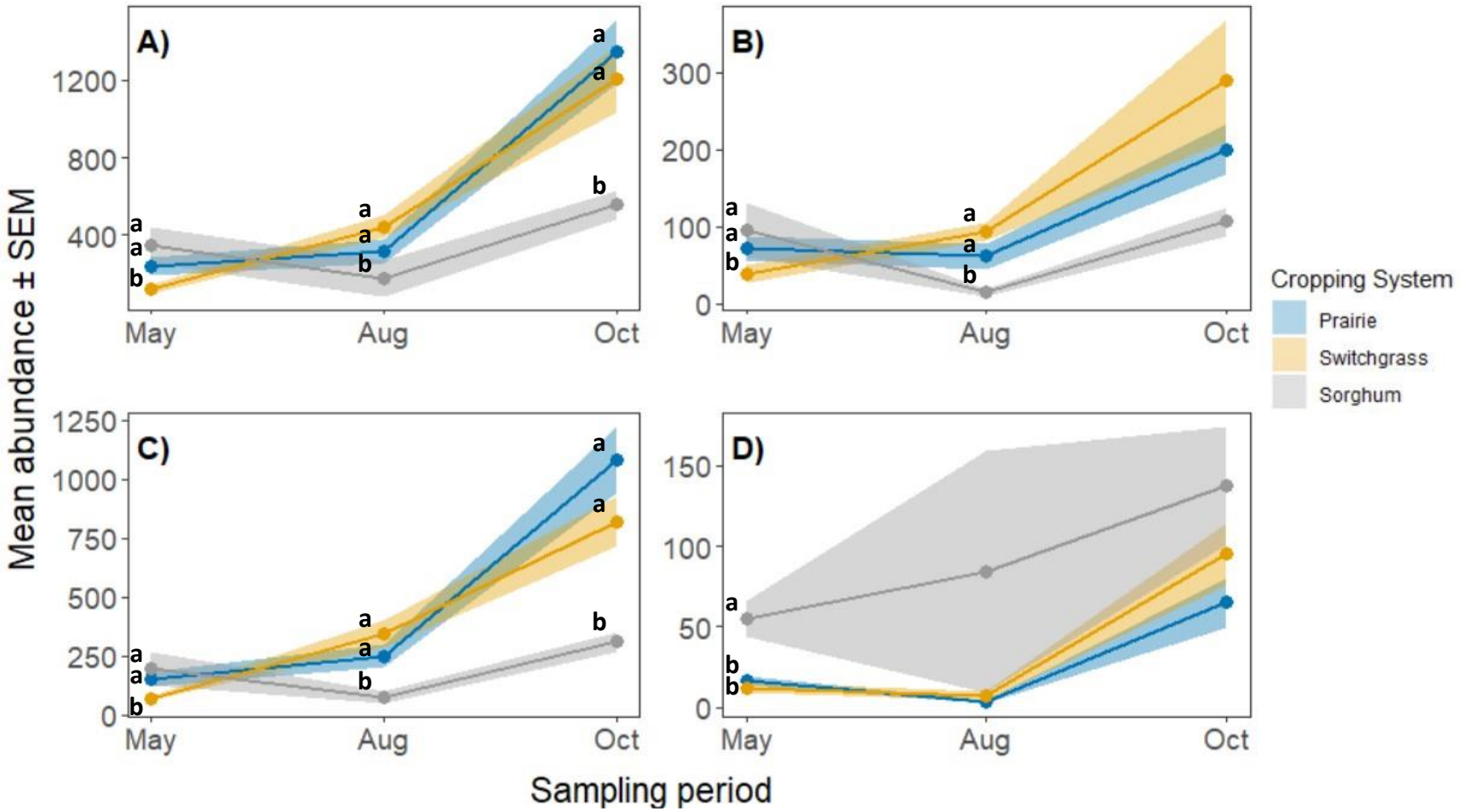

Figure 2.7. Average **A)** overall microarthropod, **B)** oribatid mite, **C)** non-oribatid mite, and **D)** collembola abundances collected from soil and litter in 2019. Shading around the points (sampling round) indicates the standard error of the mean. Different letters next to symbols indicate significant treatment differences ($p < 0.05$, Tukey's *post hoc*).

APPENDIX B: MORPHOSPECIES LIST

The following tables report the full list of **A)** collembola and **B)** oribatid mite morphospecies collected and characterized throughout dissertation research Chapters 2-4. Note that taxonomic identifications when provided remain to be verified by an expert taxonomist.

Table 2.7. Collembola morphospecies collected throughout the duration of this dissertation.

ID *elongate*	**Description**	**Collected in:** Ch. 2	Ch. 3	Ch. 4
E1	Uniform pale purple coloration, body length ≤ 500 µm, distinct eye spots, antennae < ½ body length, reduced furcula. Hypogastruroidea.	X		
E2	Uniform dark purple coloration w/ hairs, body length ~1000 µm with ABD IV elongated, distinct eye spots, antennae < ½ body length long furcula. Entomobryidae.	X	X	X
E3	Uniform pinkish purple coloration with short hairs, distinct dot at top of head, body length ≤ 500 µm, distinct eye spots, antennae < ½ body length, long furcula. Entomobryomorpha.	X	X	
E4	Uniform white coloration, body length ~1000 µm with ABD IV elongated, lack ocelli, antennae < ½ body length, long furcula. Entomobryidae.	X	X	X
E5	Uniform white coloration, body length ≤ 500 µm, lack ocelli, antennae < ½ body length, lack a furcula. Onychiuridae or Proisotominae.	X	X	X
E6	Uniform white coloration, body length ≥ 500 µm, 2 ocelli per side, antennae < ½ body length, reduced furcula. Isotomidae, *Folsomia sp.*	X	X	X
E7	Uniform grey coloration, body length ~500 µm, distinct eye spots, antennae < ½ body length, reduced furcula. Isotomidae, *Folsomia sp.*	X	X	X
E10	Uniform pale purple coloration, body length < 500 µm, distinct eye spots, antennae < ½ body length, reduced furcula. Entomobryomorpha.	X		
E12	Uniform white coloration, body length ~500 µm, single ocelli per side, antennae < ½ body length, lack a furcula. Isotominae.	X	X	

Table 2.7 (cont'd)

ID *elongate*	Description	Collected in: Ch. 2	Ch. 3	Ch. 4
E12	Uniform white coloration, body length ~500 µm, single ocelli per side, antennae < ½ body length, lack a furcula. Isotominae.	X	X	
E14	Uniform purple coloration, body length 500-1000 µm with ABD IV elongated, distinct eye spots, antennae ½ body length, long furcula. Entomobryidae.	X		X
E15	Pale coloration with longitudinal purple line spanning from eye spots down along the abdomen and long hairs, body length ≥ 1000 µm with ABD IV elongated, distinct eye spots, antennae ≥ ½ body length, long furcula. Entomobryidae.	X	X	X
E16	Uniform grey-brown coloration, body length < 500 µm, distinct eye spots, antennae < ½ body length, reduced furcula. Hypogastruroidea.	X		
E17	Uniform pale tan coloration, body length < 500 µm, distinct eye spots, antennae < ½ body length, reduced furcula. Hypogastruroidea.	X		
E20	Uniform grey coloration with short hairs, body length ≤ 500 µm with ABD IV elongated, distinct eye spots, antennae < ½ body length, reduced furcula. Entomobryidae.	X		
E21	Pale coloration with purple bordering segments and long hairs, body length ≥ 1000 µm with ABD IV elongate and purple-speckled, distinct eye spots, antennae ≥ ½ body length, long furcula. Entomobryidae.	X	X	X
E22	Uniform brownish coloration, body length < 500 µm, distinct eye spots, antennae < ½ body length, reduced furcula. Entomobryomorpha.	X		
E24	Uniform dull yellow coloration, body length ~1000 µm, distinct eye spots, antennae < ½ body length, long furcula with curled dens. Entomobryomorpha.	X		
E25	Brown coloration with dull white speckles and short hairs, body length > 1250 µm, distinct eye spots, antennae ≥ ½ body length, long furcula. Entomobryomorpha.	X	X	X
E30	Uniform dark purple coloration with hairs and scales, body length ~1000 µm with ABD IV elongated, distinct eye spots, antennae < ½ body length, long furcula. Entomobryidae.	X	X	

Table 2.7 (cont'd)

ID *elongate*	Description	Collected in: Ch. 2	Ch. 3	Ch. 4
E31	Uniform navy-blue coloration, body length ≤ 500 µm, distinct eye spots, antennae < ½ body length, reduced furcula. Hypogastruroidea.	X	X	X
E33	Uniform navy-blue coloration with short setae, body length ~500 µm with ABD IV elongate, distinct eye spots, antennae < ½ body length, long furcula. Entomobryidae.			X
E34	Uniform white coloration, body length ≤ 500 µm, single ocelli per side, antennae < ½ body length, lack a furcula. Poduromorpha.	X		
E36	Uniform dark purple coloration with setae on thorax/upper abdomen, body length ≥ 1000 µm with ABD IV elongate, distinct eye spots, antennae < ½ body length, humped thorax with a length approximately that of the head, long furcula. Entomobryidae.	X		
E37	Uniform pale tan coloration with reddish purple antennae and scales along the body, body length ≥ 1000 µm, distinct eye spots, antennae > ½ body length with 3rd segment much longer than all others, long furcula. Entomobryomorpha.	X		
E40	Uniform pale greyish blue with purple antennae and short setae, body length ≤ 500 µm with ABD IV elongate, distinct eye spots, antennae < ½ body length, long furcula. Entomobryidae.	X		
E41	Entomobryomorpha. [single specimen recorded, unable to be photographed or receive description before specimen was lost]	X		
E42	Uniform pale whitish grey coloration, body length ≤ 250 µm, distinct eye spots, antenna < ½ body length, reduced furcula. Entomobryomorpha.	X		
E43	Uniform grey coloration with medial dark dot on head, body length ≥ 500 µm, distinct eye spots, antennae < ½ body length, reduced furcula. Entomobryomorpha.	X		
E44	Uniform dark grey coloration with white speckling, body length ≤ 500 µm, distinct eye spots, antennae < ½ body length, reduced furcula. Hypogastruroidea.	X		

Table 2.7 (cont'd)

ID	Description	Collected in:		
elongate		Ch. 2	Ch. 3	Ch. 4
E45	Uniform pale tan coloration with purple antennae and short setae on the thorax/upper abdomen, body length ≥ 500 µm with ABD IV elongate, distinct eye spots, antennae ≥ ½ body length, , long furcula. Entomobryidae.	X		
E46	Uniform pale pinkish purple coloration with darker purple segment borders and medial dark spot between eye spots, body length ~500 µm with ABD IV elongate and somewhat diamond-shaped, distinct eye spots, antennae < ½ body length, long furcula. Entomobryidae.	X		
E47	Uniform dull brownish grey coloration with darker pigmentation along segment borders, body length ~500 µm with ABD IV elongate, distinct eye spots, antennae ≥ ½ body length, long furcula. Entomobryidae.	X	X	
E48	Uniform whitish coloration with short setae, body length ≤ 500 µm with ABD IV elongate, distinct eye spots, antennae < ½ body length, long furcula. Entomobryidae.		X	
E49	Uniform yellowish tan coloration with medial dark dot between eye spots , body length ≤ 500 µm with ABD IV elongate, distinct eye spots, antennae < ½ body length, long furcula. Entomobryidae.		X	
E50	Uniform rusty red coloration with short setae, body length < 500 µm with ABD IV elongate, distinct eye spots, antennae < ½ body length, long furcula. Entomobryidae.		X	
E51	Uniform pale coloration with eye spots , body length < 500 µm, distinct eye spots, antennae < ½ body length, long furcula. Entomobryomorpha.		X	
globular				
G1	Dark brown to tan abdomen w/ lighter head bearing a dark spot between eye spots, body length ~500 µm, distinct eye spots, antennae ≥ ½ body length, long furcula. Sminthuridae.	X		
G2	Dark bluish brown coloration with lighter colored speckles, head uniformly dull yellow, body length < 500 µm, distinct eye spots, antennae ≥ ½ body length, long furcula. Sminthuridae.	X		

Table 2.7 (cont'd)

ID *globular*	Description	Collected in: Ch. 2	Ch. 3	Ch. 4
G3	Uniform purple coloration, body length ≤ 250 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.	X		
G5	Uniform pale purple coloration, body length ≤ 250 µm, distinct eye spots, antennae ≥ ½ body length, long furcula. Sminthuridae.	X		X
G7	Pale purple coloration with increased pigment towards the sides and 1-2 dark spots on head, body length ≤ 500 µm, distinct eye spots, antennae ≥ ½ body length, long furcula. Sminthuridae.	X		X
G8	Uniform dull pink coloration with head paler than body, body length ~250 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.	X		
G10	Uniform dark brown coloration with pale white head with brown markings and medial dark spot, body length ≥ 500 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.	X		
G13	Uniform purple coloration with medial dark spot on head, body length ~250 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.	X		X
G14	Uniform yellow coloration, body length ~500 µm, distinct eye spots, antennae ≥ ½ body length with 4th segment annulated, long furcula. Sminthuridae.	X		
G15	Dull tan coloration with brown pigmentation towards the sides with brown markings and a medial dark spot on head, body length ≤ 500 µm, distinct eye spots, antennae ≥ ½ body length, long furcula. Sminthuridae.	X		
G16	Dull white coloration with black coloration on segment borders with black stripe markings and 1-2 dark spots on head, body length ≤ 500 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.	X		X
G17	Uniform tan coloration with dull brownish accents towards sides and 2 dark spots on head, body length > 500 µm, distinct eye spots, < ½ body length, long furcula. Sminthuridae.	X		X

Table 2.7 (cont'd)

ID *globular*	Description	Collected in: Ch. 2	Ch. 3	Ch. 4
G18	Dark brown body with lighter colored head, body length ~250 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.	X		
G20	Uniform dark brown coloration with pale speckled marking on head, body length ~500 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.	X		
G21	Uniform white coloration sometimes with reddish speckling on the abdomen, body length ≤ 500 µm, lack ocelli, antennae < ½ body length, long furcula. Arrhopalitidae.	X		X
G23	Dull white coloration with black coloration on segment borders and medial dark spot on head, body length ~250 µm, distinct eye spots, antennae ≥ ½ body length with 4th segment annulated, long furcula. Sminthuridae.	X		
G24	Dark bluish brown coloration dark mottling on limbs and antennae with yellowish head, body length > 500 µm, distinct eye spots, antennae ≥ ½ body length, long furcula. Sminthuridae.	X		
G25	Uniform dull yellowish-brown coloration with medial dark spot on head, body length ≤ 500 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.	X		
G26	Uniform pale pinkish purple coloration with dark markings on head, body length ≥ 500 µm, distinct eye spots, antennae ≥ ½ body length, long furcula. Sminthuridae.	X		
G27	Uniform pale grey coloration with dark markings on head, body length distinct eye spots, antennae ≥ ½ body length ≥ 500 µm, long furcula. Sminthuridae.	X		
G28	Uniform pink coloration, body length ~250 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.	X		
G29	Uniform purple coloration, body length ≤ 500 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.	X		
G30	Dull white coloration with black coloration on segment borders, body length ~500 µm, distinct eye spots, antennae < ½ body length, long antennae, long furcula. Sminthuridae.	X		

Table 2.7 (cont'd)

ID *globular*	Description	Collected in: Ch. 2	Ch. 3	Ch. 4
G31	White coloration with longitudinal black lines spanning the abdomen and dark stripe on head, body length ~250 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.	X		
G34	Body yellow medially with increasingly pinkish purple coloration towards the sides and head uniformly pink with small medial dark spot, body length ~500 µm, distinct eye spots, antennae ≥ ½ body length, long furcula. Sminthuridae.	X		
G35	Uniform dark brown coloration, body length ~250 µm, distinct eye spots, antennae ≥ ½ body length, long furcula. Sminthuridae.	X		
G38	Uniform greyish purple coloration with dark borders around segments, body length ≤ 500 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.	X		
G39	Uniform dull yellow to brown coloration, body length ≤ 500 µm, distinct eye spots, antennae ≥ ½ body length with 4th segment annulated, long furcula. Sminthuridae.	X		
G41	Uniform dark greyish purple coloration with mottling on the head and abdomen, body length ~250 µm, distinct eye spots, antennae ≥ ½ body length with 4th segment annulated, long furcula. Sminthuridae.	X		
G42	Uniform bluish-purple coloration, body length ~250 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.	X		
G43	Uniform dull yellowish brown coloration w/ darker mottling on head and abdomen, body length ≤ 500 µm, distinct eye spots, antennae > ½ body length with 4th segment annulated, long furcula. Sminthuridae.	X		
G44	Uniform pale purplish pink coloration, body length ≤ 500 µm, distinct eye spots, antennae < ½ body length, long furcula. Sminthuridae.		X	
G61	Dull yellow coloration dorsally that gradually becomes a dark greyish distally and dull yellow head with slight darker mottling, body length ≤ 500 µm, distinct eye spots, antennae ≥ ½ body length, long furcula. Sminthuridae.			X

Table 2.7 (cont'd)

ID *globular*	Description	Collected in: Ch. 2	Ch. 3	Ch. 4
G62	Uniform dull yellow coloration, body length ≤ 250 µm, distinct eye spots, antennae ≥ ½ body length, long furcula. Sminthuridae.			X

Table 2.8. Oribatid mite morphospecies collected throughout the duration of this dissertation.

ID *adults*	Description	Collected in: Ch. 2	Ch. 3	Ch. 4
O1	Uniform orange coloration with weak sclerotization, body length < 250 µm, body dichoid with two notogastral scissures, dorsum possesses two small posterior bumps. Brachychthoniidae, possibly *Liochthonius sp.*	X		
O2	Uniform brown coloration with strong sclerotization, body length < 500 µm, body holoid with triangular prodorsum and ovular notogaster, anal and genital plates at opposite ends of the ventral plate. Oppiidae.	X	X	X
O3	Uniform brown coloration with strong sclerotization, body length ~250 µm, body holoid with rounded circular notogaster, prodorsal lamella with cusps very close together, anal and genital plates very close together appearing nearly touching. Astegistidae, possibly *Cultroribula sp.*	X	X	X
O4	Uniform brown to dark brown coloration with strong sclerotization, body length > 500 µm, body holoid with triangular prodorsum and rounded notogaster with weakly sclerotized anterior-facing lateral protrusions, anal and genital plates at opposite ends of the ventral plate. Scheloribatidae, *Scheloribates moestus*.	X	X	X
O5	Uniform brown coloration with strong sclerotization and granular cuticle, body length < 500 µm, body holoid with somewhat triangular prodorsum and rounded notogaster that extends forward anteriorly and bluntly over prodorsum, anal and genital plates close but clearly separated by gap. Tectocepheidae, possibly *Tectocepheus velatus*.	X	X	X
O6	Uniform brown to dark brown coloration with strong sclerotization, body length < 500 µm, body holoid with articulating pteromorphs present, anal and genital plates distant from each other. Galumnidae.	X	X	X
O10	Uniform pale brown coloration with strong sclerotization, body length > 500 µm, body ptychoid with setae clearly visible on prodorsum and notogaster. Euphthiracaridae, possibly *Acrotritia ardua*.	X	X	X
O11	Uniform pale coloration with weak sclerotization and pigmentation, body length < 500 µm, body holoid or dichoid with long setae covering the prodorsum and notogaster.	X		

Table 2.8 (cont'd)

ID	Description	Collected in:		
adults		Ch. 2	Ch. 3	Ch. 4
O13	Uniform brown coloration with strong sclerotization, body length ~500 µm, body dichoid with elongate notogaster, apodemes directed medially towards sejugal furrow, chelicerae covered dorsally by weakly sclerotized rostral tectum. Eulohmanniidae, possibly *Eulohmannia sp*.	X	X	X
O22	Uniform dark brown coloration with strong sclerotization and leathery cuticle, body length > 500 µm, body holoid with distinct club-shaped setae extending posteriorly from notogaster. Nothridae.	X		
juveniles				
O7	Gnathosoma and legs brown in coloration and weakly sclerotized while the rest of the body is very weakly sclerotized and unpigmented with lateral spots towards the posterior of the dorsum sometimes present, body length < 500 µm, body elongate, distinct club-shaped trichobothria. Brachypylina, possibly *Scheloribates moestus.*	X	X	X
O8	Uniform pale brown coloration with weak sclerotization and pigmentation, body length ~250 µm, body plicate with dorsoventrally flat notogaster with a distinct scissure, distinct club-shaped trichobothria. Brachypylina.	X	X	X
O12	Very weak sclerotization and unpigmented, body length < 500 µm, body elongate with setae sometimes present on notogaster.	X	X	X
O14	Uniform pale coloration with very weak sclerotization and pigmentation, body length < 250 µm, body elongate with short setae on notogaster.	X		
O18	Uniform pale brown coloration with weak sclerotization and pigmentation, body length < 500 µm, body round, distinct club-shaped trichobothria.	X	X	X
O20	Very weak sclerotization and unpigmented except for two lateral dark spots towards the posterior of the dorsum, body length < 250 µm, body elongate.	X	X	X
O23	Uniform pale brown coloration with weak sclerotization and pigmentation, leathery cuticle, body length > 500 µm, body box-shaped with two posterior bumps. Possibly Nothridae.	X		

Table 2.8 (cont'd)

ID *adults*	Description	Collected in:		
		Ch. 2	Ch. 3	Ch. 4
O24	Very weak sclerotization and unpigmented, body length ~250 µm, body elongate.	X		

APPENDIX C: RECORD OF DEPOSITION OF VOUCHER SPECIMENS

FORM 1

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2023-11

Author and Title of thesis:

Allison Zahorec

MICROARTHROPOD-MICROBE INTERACTIONS ON SOIL CARBON DYNAMICS IN BIOENERGY CROPPING SYSTEMS

Museum(s) where deposited:

Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Morphospecies	Taxa (subclass or order)	Life Stage	Quantity	Preservation
E1	Collembola	adult	10	95% ethanol
E2	Collembola	adult	10	95% ethanol
E3	Collembola	adult	10	95% ethanol
E4	Collembola	adult	10	95% ethanol
E5	Collembola	adult	10	95% ethanol
E6	Collembola	adult	10	95% ethanol
E7	Collembola	adult	10	95% ethanol
E10	Collembola	adult	3	95% ethanol
E12	Collembola	adult	10	95% ethanol
E14	Collembola	adult	10	95% ethanol
E15	Collembola	adult	10	95% ethanol
E16	Collembola	adult	3	95% ethanol
E17	Collembola	adult	1	95% ethanol
E20	Collembola	adult	1	95% ethanol
E21	Collembola	adult	3	95% ethanol
E22	Collembola	adult	3	95% ethanol
E24	Collembola	adult	1	95% ethanol

Specimens (cont'd):

Morphospecies	Taxa (subclass or order)	Life Stage	Quantity	Preservation
E25	Collembola	adult	3	95% ethanol
E30	Collembola	adult	5	95% ethanol
E31	Collembola	adult	10	95% ethanol
E33	Collembola	adult	1	95% ethanol
E34	Collembola	adult	1	95% ethanol
E36	Collembola	adult	1	95% ethanol
E37	Collembola	adult	1	95% ethanol
E40	Collembola	adult	1	95% ethanol
E42	Collembola	adult	1	95% ethanol
E43	Collembola	adult	10	95% ethanol
E44	Collembola	adult	1	95% ethanol
E45	Collembola	adult	1	95% ethanol
E46	Collembola	adult	1	95% ethanol
E47	Collembola	adult	1	95% ethanol
E48	Collembola	adult	1	95% ethanol
E49	Collembola	adult	1	95% ethanol
E50	Collembola	adult	1	95% ethanol
E51	Collembola	adult	1	95% ethanol
G1	Collembola	adult	1	95% ethanol
G2	Collembola	adult	3	95% ethanol
G3	Collembola	adult	5	95% ethanol
G5	Collembola	adult	5	95% ethanol
G7	Collembola	adult	5	95% ethanol
G8	Collembola	adult	5	95% ethanol
G10	Collembola	adult	3	95% ethanol
G13	Collembola	adult	10	95% ethanol
G14	Collembola	adult	1	95% ethanol
G15	Collembola	adult	3	95% ethanol
G16	Collembola	adult	5	95% ethanol
G17	Collembola	adult	3	95% ethanol
G18	Collembola	adult	5	95% ethanol
G20	Collembola	adult	1	95% ethanol
G21	Collembola	adult	5	95% ethanol
G23	Collembola	adult	1	95% ethanol
G24	Collembola	adult	1	95% ethanol
G25	Collembola	adult	3	95% ethanol
G26	Collembola	adult	3	95% ethanol
G27	Collembola	adult	1	95% ethanol
G28	Collembola	adult	5	95% ethanol
G29	Collembola	adult	1	95% ethanol
G30	Collembola	adult	1	95% ethanol
G31	Collembola	adult	3	95% ethanol

Specimens (cont'd):

Morphospecies	Taxa (subclass or order)	Life Stage	Quantity	Preservation
G34	Collembola	adult	1	95% ethanol
G35	Collembola	adult	1	95% ethanol
G38	Collembola	adult	1	95% ethanol
G39	Collembola	adult	1	95% ethanol
G41	Collembola	adult	1	95% ethanol
G42	Collembola	adult	1	95% ethanol
G43	Collembola	adult	1	95% ethanol
G44	Collembola	adult	1	95% ethanol
G61	Collembola	adult	1	95% ethanol
O1	Oribatida	adult	10	95% ethanol
O2	Oribatida	adult	10	95% ethanol
O3	Oribatida	adult	10	95% ethanol
O4	Oribatida	adult	10	95% ethanol
O5	Oribatida	adult	10	95% ethanol
O6	Oribatida	adult	10	95% ethanol
O7	Oribatida	juvenile	10	95% ethanol
O8	Oribatida	juvenile	10	95% ethanol
O10	Oribatida	adult	10	95% ethanol
O11	Oribatida	adult	3	95% ethanol
O12	Oribatida	juvenile	10	95% ethanol
O13	Oribatida	adult	10	95% ethanol
O14	Oribatida	juvenile	3	95% ethanol
O18	Oribatida	juvenile	10	95% ethanol
O20	Oribatida	juvenile	10	95% ethanol
O22	Oribatida	adult	3	95% ethanol
O23	Oribatida	juvenile	3	95% ethanol
O24	Oribatida	juvenile	3	95% ethanol

CHAPTER 3: MICROARTRHOPOD EFFECTS ON MICROBIAL CARBON USE EFFICIENCY

ABSTRACT

Understanding the forces driving soil carbon accrual in managed agroecosystems is necessary to help offset further carbon emissions. There is great promise for bioenergy crop production to make significant contributions by replenishing soil carbon stocks, yet uncertainty in the true potential for bioenergy cropping systems, even of perennial systems, remains a substantial barrier. Soil organic carbon formation, stabilization, and turnover is directly controlled largely by the activity of soil microbes, yet the potential for microarthropod activity to regulate key microbial processes critical for soil carbon accrual and stabilization has received little attention. Through their important role in organic matter decomposition or by altering microbial community structure or activity rates, microarthropods have the potential to regulate microbial carbon use efficiency via their diverse and indirect interactions with microbes. To attempt to better understand the potential for microarthropods to influence carbon use efficiency in the context of bioenergy cropping systems, I used an ^{18}O-water stable isotope method to estimate carbon use efficiency in greenhouse mesocosms maintained in the presence of distinct microarthropod communities for ~6 mo. The microarthropod communities introduced to mesocosms were collected from switchgrass and energy sorghum bioenergy cropping systems to assess the potentially varying influence of more abundant, mite-dominated communities supported by perennial cropping systems compared to that from an annual monoculture. While I did not find evidence that microarthropods from either system exert a significant influence on carbon use efficiency, I was able to glean important insights into the use of field-collected microarthropod communities in longer duration mesocosms to investigate the net impacts of fauna-microbe interactions on key ecosystem processes. Further, I address some of the caveats of investigating carbon use efficiency at increasingly larger spatial and temporal scales and the need to further adapt methodologies that allow researchers to better account for the long-term implications of carbon use efficiency being driven by short-term dynamics.

INTRODUCTION

There is growing consensus that implementing management strategies to promote soil organic carbon (SOC) accrual will be fundamental to mitigate rising C emissions globally (Field et

al., 2020; Paustian et al., 2019). A substantial proportion of global C emissions over the last century has come from agricultural land-use, with the conversion of natural lands and associated intensive agro-management practices causing SOC depletions in most agroecosystems (Lal, 2004). With much of Earth's terrestrial surface managed for crop production, including the production of bioenergy crops, replenishing SOC stocks in these systems will be increasingly necessary to help meet climate goals. There is strong evidence that SOC accrual and stability is strongly dependent on micro-scale interactions between the soil environment, microbes, and plants (Cotrufo et al., 2019; Kravchenko et al., 2019) and that microbes are the primary drivers of SOC formation and transformations (Dungait et al., 2012; Schimel & Schaeffer, 2012). Traditionally believed to be driven largely by the chemical recalcitrance of soil organic matter (SOM), SOC gains and stability are now known to be controlled primarily by microbial physiology and access to SOM (Zhang et al., 2020; Wieder et al., 2014). However, there has thus far been little effort to incorporate the contributions of soil heterotrophs in soil C models, including the roles of microarthropods.

Despite their relatively small contributions to direct SOC gains and losses, microarthropods exert a broad range of indirect effects on SOC which have potentially strong implications for SOC accrual (Filser et al., 2016; Grandy et al., 2016; Soong & Nielsen, 2016). Perhaps most important of these are microarthropod interactions with microbes. While filling a diversity of trophic niches, microarthropods are predominantly characterized as detritivores and/or microbivores, with generalist fungivory assumed to be the dominant functional feeding group (Potapov et al., 2022; Pollierer & Scheu, 2021). Interactions between microarthropods and microbes have been widely documented, with microarthropods found to influence microbes primarily through direct trophic interactions, by changing SOM quality or availability, as well as indirectly such as by modifying the soil environment (Crowther et al., 2012; Lussenhop, 1992). Studies have found these interactions to alter microbial activity (Gange, 2000; Siepel & Maaskamp, 1994; Teuben, 1991), growth (Moore, 1988), and community composition (Janoušková et al., 2018; Maboreke et al. 2017; Parkinson, 1983). Microarthropods therefore have the potential to indirectly influence SOC dynamics by regulating microbial community dynamics and function, quantifying the effects on SOC accrual

remains a challenge. This is in part because SOC accrual is a long-term process, with time spans of decades or centuries needed to measure changes in SOC storage and stabilization. Instead, I therefore propose the necessity of investigations into the potential roles of microarthropods on the microbial factors driving SOC accrual; in particular, microbial carbon use efficiency.

Microbial carbon use efficiency (CUE) is the proportion of metabolized C that becomes assimilated into microbial biomass or other bioproducts relative to that used up for cellular metabolism and respired as CO_2. As such, CUE has been identified as a critical regulator of SOC accrual (Kallenbach et al., 2016). Soils with high SOC stocks have been associated with microbial communities with high CUE, particularly those dominated by fungi (Macdonald et al., 2018; Kallenbach et al., 2016). CUE is influenced by a wide range of intrinsic microbial and external factors, the latter of which become more important for considering the CUE of natural microbial communities. At this scale, CUE is primarily driven by environmental factors, the degree to which microbial exudates and necromass can be reutilized by living microbes, and microbial turnover (Geyer et al., 2016). In theory, microarthropods could influence CUE both by regulating the recycling rates of microbe-derived SOC (i.e., necromass) as well as microbial turnover rates (Chapter 1: **Fig. 1.1**). Microarthropods therefore have the potential to indirectly influence SOC dynamics by regulating microbial community dynamics and activity in ways that increase CUE. However, there has been very little research into the role of trophic interactions in influencing the CUE of natural microbial communities. In one of the few studies to do so, Frey et al. (2001) found evidence that increasing grazing intensity by protozoans can change CUE, though the direction in which CUE changed depended on the calculation used. Indeed, there is a diversity of methods used to measure and calculate CUE, each of which can produce widely differing estimates (Geyer et al., 2019; Geyer et al., 2016). Today, there is consensus that stable isotope tracer methods offer the greatest ability to estimate the CUE of natural microbial communities or *in situ* CUE (Geyer et al., 2019; Geyer et al., 2016).

Stable isotope tracing is a technique in which a naturally rare isotope (often ^{13}C or ^{15}N) is introduced into a system where it can be traced into distinct pools of varying sizes and stability (Fry, 2006). Stable isotope tracing is increasingly being used to investigate the partitioning of SOC across biotic and abiotic pools following SOC transformations to identify the major

mechanisms and drivers of SOC accrual across ecosystems, including in studies of CUE (Geyer et al., 2019). This technique has been particularly useful in investigating SOC dynamics over the last few decades as unlike traditional methods that account for SOC transformations alone (i.e., litter bag studies), it also accounts for the subsequent fate of SOC following these transformations. Stable isotope tracing has been used to quantify the role of microarthropods on SOC accrual, in which Soong et al. (2016) tracked the fate of C and N from enriched grass litter over 3 yr as it decomposed in the presence or absence of microarthropods. Despite having no impact on the overall amount of C and N mobilized from litter, this study found that microarthropods significantly altered the timing and availability of these nutrients that, when incorporated into the DayCent ecosystem model, resulted in an 11% increase in SOC over two centuries (Soong et al., 2016). This study highlights the promise of stable isotope tracer methods as a means for quantifying the community-level effects of trophic interactions on SOC dynamics, such as the potential role of microarthropods in shaping the CUE of natural soil ecosystems.

In this study, I designed a greenhouse mesocosm experiment to evaluate the potential for microarthropods to regulate CUE using an ^{18}O stable isotope tracer method, in which soils are amended with ^{18}O-labeled water to track its incorporation into microbial DNA (Blazewicz & Schwartz, 2011; Spohn et al., 2016). This method is preferable for measuring *in situ* CUE as it does not require introducing an organic tracer and produces a relatively stable CUE estimation over time (Geyer et al., 2019). This experiment was specifically conducted in the context of bioenergy cropping systems established on formerly agricultural land, as such soil ecosystems are assumed to be depleted in SOC from food crop cultivation and present an opportunity to assess how bioenergy crop production and management decisions may promote SOC accrual (Guo & Gifford, 2002, Hansen, 1993). In particular, I utilized soils collected from the Biofuel Cropping System Experiment (BCSE) site, allowing me to measure the CUE of the native microbial community of this ecosystem in the presence and absence of field-collected microarthropod communities. This was done to account for the wide range of microarthropod-microbe species-specific interactions simultaneously operating at the community level. As empirical studies find that perennial cropping systems generally store more C and for longer

timespans than annual cropping systems, I introduced microarthropod communities from either perennial (switchgrass) or annual (energy sorghum) cropping systems. As with microbes, microarthropod abundance and community structure are strongly affected by perenniality (Chapter 2). However, there lacks clear evidence associating these effects on community dynamics to changes in functions such as SOM decomposition rate or microbial biomass turnover that may be important for determining the CUE of a native microbial community. Due to the association between higher CUE in soils with increased fungal dominance as well as the prevalence of both trophic and indirect interactions between fungi and microarthropods, I hypothesized that CUE would be greater in mesocosms in which microarthropods were added as compared to those that did not receive microarthropods. Additionally, I hypothesized that the microarthropod community collected from switchgrass would have a more positive effect on CUE than that collected from energy sorghum due to the greater number of total microarthropods and relative abundance of mites, especially oribatid mites.

METHODS

Mesocosm experiment design

Mesocosms were constructed from Anderson #1 (Portland, OR USA) deep nursery pots (**Fig. 3.1**). To prevent microarthropod escape from mesocosms without impeding water drainage, ~14.5 cm^2 squares of woven stainless steel 200 mesh (74 µm openings) were secured over drainage holes from the outside with hot melt glue. To reduce arthropod movement in or out of mesocosms from above, plastic shields were affixed with hot melt glue to the interior top diameter of pots. Constructed from 34 cm tall cylinders of ~0.008 cm thick ultra-clear Grafix Dura-Lar (Maple Heights, OH USA) with a circumference of 49 cm, the plastic shields extended 30 cm above the top of the pots. Mesocosm substrate was composed of soil (Kalamazoo loam; fine-loamy, mixed, mesic Typic Hapludalfs) collected from the BCSE on September 7, 2020. Soil was collected from the grass alleyway to a 15 cm depth to represent the site's soil type unconditioned by specific bioenergy crops. The soil was then sieved (~8 cm) before undergoing three freeze-thaw cycles (24 h at -20 °C followed by 24 h at room temperature) to eliminate soil meso- and microfauna while keeping the microbial community relatively intact (Huhta et al., 1989). Following defaunation, the soil was mixed with sand in a 1:1 ratio by volume and

homogenized. As preliminary testing indicated low AMF presence in soil from the grass alleyway, I augmented mesocosms with field-collected switchgrass roots assumed to harbor AMF to allow collaborators to address AMF-related research questions. Prior to adding to mesocosms, roots were cut into ~1 cm pieces, surface sterilized in 1% bleach solution for 15 s, repeatedly rinsed with sterile water, and oven dried at 60 °C. Switchgrass rhizomes were collected from the Agronomy Farm on Michigan State University campus. After rinsing off any soil present, the roots were surface sterilized and rinsed as above prior to planting in mesocosms on October 8, 2020.

Microarthropod community treatments were created by collecting microarthropods from switchgrass (*Panicum virgatum* L. cv. Cave-in-Rock) and energy sorghum (*Sorghum bicolor* L. Moench) replicate field plots at the BCSE site (**Table 3.1**). These cropping systems were chosen as previous surveys conducted at the BCSE site found strong differences in microarthropod abundance and community composition between switchgrass and energy sorghum plots. In particular, switchgrass consistently supported higher overall microarthropod abundances largely due to high mite densities while sorghum tended to have lower microarthropod abundances with a greater prevalence of collembola (Chapter 2). Microarthropods were collected from both soil and surface litter samples. Soil-dwelling microarthropods were collected by taking two soil core subsamples (3 cm diameter, 15 cm depth) using cone-shaped soil corers (Chapter 2: **Fig. 2.1**) near previously established sampling stations (n = 3 sampling stations per replicate plot per treatment). Due to potentially high spatial variability of microarthropods (Badejo & Tian, 1999; Santo et al., 1978; Wallwork, 1976), subsamples were composited to create a single soil sample. Litter-dwelling microarthropods were collected by harvesting surface litter within 625 cm^2 square PVC quadrats at each sampling station. Soil and litter samples were kept in refrigerated storage (~6 °C) prior to microarthropod extraction via Tullgren funnels (Chapter 2: **Fig. 2.2**). To collect sufficient numbers of soil-dwelling microarthropods to ensure mesocosm colonization, soil core sampling occurred on five occasions in 2020: September 25, October 5, October 13, October 28, and November 5. Due to their greater abundance (Chapter 2), litter-dwelling microarthropods were collected on a single occasion on October 20, 2020. All microarthropod collection occasions

occurred within 7-10 d of each other to minimize temporal dynamics in microarthropod community structure.

Soil- and litter-dwelling microarthropods were extracted alive using Tullgren funnels into collection cups containing a shallow layer of water (~1 cm) at the bottom. An additional subset of randomly selected samples were extracted into 70% propylene glycol to assess microarthropod field abundance and composition at each sampling occasion. Soil samples were gently broken up prior to placing into Tullgren funnels and given 5 d to complete the extraction while litter samples were given 3 d. Every 18-36 h during extraction, collection cups were removed from under the Tullgren funnels and replaced. The contents of collection cups were then visually inspected with any visible insects, spiders, and any other non-microarthropod fauna removed before the contents of the cups were placed into the mesocosms. In this way, the abundance and composition of microarthropods entering the mesocosms most closely reflected that of microarthropod communities in the field at that time. Once litter-dwelling microarthropods were introduced to mesocosms, ~3 cm layer of switchgrass litter which had been harvested from the BCSE site in 2019 and ground to 2 mm pieces was added to the soil surface. The experiment began once the last of the field-collected microarthropods were added into the mesocosms.

Mesocosms were randomly designated as receiving microarthropods from either switchgrass, energy sorghum, or as controls in which no microarthropods were introduced, henceforth referred to as switchgrass microarthropod (n = 20), sorghum microarthropod (n = 20), and control mesocosms (n = 12), respectively. A set of switchgrass microarthropod (n = 8) and sorghum microarthropod (n = 8) mesocosms were maintained in the greenhouse to monitor microarthropod survival and composition following their introduction. All 52 mesocosms were kept in the greenhouse at 27 °C with a 12 h photoperiod. A drip irrigation system was used to water mesocosms with emitters activated for 2 min every 3 d for the first 3 mo of the experiment. Following a drip irrigation system malfunction, mesocosms were manually watered throughout the remainder of the experiment.

Microarthropod survival monitoring mesocosms were destructively harvested at two different times throughout the experiment. The first microarthropod survival monitoring

mesocosms (n = 2 per microarthropod community treatment) were harvested in early February 2021, approximately one week after the drip irrigation system malfunction, to assess microarthropod survival in mesocosms following ~41 hr of flooding conditions. The remaining survival mesocosms (n = 6 per treatment) were harvested at the end of the experiment in May 2021 to determine the final abundance and composition of microarthropod communities in mesocosms following their ~6 mo duration in the greenhouse. Microarthropods were extracted from both litter and soil substrate into 70% propylene glycol over a period of 5 d before being rinsed and transferred into vials of 95% ethanol. Additionally, the litter layer and remaining soil from a subset of control mesocosms (n = 6) were collected to check for the presence of microarthropods that were unintentionally introduced. These samples were similarly placed separately into Tullgren funnels with microarthropods extracted as above. As the majority of microarthropods recovered from mesocosms were extracted from litter, litter- and soil-dwelling microarthropods were combined to produce a single mesocosm community for simplicity. For further details on the treatment designation and analyses conducted across mesocosm types, see **Table 3.2**.

^{18}O-water tracing to estimate CUE

The mesocosms were maintained in the greenhouse for approximately 6 mo before being destructively harvested between May 4 and 17, 2021. Mesocosms were harvested as follows: 4 per treatment on May 4, 2 per treatment on May 5, 3 per treatment on May 15, and 3 per treatment on May 17. After removing the litter layer, aboveground switchgrass tissue was harvested, oven dried at 65 °C, and weighed to determine switchgrass shoot biomass. Following this, the soil substrate was removed from pots, sieved (2 mm), and gently homogenized with a metal spatula before aliquots were taken to conduct the following analyses (**Fig. 3.2**).

To determine soil water content prior to ^{18}O-water amendment, three replicate aliquots of 5-10 g soil per mesocosm ("sample" will be used henceforth to refer to a single mesocosm) were weighed into aluminum weigh tins. After recording fresh soil mass, tins were placed in a drying oven at 65 °C and reweighed after 24 h to determine dry soil mass. The gravimetric soil moisture for the sample was calculated by subtracting the dry soil mass from fresh soil mass and then averaging the value of the three subsamples. Following this, the dried soil samples

were mixed with water to produce a slurry to measure soil pH.

Two 4 g soil samples were placed into 30 mL Wheaton glass serum bottles (Millville, NJ USA) which were randomly assigned to receive ^{18}O-enriched or unenriched water (henceforth "enriched subsample" and "natural abundance subsample", respectively). Sterile water was added to soils to raise the soil moisture content to 48% water holding capacity (WHC), after which 160 µL 97 at% ^{18}O-water was added to enriched subsamples while natural abundance subsamples received 160 µL sterile water. Following these amendments, the soil moisture of subsamples was approximately 60% the WHC of BCSE soil with enriched subsamples being enriched by ~20 at%. Bottles were sealed immediately after soil amendment, beginning a 24 h incubation period where they were kept at room temperature and left in the dark. Microbial respiration was measured at the beginning and end of the incubation period by taking 13 mL headspace samples which were later analyzed using a gas chromatograph. Bottles were kept at -80 °C prior to performing DNA extractions for assessing ^{18}O-enrichment. I performed chloroform fumigation extraction to determine microbial biomass, which was necessary to calculate microbial growth. For this, I collected six 8 g soil aliquots per sample, with half of the aliquots randomly chosen to undergo chloroform fumigation with the remaining half designated as unfumigated controls. Aliquots received 40 mL 0.5 M potassium sulfate before the extracts were filtered and collected after being placed on an orbital shaker for 1 h at 200 rpm, with one pair of the replicates being fumigated with 2-3 mL chloroform for 24 h prior to extraction. Extracts were analyzed for total C and total bound N using a vario TOC cube (Ronkonkoma, NY USA) to calculate microbial biomass C (MBC) and N (MBN).

DNA was extracted four times for both enriched and natural abundance subsamples using Qiagen's PowerSoil Pro kits (Venlo, NL). In addition to the protocol modifications used by Geyer et al. (2019), I increased the amount of soil utilized for each extraction to 50 mg and heated soil and cell lysis solution mixtures at 65-70 °C prior to bead-beating to maximize DNA extraction for enriched subsamples. PicoGreen-based DNA quantification was performed to determine the DNA concentration of extracts. Due to the relatively low concentration of DNA extracted, natural abundance subsamples were pooled to produce four natural abundance extracts per treatment comprised of three pooled replicates and all extracts were spiked with

80 µg ultrapure salmon sperm DNA (Invitrogen; Waltham, MA USA) before being shipped to the University of California Davis's Stable Isotope Facility for ^{18}O-enrichment analysis via TC/EA-IRMS.

CUE was estimated using calculations based on Geyer et al. (2019) and Spohn et al. (2016). The at% ^{18}O of enriched and natural abundance subsamples were calculated using two-pool mixing models (Equations 1 and 2, respectively):

Equation 1: $$at\%\ O_{enriched} = \frac{(at\%\ O_{enriched+salmon} * O_{enriched+salmon}) - (at\%\ O_{salmon} * O_{salmon})}{O_{enriched}}$$

Equation 2: $$at\%\ O_{natural} = \frac{(at\%\ O_{natural+salmon} * O_{natural+salmon}) - (at\%\ O_{salmon} * O_{salmon})}{O_{natural}}$$

The at% O_x is the atomic ratio ^{18}O to ^{16}O and O_x is the mass (µg) of oxygen. Subscripts refer to enriched and natural abundance subsample and salmon sperm DNA extracts. The difference in at% ^{18}O (at% O excess) between paired enriched and natural abundance subsamples was calculated using Equation 3:

Equation 3: $$at\%\ O\ excess = at\%\ O_{enriched} - at\%\ O_{natural}$$

Total microbial growth in terms of O mass (µg) was calculated using Equation 4 using the at% O of the final soil moisture post-^{18}O-amendment (at% O_{post}) to correct for the dilution of soil water present prior to the amendment (Equation 5):

Equation 4: $$microbial\ growth\ O = (O_{enriched} * \frac{at\%\ O\ excess}{100}) * \frac{100}{at\%\ O_{post}}$$

Equation 5: $$at\%\ O_{post} = \left(\frac{amendment}{moisture_{post}} * at\%\ O\ 97\right) + \left(\frac{moisture_{pre}}{moisture_{post}} * at\%\ O\ 0.205\right)$$

The term "amendment" refers to the 160 µL ^{18}O-water given to all enriched subsamples, "$moisture_{pre}$" and "$moisture_{post}$" give the total soil moisture (µL) of enriched subsamples before and after the amendment. Microbial growth was then scaled to µg C g^{-1} soil using Equation 6:

Equation 6: $$microbial\ growth\ C = microbial\ growth\ O * \frac{1}{31} * \frac{MBC}{DNA} * \frac{1}{soil\ mass}$$

In this equation, 0.31 is the ratio of oxygen to DNA by mass, "MBC" is MBC determined from chloroform fumigation extraction, and "DNA" is the mass of DNA extracted (µL g^{-1} soil). Taken together, CUE is calculated as microbial growth C divided by the sum of microbial growth C and cumulative respiration (Equation 7):

Equation 7: $$CUE = \frac{microbial\ growth\ C}{microbial\ growth\ C + cumulative\ respiration}$$

Statistical analysis

All analyses were performed in R ver. 4. 2. 1. (R Core Team, 2018). Total microarthropod, broad taxonomic groups, and collembola and oribatid mite morphospecies abundance were determined to assess the abundance and community structure of microarthropods recovered from survival monitoring mesocosms as well as those present in a subset of control mesocosms (n = 6). Prior to performing any analyses, litter- and soil-extracted microarthropod count data was summed for each individual mesocosm. The abundance of microarthropods extracted from switchgrass and sorghum microarthropod survival mesocosms, as well as those found to be present in the subset of control mesocosms checked, were compared using the Kruskal-Wallis test, with Dunn's test (R package: *FSA*) with the Holm method for p-value adjustment used for *post-hoc* pairwise comparisons (Ogle et al., 2022). Differences in microarthropod community composition across treatments were visualized with NMDS using the *vegan* package (Oksanen et al., 2022). Significant differences in composition were assessed via PERMANOVA.

Correlation analyses were conducted to explore the relationships between soil, microbial, and switchgrass response variables measured following destructive mesocosm harvesting. Prior to this, variables not meeting the assumption of normality were transformed using the log (performed on pH, cumulative respiration, microbial growth, DNA presence in soil) or log + 1 transformations (performed on CUE, gravimetric soil moisture). The correlations between these variables, after scaling and centering the data, were then assessed after using the R package *corrplot* with the Pearson method (Wei & Simko, 2021). As mesocosms were harvested on four occasions over a period of 11 days, collinearity between soil moisture and harvest time was thought to potentially be influencing the observed correlations between variables. To account for this, the pcor.test function (R package: *ppcor*; Seongho, 2015) using the Pearson method was used to assess partial correlations in two ways: first to check the partial correlation between soil moisture and other variables given harvest time, grouping mesocosms harvested May 4-5 as opposed to those harvested May 15-17. This was followed by evaluating the partial correlations between variables indicated in the original correlation analysis to be significantly related given soil moisture and harvest time. From these, harvest

time was found to have a correlative effect with all response variables except for pH and switchgrass shoot biomass.

Means comparisons were performed to assess differences in the means between treatments for response variables. Treatment-level differences were assessed using linear mixed effects models (R package: *lme4*) for all variables found to be correlated with harvest time, in which harvest time was included as a random effect, whereas linear models were used for pH and switchgrass shoot biomass means comparisons (Bates et al., 2015). Pairwise-comparisons across treatment were evaluated using the *emmeans* function (R package: *emmeans*) with a Tukey adjustment (Lenth, 2022; Searle et al., 1980). Median based linear models (R package: *mblm*) with the Siegel repeated medians method were then used to assess potential relationships between these variables and microarthropod taxonomic group abundances (Komsta, 2019). However, this correlation analysis was meant to be exploratory rather than explanatory as it was conducted for a subset of control mesocosms (n = 6) where both soil and microbial community analyses were collected. Associations were assessed for the following microarthropod abundances: total microarthropods, collembola, prostigmatid mites, and mesostigmatid mites. Associations with oribatid and astigmatid mite abundances were not included due to their low recovery from these mesocosms.

RESULTS

Microarthropod abundance and community structure

Microarthropods communities were recovered from switchgrass and sorghum microarthropod survival mesocosms following harvest, indicating that microarthropod treatments were able to successfully colonize and subsist within mesocosms over the duration of the experiment. However, it was also found that microarthropods were present in all of the control mesocosms checked (n = 6). Switchgrass microarthropod survival mesocosms tended to have higher overall microarthropod abundances, though this difference was not significant (χ^2 = 1.347, p = 0.510) (**Table 3.3**). Of the 32,752 total microarthropods recovered from switchgrass survival mesocosms, mites made up 73% of the total abundance. Prostigmatid mites were the most abundant group recovered, followed by oribatid mites, collembola, and mesostigmatid mites. Astigmatid mites were present but contributed less than 2% to the total abundance. In

comparison, collembola contributed 43% to the 24,693 microarthropods recovered from energy sorghum survival mesocosms. Prostigmatid mites were the next most abundant group, followed by mesostigmatid mites, oribatid mites, and astigmatid mites (<1%). The communities recovered from control mesocosms were even more collembola dominated, comprising 55% of the total abundance. Across treatments, oribatid mites were significantly more abundant in switchgrass microarthropod survival mesocosms (χ^2 = 11.315, p = 0.003), with other microarthropod taxa exhibiting similar abundances. This was partly due to the high variability observed from both microarthropod abundance and composition of major taxa (**Fig. 3.3**). Despite this, the communities extracted from switchgrass and sorghum microarthropod survival mesocosms exhibited strong differences in community structure ($F_{[2,19]}$: 3.234, p = 0.001) (**Fig. 3.4**). From switchgrass microarthropod survival mesocosms, 13 collembola and 10 oribatid mite morphospecies were recovered compared to 14 collembola and 10 oribatid mite morphospecies recovered from sorghum microarthropod survival mesocosms (**Tables 3.4 & 3.5**). Control mesocosms had the lowest number of morphospecies overall, with 6 collembola and 6 oribatid mite morphospecies recovered.

Effects on switchgrass, soil variables, and microbial CUE

At the time of harvest, most switchgrass plants were in the flowering or post-flowering stage. Switchgrass shoot biomass was similar across treatments (F = 0.547, p = 0.584) and exhibited relatively high within-treatment variability (**Fig. 3.5**). In contrast, there was a slight but significant difference at the treatment level for soil pH (F = 3.865, p = 0.031), with soil in sorghum microarthropod mesocosms tending to be more basic compared to that in switchgrass microarthropod and control mesocosms (**Fig. 3.6**). Across treatments, average soil pH in mesocosms ranged between 7.201 and 7.425, which is similar to the average pH previously reported from the BCSE site (Robertson, 2020; unpublished data). Gravimetric soil moisture at the time of harvest was similar across treatments (χ^2 = 0.287, p = 0.866). Harvest timing was found to strongly influence soil moisture, with mesocosms harvested between May 4-5 found to be significantly drier than those harvested between May 15-17 (χ^2 = 24.058, p < 0.001).

As with switchgrass shoot biomass and soil moisture, microbial activity and growth measures were similar across and variable within treatment mesocosms (**Table 3.6**). Neither

cumulative respiration nor microbial growth, the principal variables in calculating CUE, exhibited treatment-level differences (respiration: $\chi^2 = 0.521$, $p = 0.777$; microbial growth: ; $\chi^2 = 0.944$, $p = 0.624$). As such, CUE was also found to be similar across treatments ($\chi^2 = 1.001$, $p = 0.577$), where it ranged from 0.19 to 0.23 (**Fig. 3.7**). When just comparing switchgrass microarthropod and sorghum microarthropod treatments, I did not find any evidence that including the control mesocosms was masking treatment-level differences for any of the microbial variables, including CUE (**Table 3.7**).

Correlation analysis identified multiple significant relationships between response variables (**Fig. 3.8**). Similarities in the effect size and direction of correlations of both soil moisture and harvest time between a number of my response variables further supported collinearity between these factors. As mesocosm watering was standardized for all mesocosms, partial correlation analyses were performed to determine whether there were correlations with soil moisture independent of harvest time. It was determined from these analyses that harvest time, with mesocosms harvested between May 4-5 being significantly drier than those harvested between May 15-17, was responsible for the significance of these correlations (**Table 3.8**). In contrast, partial correlations remained significant for all but one of the significant associations identified in **Fig. 3.8** (**Table 3.9**). CUE was positively correlated with microbial growth and MBC in addition to harvest time and negatively correlated with respiration. Microbial growth and MBC, strongly positively correlated with each other, were both positively associated with harvest time as well. Microbial growth was also negatively related to microbial turnover time, which itself was positively associated with MBN, DNA presence in soil, and harvest time. Other than with soil moisture, harvest time was most strongly positively correlated with MBN, whereas respiration was the only response variable negatively correlated with harvest time. Neither pH nor microarthropod treatment were significantly correlated with any response variables.

Despite the low sample number, nonparametric linear regression indicated several relationships of interest between the variables above and the abundances of microarthropods recovered from mesocosms. There was a positive association between turnover time and total microarthropod abundance that was driven by collembola ($RSE_{[4]} = 15.46$, MAD = 0.003, p =

0.036) and prostigmatid mites ($RSE_{[4]}$ = 12.31, MAD = 0.010, p = 0.036), the two numerically dominant microarthropods recovered from mesocosms. Further, prostigmatid mite abundance had a marginally negative relationship with microbial growth, though this was not statistically significant ($RSE_{[4]}$ = 7.08, MAD = 0.004, p = 0.059). While greater turnover time and lower microbial growth could indicate lower CUE, there was no relationship found between CUE and total microarthropod abundance, nor the abundance of any microarthropod group (**Fig. 3.9**). Lastly, switchgrass shoot biomass was positively related to mesostigmatid mite abundance ($RSE_{[4]}$ = 1.76, MAD < 0.001, p = 0.036).

DISCUSSION

The ability of microarthropods to influence soil microbial community dynamics and function, primarily via direct and indirect trophic interactions and their involvement in SOM decomposition, has been well documented. However, the diversity of possible mechanisms involved, as well as the high degree of context dependency, has precluded finding general patterns in the regulatory effects of microarthropods on microbes. This has hampered efforts to identify potential connections between microarthropod-microbe interactions and SOC dynamics, despite the potential for these interactions to alter microbial dynamics in ways known to be important drivers of SOC accrual and stability. I attempted to overcome this by investigating the potential for a diverse assemblage of field-collected microarthropods to influence the CUE of a native microbial community in a greenhouse mesocosm experiment using ^{18}O-water stable isotope tracing. By utilizing whole microarthropod and microbial communities with relatively minimal manipulation upon starting the greenhouse experiment, I attempted to capture the net impact of microarthropods with the understanding that overall CUE would be influenced by a number of species-specific interactions and mechanisms that could not be individually manipulated. Further, I utilized two distinct microarthropod communities as treatments from a perennial and annual bioenergy cropping system (switchgrass and energy sorghum, respectively). This was done for two primary reasons. First, perennial cropping systems generally contain more SOC with a greater proportion of SOC being stabilized than annual systems, resulting in increased potential for SOC accrual in these systems (Lemus & Lal, 2005; McLaughlin et al., 2002). Second, perennial cropping systems also support

higher total microbial biomass and fungi-to-bacteria ratios (Jesus et al., 2016; Liang et al., 2012) in addition to harboring more abundant and mite-dominated microarthropod communities (Chapter 2). By incorporating microarthropod communities from both perennial and annual systems, I attempted to account for the ways in which cropping system perenniality, which strongly determines the abundance and composition of microarthropod communities, may influence the direction and strength of microarthropod effects on microbial CUE.

By including an additional subset of mesocosms to monitor microarthropod survival following mesocosm introduction, I was able to confirm that the microarthropod communities collected from switchgrass and energy sorghum cropping systems were able to successfully colonize mesocosms. Across all mesocosms, collembola and prostigmatid mites tended to be the most abundant microarthropod groups recovered from mesocosm substrates, though oribatid and mesostigmatid mites occasionally reach high relative abundances. This can partly be attributed to general life history differences between microarthropod groups, with collembola and numerous prostigmatid groups tending to be both faster growing and more disturbance tolerant than oribatid mites (Behan-Pelletier, 1999; Dindal, 1990). Despite high variability in both abundance and composition across replicates, surviving microarthropods communities retained characteristic differences depending on their origin. Specifically, mesocosms given microarthropod communities collected from switchgrass had significantly more oribatid mites on average, and while not statistically significant, collembola made up a greater proportion of the microarthropods recovered from mesocosms given energy sorghum microarthropods communities. That said, the structure of microarthropod communities recovered from mesocosms are not presumed to be equivalent to those present in the field, instead likely favoring those species which are more resilient and/or better able to rebound quickly from disturbance events. I found observational evidence lending support to this from surviving collembola. In particular, a single morphospecies (E31: see Chapter 2, **Table 2**) made up 78% and 95% of the total collembola recovered from switchgrass microarthropod survival and control mesocosms, respectively. In the more collembola dominated energy sorghum survival mesocosms, which had a greater total number and more even distribution of collembola morphospecies present, this morphospecies only contributed 16% to total

collembola abundance. Furthermore, this morphospecies was only present in 5 mesocosms, 3 control mesocosm and 1 mesocosm for both switchgrass and energy sorghum microarthropod survival. In microarthropod surveys conducted at the BCSE in 2019, this morphospecies was collected infrequently and at low abundance except for in a single energy sorghum field plot, in which it was so numerous that it accounted for 26% of the total collembola abundance (Chapter 2). These observations underscore the need for further research to better understand these community dynamics and their implications for use in mesocosm studies, especially as microarthropod research continues to move away from highly simplified and/or low diversity microcosm studies.

Contrary to my initial hypotheses, I did not find conclusive evidence that microarthropods affect CUE. While CUE was more similar between mesocosms that received switchgrass or energy sorghum microarthropod communities (mean CUE = 0.191 and 0.199, respectively) compared to control mesocosms (mean CUE = 0.234), these differences were not statistically significant. These values are similar to other reported ecosystem-scale CUE values as well as those which were measured using the ^{18}O-water tracer method (Geyer et al., 2019; Geyer et al., 2016). However, none of the component measures necessary for calculating CUE (i.e., MBC, microbial growth, respiration) exhibited treatment-level differences. The only measured variable found to respond to microarthropod addition treatment was soil pH, with energy sorghum microarthropod community mesocosms having the highest pH though this increase was slight. In comparison, harvest timing, through its effect on gravimetric soil moisture, was found to have had a significant effect on CUE. The half of mesocosms harvested between May 15-17 had more recently received water relative to those harvested between May 4-5, which were significantly drier irrespective of microarthropod treatment. Correlation analysis revealed that together, later harvest and subsequent wetter soils were associated with increased MBC and reduced microbial respiration, both of which were reflective of higher CUE.

One factor that affected my ability to find significant treatment-level differences was the high degree of within-treatment variability in my data for CUE, as well as the factors used to calculate and those calculated from CUE (e.g., microbial turnover time). Though it varied by measurement, within-treatment variability tended to be high for all treatments. One probable

explanation for this greater than expected within-treatment variability is that the structure of the microarthropod communities that colonized mesocosms showed similarly high variability across replicates. Thus, any potential community-level treatment effects were obscured by the variability in microarthropod abundance, morphospecies richness, and community composition between mesocosms. Exploratory correlation analysis indicated a possible positive relationship between both collembola and prostigmatid mite abundance with microbial turnover time as well as a marginally negative correlation between prostigmatid mite abundance and microbial growth. While the microarthropod communities from switchgrass and energy sorghum retained distinctly different compositions following mesocosm colonization, total microarthropod abundance, as well as the abundance for collembola and prostigmatid mites, was similar between these communities. However, due to the small number of replicates in which this correlations analysis could be performed, this remains speculative.

By obtaining the majority of microarthropod community composition data from survival mesocosms in which soil and microbial analyses were not conducted, I was unable to assess potential patterns between response variables and microarthropods aside from general community structure, precluding identifying potentially influential effects by specific microarthropod taxa. My ability to assess potential microarthropod effects on CUE was further hindered as control mesocosms were found to have accrued microarthropods over time despite efforts to mitigate contamination. The presence of unintentionally introduced microarthropods to microarthropod-free controls is not uncommon in mesocosm experiments, particularly those with multi-month durations. For example, in a 6 mo field mesocosm experiment, defaunated soil monoliths wrapped in 35 µm mesh to exclude microarthropod recolonization contained 10% and 23% the average number of collembola and mites, respectively, that were found in monoliths accessible to microarthropods (Kandeler et al., 1999). Similarly, Janoušková et al. (2018) reported finding microarthropods in 40% of control mesocosms after 22 weeks in a greenhouse, with average abundances in controls approximately 17% and 12% of that in treatment pots for collembola and mites, respectively. Both studies attributed microarthropod presence in controls to the use of freeze-thaw defaunation to initially exclude microarthropods from mesocosm substrates. Though repeated freeze-thaw cycles are generally highly effective

in defaunating soils without inducing also substantial changes to the soil microbiome or physiochemical conditions, this method is known to be imperfect for complete microarthropod elimination especially for highly resistant species and eggs (Gergócs & Hufnagel, 2011; Bruckner et al., 1995). While the average number of total microarthropods recovered from control mesocosms in this experiment was lower than for mesocosms in which microarthropods were intentionally added, this decrease was not statistically significant with oribatid mites the only microarthropod group found to be present at significantly lower abundance in control mesocosms. Though imperfect defaunation is likely to have had a role in this, a mesocosm flooding event midway through the experiment caused by a drip irrigation system malfunction may have potentially allowed for microarthropod immigration into control mesocosms. Microarthropod survival mesocosms harvested following the flooding event did not indicate substantial mortality, suggesting that most microarthropods were able to get to the substrate surface and float on the water surface until the malfunction was noticed. This disturbance therefore created a potential short-term window of time (<48 hr) in which the plastic barriers extending above the tops of the greenhouse pots would have reduced effectiveness in mitigating microarthropod immigration/emigration, particularly for more mobile groups like collembola.

Aside from the methodological challenges and caveats of utilizing microarthropod community treatments, this study highlights the need for further research into the ways CUE is studied. As previously stated, there exists a wide variety of methods utilized for measuring CUE from the individual microbial colony to ecosystem scale, each having their own unique underlying assumptions, context-specific considerations, and potential limitations (Geyer et al., 2019; Geyer et al., 2016). Beyond the specific methodology used, perhaps a more fundamental challenge in CUE research is the knowledge gaps regarding the long-term processes influencing CUE, including at which time scales these processes become important and how they vary over time (Geyer et al., 2016). Most CUE studies conducted to date have occurred over relatively short time scales (i.e., days as opposed to weeks or months), with these investigations indicating that CUE, as are the microbial processes underlying it, is highly dynamic over short time scales (Frey et al., 2001). However, it can be assumed that what will ultimately be most

important in terms of the relationship between CUE and SOC accrual, itself a long-term process, will be understanding how the net effect of these short-term CUE dynamics produce a long-term average CUE for natural systems. To obtain this "big picture" view of CUE, it will be important that investigations of CUE "scale up" to account for the long-term factors affecting CUE as well as expanding the time spans in which CUE is being measured. In this current study, mesocosms were established for approximately 6 mo. This was done to allow microarthropod communities time to stabilize after being introduced to mesocosms as well as to allow adequate time for litter decomposition and switchgrass growth to occur, both of which were expected to have important impacts on CUE. However, logistical constraints limited my ability to measure CUE for mesocosms to a single time period at the time of destructive harvesting. Given the impact of harvest timing and soil moisture on CUE and expected changing dynamics in microarthropod community structure following mesocosm introduction, performing multiple ^{18}O-water incubations would have allowed me to account for potential changes in CUE as the conditions in mesocosms changed. This could be important for considering the long-term dynamics of CUE even if there was no difference between treatments by the end of the experiment. With the understanding that CUE is a crucial determinant of SOC accrual and stabilization, future research into the methodological "best practices" for measuring CUE, with particular focus on clarifying the relationship between short-term changes and long-term implications, will be a critical next step in elucidating the potential for microarthropod-mediated processes and their interactions with microbes to regulate CUE in natural soil ecosystems.

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REFERENCES

Badejo, M. A., & Tian, G. (1999). Abundance of soil mites under four agroforestry tree species with contrasting litter quality. *Biology and Fertility of Soils*, *30*, 107-112.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1-48.

Behan-Pelletier, V. M. (1999). Oribatid mite biodiversity in agroecosystems: Role for bioindication. *Agriculture, Ecosystems & Environment*, *74*(1-3), 411-423.

Bruckner, A., Wright, J., Kampichler, C., Bauer, R., & Kandeler, E. (1995). A method of preparing mesocosms for assessing complex biotic processes in soils. *Biology and Fertility of Soils*, *19*, 257-262.

Cotrufo, M. F., Ranalli, M. G., Haddix, M. L., Six, J., & Lugato, E. (2019). Soil carbon storage informed by particulate and mineral-associated organic matter. *Nature Geoscience*, *12*(12), 989-994.

Crowther, T. W., Boddy, L., & Jones, T. H. (2012). Functional and ecological consequences of saprotrophic fungus–grazer interactions. *ISME Journal*, *6*(11), 1992-2001.

Dindal, D. L. (1990). *Soil biology guide*. Wiley.

Dungait, J. A., Hopkins, D. W., Gregory, A. S., & Whitmore, A. P. (2012). Soil organic matter turnover is governed by accessibility not recalcitrance. *Global Change Biology*, *18*(6), 1781-1796.

Field, J. L., Richard, T. L., Smithwick, E. A., Cai, H., Laser, M. S., LeBauer, D. S., Long, S. P., Paustian, K., Qin, Z., Sheehan, J. J., Smith, P., Wang, M. Q., & Lynd, L. R. (2020). Robust paths to net greenhouse gas mitigation and negative emissions via advanced biofuels. *Proceedings of the National Academy of Sciences*, *117*(36), 21968-21977.

Filser, J., Faber, J. H., Tiunov, A. V., Brussaard, L., Frouz, J., de Deyn, G. D., Uvarov, A. V., Berg, M. P., Lavelle, P., Loreau, M., Wall, D. H., Querner, P., Eijsackers, H., & Jiménez, J. J. (2016). Soil fauna: Key to new carbon models. *Soil*, *2*(4), 565-582.

Frey, S. D., Gupta, V. V. S. R., Elliott, E. T., & Paustian, K. (2001). Protozoan grazing affects estimates of carbon utilization efficiency of the soil microbial community. *Soil Biology & Biochemistry*, *33*(12-13), 1759-1768.

Fry, B. (2006). *Stable isotope ecology*. Springer New York.

Gange, A. (2000). Arbuscular mycorrhizal fungi, Collembola and plant growth. *Trends in Ecology & Evolution*, *15*(9), 369-372.

Gergócs, V., & Hufnagel, L. (2009). Application of oribatid mites as indicators. *Applied Ecology and Environmental Research*, *7*(1), 79-98.

Geyer, K. M., Dijkstra, P., Sinsabaugh, R., & Frey, S. D. (2019). Clarifying the interpretation of carbon use efficiency in soil through methods comparison. *Soil Biology & Biochemistry*,

128, 79-88.

Geyer, K. M., Kyker-Snowman, E., Grandy, A. S., & Frey, S. D. (2016). Microbial carbon use efficiency: Accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. *Biogeochemistry*, *127*, 173-188.

Grandy, A. S., Wieder, W. R., Wickings, K., & Kyker-Snowman, E. (2016). Beyond microbes: Are fauna the next frontier in soil biogeochemical models?. *Soil Biology & Biochemistry*, *102*, 40-44.

Guo, L. B., & Gifford, R. M. (2002). Soil carbon stocks and land use change: A meta analysis. *Global Change Biology*, *8*(4), 345-360.

Hansen, E. A. (1993). Soil carbon sequestration beneath hybrid poplar plantations in the north central United States. *Biomass & Bioenergy*, *5*(6), 431-436.

Huhta, V., Wright, D. H., & Coleman, D. C. (1989). Characteristics of defaunated soil: I. A comparison of three techniques applied to two different forest soils. *Pedobiologia*, *33*(6), 417-426.

Janoušková, M., Kohout, P., Moradi, J., Doubková, P., Frouz, J., Vosolsobě, S., & Rydlová, J. (2018). Microarthropods influence the composition of rhizospheric fungal communities by stimulating specific taxa. *Soil Biology & Biochemistry*, *122*, 120-130.

Jesus, E. D. C., Liang, C., Quensen, J. F., Susilawati, E., Jackson, R. D., Balser, T. C., & Tiedje, J. M. (2016). Influence of corn, switchgrass, and prairie cropping systems on soil microbial communities in the upper Midwest of the United States. *GCB Bioenergy*, *8*(2), 481-494.

Kallenbach, C. M., Frey, S. D., & Grandy, A. S. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications*, *7*(1), 1-10.

Kandeler, E., Kampichler, C., Joergensen, R. G., & Mölter, K. (1999). Effects of mesofauna in a spruce forest on soil microbial communities and N cycling in field mesocosms. *Soil Biology & Biochemistry*, *31*(13), 1783-1792.

Kim, S. (2015). Ppcor: An R package for a fast calculation to semi-partial correlation coefficients. *Communications for Statistical Applications and Methods*, *22*(6), 665.

Komsta, L (2019). Mblm: Median-based linear models. R package version 0.12.1, <https://CRAN.R-project.org/package=mblm>.

Kravchenko, A. N., Guber, A. K., Razavi, B. S., Koestel, J., Quigley, M. Y., Robertson, G. P., & Kuzyakov, Y. (2019). Microbial spatial footprint as a driver of soil carbon stabilization. *Nature Communications*, *10*(1), 3121.

Lal, R. (2004). Soil carbon sequestration impacts on global climate change and food security. *Science*, *304*(5677), 1623-1627.

Lemus, R., & Lal, R. (2005). Bioenergy crops and carbon sequestration. *Critical Reviews in Plant*

Sciences, *24*(1), 1-21.

Lenth, R (2022). Emmeans: Estimated marginal means, aka least-squares means. R package version 1.7.5, <https://CRAN.R-project.org/package=emmeans>.

Liang, C., Jesus, E. D. C., Duncan, D. S., Jackson, R. D., Tiedje, J. M., & Balser, T. C. (2012). Soil microbial communities under model biofuel cropping systems in southern Wisconsin, USA: Impact of crop species and soil properties. *Applied Soil Ecology*, *54*, 24-31.

Liebig, M. A., Schmer, M. R., Vogel, K. P., & Mitchell, R. B. (2008). Soil carbon storage by switchgrass grown for bioenergy. *BioEnergy Research*, *1*, 215-222.

Lussenhop, J. (1992). Mechanisms of microarthropod-microbial interactions in soil. In M. Begon & A. H. Fitter (Eds.). *Advances in ecological research* (Vol. 23, pp. 1-33). Academic Press.

Maboreke, H. R., Graf, M., Grams, T. E. E., Herrmann, S., Scheu, S., & Ruess, L. (2017). Multitrophic interactions in the rhizosphere of a temperate forest tree affect plant carbon flow into the belowground food web. *Soil Biology & Biochemistry*, *115*, 526-536.

Macdonald, C. A., Delgado-Baquerizo, M., Reay, D. S., Hicks, L. C., & Singh, B. K. (2018). Soil nutrients and soil carbon storage: Modulators and mechanisms. In B. K. Singh (Ed.), *Soil carbon storage* (pp. 167-205). Academic Press.

McGowan, A. R., Nicoloso, R. S., Diop, H. E., Roozeboom, K. L., & Rice, C. W. (2019). Soil organic carbon, aggregation, and microbial community structure in annual and perennial biofuel crops. *Agronomy Journal*, *111*(1), 128-142.

McLaughlin, S. B., de la Torre Ugarte, D. G., Garten, C. T., Lynd, L. R., Sanderson, M. A., Tolbert, V. R., & Wolf, D. D. (2002). High-value renewable energy from prairie grasses. *Environmental Science & Technology*, *36*(10), 2122-2129.

Moore, J. C. (1988). The influence of microarthropods on symbiotic and non-symbiotic mutualism in detrital-based below-ground food webs. *Agriculture, Ecosystems & Environment*, *24*(1-3), 147-159.

Ogle, D. H., Doll, J. C., Wheeler, P., & Dinno, A. (2022). FSA: Fisheries stock analysis. R package version 0.9.3, <https://github.com/fishR-Core-Team/FSA>.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., de Caceres, M., Durand, S., … Weedon, J. (2022). Vegan: Community ecology package. R package version 2.6-2, <https://CRAN.R-project.org/package=vegan>.

Parkinson, D. (1983). Functional relationships between soil organisms. In H. M. Lebrun, A. A. de Medts, C. Gregoire-Wibo, & G. Wauthy, G. (Eds.), *New trends in soil biology. Proceedings of the VIII international colloquium of soil zoology, Louvain-la-Neuve, 1982: International colloquium of soil zoology, 8th, 1982*. Universite Catholique de Louvain.

Paustian, K., Larson, E., Kent, J., Marx, E., & Swan, A. (2019). Soil C sequestration as a biological

negative emission strategy. *Frontiers in Climate*, *1*, 8.

Pollierer, M. M., & Scheu, S. (2021). Stable isotopes of amino acids indicate that soil decomposer microarthropods predominantly feed on saprotrophic fungi. *Ecosphere*, *12*(3), e03425.

Potapov, A. M., Beaulieu, F., Birkhofer, K., Bluhm, S. L., Degtyarev, M. I., Devetter, M., Goncharov, A. A., Klarner, B., Korobushkin, D. I., Liebke, D. F., Maraun, M., McDonnell, R. J., Pollierer, M. M., Schaefer, I., Shrubovych, J., Semenyuk, I. I., Sendra, A., Tuma, J., Tůmová, M., … Scheu, S. (2022). Feeding habits and multifunctional classification of soil-associated consumers from protists to vertebrates. *Biological Reviews*, *97*(3), 1057-1117.

Robertson, G. P. (2020). GLBRC soil pH (2008 to 2013) (GLBRC008) [Unpublished data set]. Kellogg Biological Station Long-Term Ecological Research (LTER) Program.

Santos, P. F., DePree, E., & Whitford, W. G. (1978). Spatial distribution of litter and microarthropods in a Chihuahuan desert ecosystem. *Journal of Arid Environments*, *1*(1), 41-48.

Schimel, J. P., & Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Frontiers in Microbiology*, *3*, 348.

Searle, S. R., Speed, F. M., & Milliken, G. A. (1980). Population marginal means in the linear model: An alternative to least squares means. *American Statistician*, *34*(4), 216-221.

Siepel, H., & Maaskamp, F. (1994). Mites of different feeding guilds affect decomposition of organic matter. *Soil Biology & Biochemistry*, *26*(10), 1389-1394.

Soong, J. L., Vandegehuchte, M. L., Horton, A. J., Nielsen, U. N., Denef, K., Shaw, E. A., de Tomasel, C. M., Parton, W., Wall, D. H., & Cotrufo, M. F. (2016). Soil microarthropods support ecosystem productivity and soil C accrual: Evidence from a litter decomposition study in the tallgrass prairie. *Soil Biology & Biochemistry*, *92*, 230-238.

Spohn, M., Pötsch, E. M., Eichorst, S. A., Woebken, D., Wanek, W., & Richter, A. (2016). Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. *Soil Biology & Biochemistry*, *97*, 168-175.

Teuben, A. (1991). Nutrient availability and interactions between soil arthropods and microorganisms during decomposition of coniferous litter: A mesocosm study. *Biology and Fertility of Soils*, *10*, 256-266.

Wallwork, J. A. (1976). *The distribution and diversity of soil fauna*. Academic Press.

Wei, T., & Simko, V. (2021). R package 'corrplot': Visualization of a correlation matrix (Version 0.92). <https://github.com/taiyun/corrplot>.

Wieder, W. R., Grandy, A. S., Kallenbach, C. M., & Bonan, G. B. (2014). Integrating microbial physiology and physio-chemical principles in soils with the MIcrobial-MIneral Carbon Stabilization (MIMICS) model. *Biogeosciences*, *11*(14), 3899-3917.

Zhang, H., Goll, D. S., Wang, Y. P., Ciais, P., Wieder, W. R., Abramoff, R., Huang, Y., Guenet, B., Prescher, A. K., Viscarra Rossel, R. A., Barré, P., Chenu, C., Zhou, G., & Tang, X. (2020). Microbial dynamics and soil physicochemical properties explain large-scale variations in soil organic carbon. *Global Change Biology*, *26*(4), 2668-2685.

APPENDIX

CHAPTER 3 FIGURES & TABLES

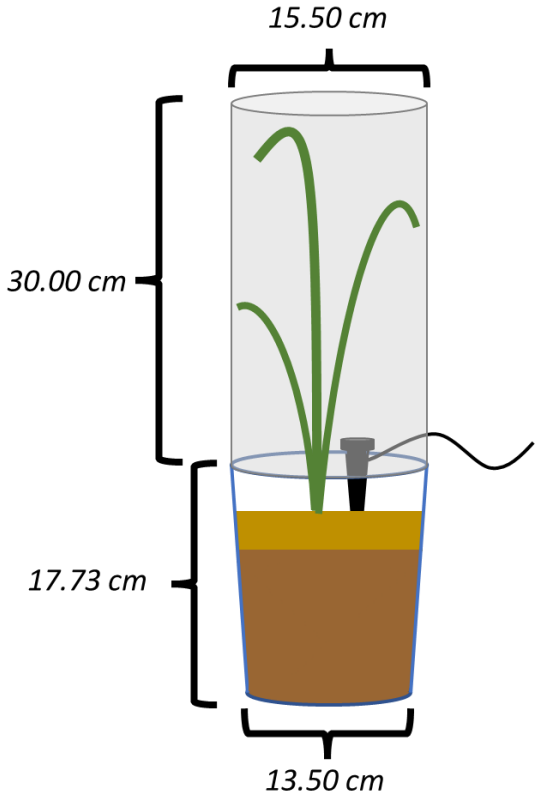

Figure 3.1. Mesocosm design. The nursery pot in the diagram is transparent to show the approximate depth of the soil (brown) and litter (yellow) layers.

Table 3.1. Agronomic management details for switchgrass and energy sorghum field plots at the BCSE. Fertilization and pest control details are specific to the 2020 growing season but are representative of the normal agronomic management for these cropping system treatments. Fertilization and pesticide amounts are given on a per hectare basis.

Cropping system	Crop details	Fertilization regime	Pest control regime
Switchgrass	Switchgrass (*Panicum virgatum*, var. Cave-in-Rock) established in 2008	25.58 L 28% N fertilizer (9.18 L of N)	No chemical pest control
Energy sorghum	Continuous energy sorghum (*Sorghum bicolor*, photoperiod-sensitive hybrid ES5200) established in 2018 with annual replanting, previously corn-soybean-canola from 2008-2011 and continuous corn with cover crop from 2012-2017	18.36 kg potash (11.01 kg of potassium oxide)	0.25 L Dual II Magnum 0.77 L Roundup Powermax 0.61 L methylated soybean seed oil 0.02 L Sharpen

Table 3.2. Mesocosm treatments as assigned for each mesocosm type, with "CUE mesocosms" referring to those in which ^{18}O-water tracing methods were performed to calculate CUE while "Survival mesocosms" refers to the subset of replicates maintained to monitor microarthropod survival in mesocosms throughout the duration of the experiment. Included are the total number of replicates for each mesocosm type and treatment cross as well as mesocosm harvest timing details.

	Mesocosm type	
Treatment	CUE mesocosms	Survival mesocosms
Switchgrass microarthropods	N = 12 • n = 6 harvested May 4-5 • n = 6 harvested May 15-17	N = 8 • n = 2 harvested in early Feb • n = 6 harvested in May
Sorghum microarthropods	N = 12 • n = 6 harvested May 4-5 • n = 6 harvested May 15-17	N = 8 • n = 2 harvested in early Feb • n = 6 harvested in May
Control (no fauna introduced)	N = 12 • n = 6 harvested May 4-5 • n = 6 harvested May 15-17 • microarthropod presence also assessed	--

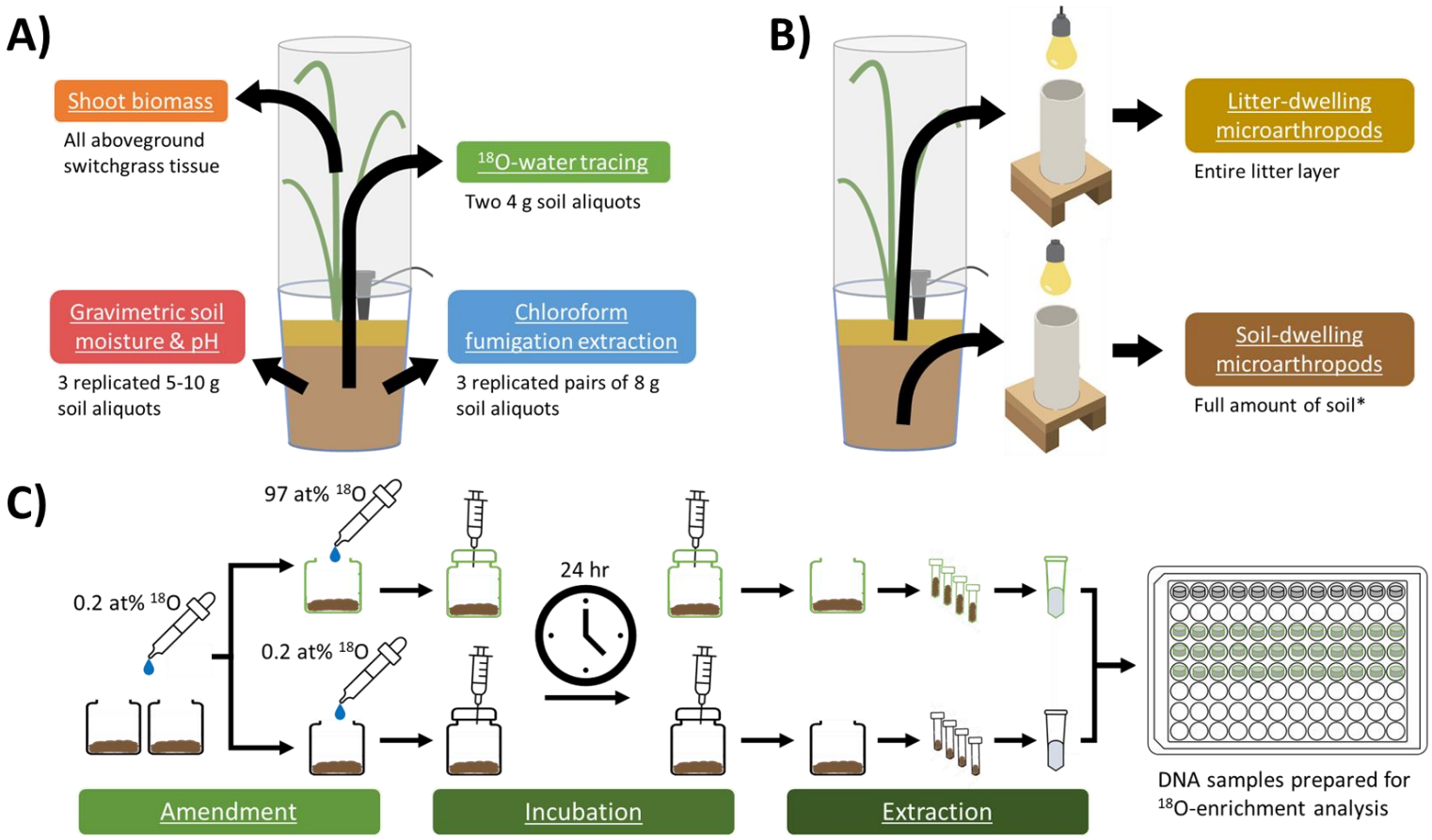

Figure 3.2. Diagram indicating the substrates harvested from and analyses performed, including the amounts of substrates used for these, on **A)** mesocosms in which CUE was assessed via ^{18}O-water tracing (n = 12 per treatment) as well as **B)** those maintained to monitor microarthropod survival following mesocosm colonization. **C)** Diagram visualizing the ^{18}O-water tracing method protocol. Paired 4 g soil subsamples were raised to 48% WHC before being amended with either sterile or ^{18}O-enriched water. Immediately following this, enriched (green) and natural abundance (black) subsamples were incubated for 24 hr, before and after which respiration assays were performed. DNA was then extracted from both subsamples, with these samples then being sent to a stable isotope facility for ^{18}O-enrichment analysis.

Figure 3.2 (cont'd)
* Only in 6 control mesocosms were both CUE and microarthropod communities assessed, with all the soil remaining after taking the necessary aliquots for measuring CUE and other soil properties placed into Tullgren funnels for microarthropod extraction.

Table 3.3. Average microarthropod abundance ± standard deviation of microarthropods recovered from mesocosms. Values were rounded to the nearest integer when necessary. Letters indicate significant differences of the means as determined via the Kruskal-Wallis test ($p < 0.05$, Dunn's test). SW survival: switchgrass microarthropod survival (n = 8); ES survival: energy sorghum microarthropod survival (n = 8); and control (n = 6) mesocosms.

	SW survival	**ES survival**	**Control**
Total	4094 ± 3273	3087 ± 2140	2255 ± 1868
Collembola	1100 ± 2341	1339 ± 1783	1242 ± 1536
Oribatid mites	1216 ± 2747 **a**	119 ± 227 **ab**	5 ± 6 **b**
Mesostigmatid mites	379 ± 546	428 ± 385	190 ± 130
Prostigmatid mites	1335 ± 965	1198 ± 1206	818 ± 530
Astigmatid mites	64 ± 98	3 ± 3	1 ± 1

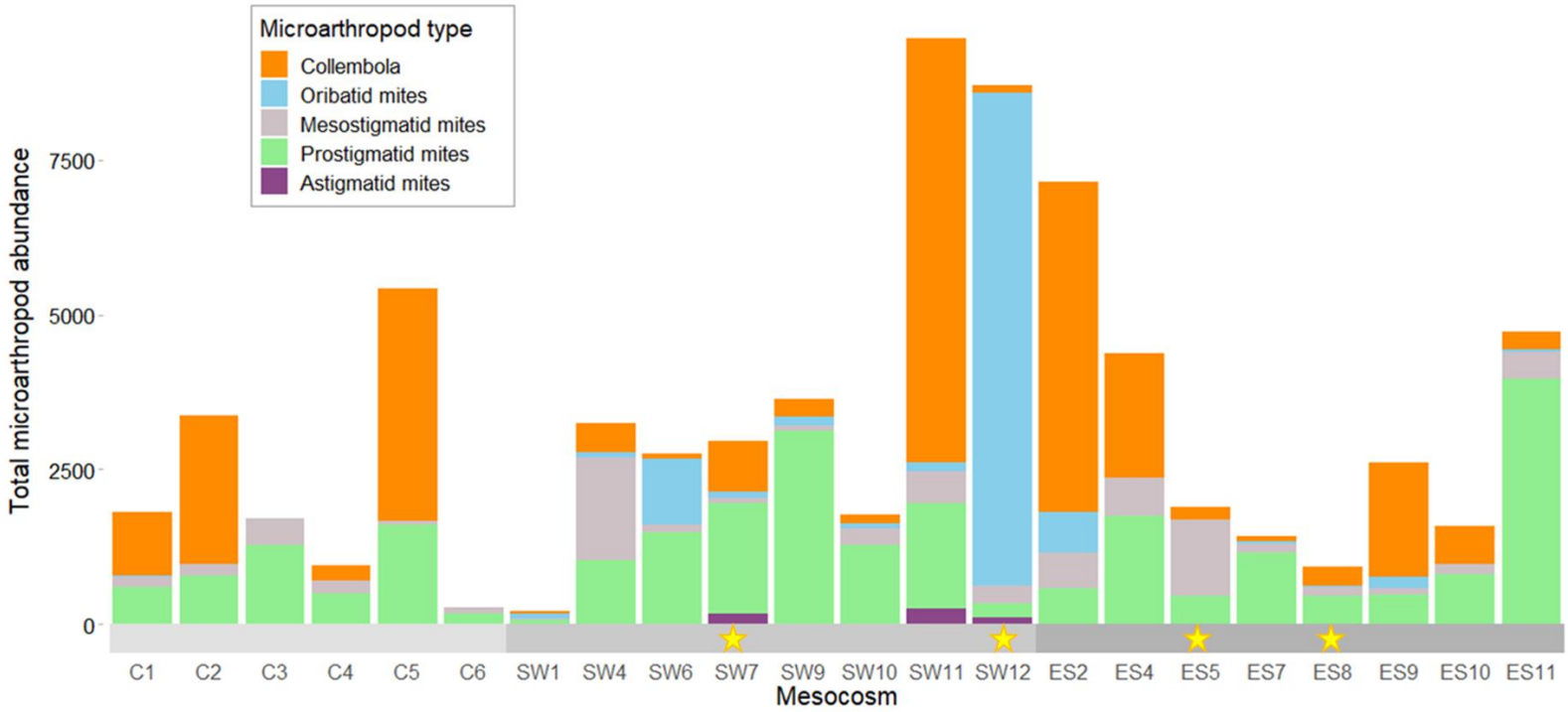

Figure 3.3. Total number and composition of microarthropods recovered from mesocosms in which microarthropod community data was collected upon harvest. Grey bars underneath the x-axis are to visualize the different mesocosm types, as do the letters in front of mesocosm ID number. From left to right: control mesocosms (C), switchgrass microarthropod survival mesocosms (SW), energy sorghum survival mesocosms (ES). Stars indicate mesocosms which were harvested in early February, with the rest all harvested at the end of the experiment duration in May.

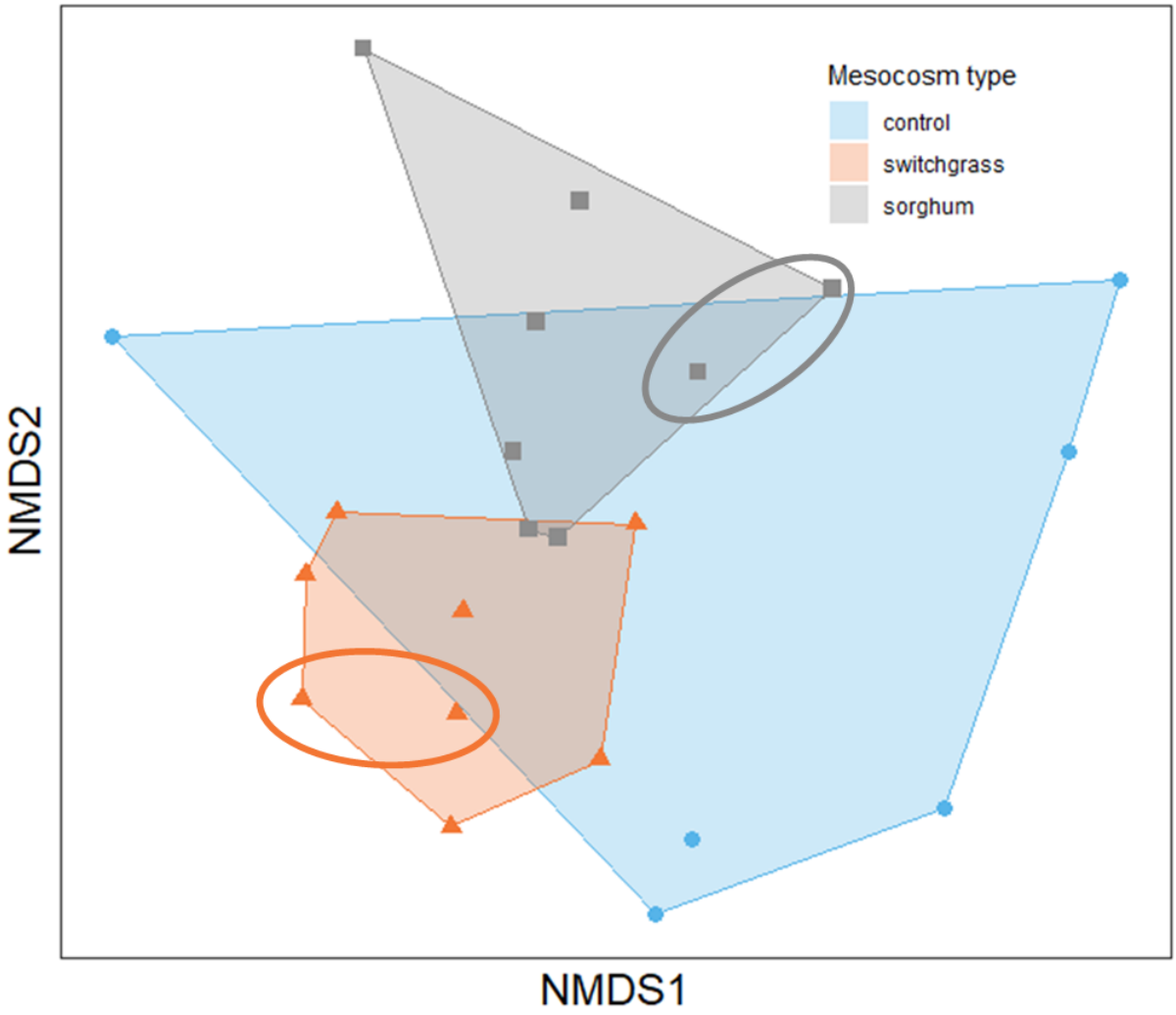

Figure 3.4. Community structure differences between microarthropods recovered from switchgrass and sorghum microarthropod survival and control mesocosms, visualized using NMDS (k = 3, stress = 0.180). Significant compositional differences by treatment were determined via PERMANOVA ($F_{[2,19]}$: 3.234, p = 0.001). Ellipses are placed around the subset of switchgrass and sorghum microarthropod survival mesocosms harvested in post-flooding conditions mid-way through the experiment in early February.

Table 3.4. Collembola morphospecies collected throughout the duration of the experiment and which samples they were extracted from, including the proportion they made up of the overall abundance for that group (% abund) as well as the number of mesocosms it was collected from for that treatment.

ID	**Switchgrass survival**		**Sorghum survival**		**Control**	
elongate	% abund	n = **8**	% abund	n = **8**	% abund	n = **6**
E2	1.71	5	8.64	6	--	--
E3	<1	1	--	--	--	--
E4	12.12	3	5.11	5	--	--
E5	<1	4	7.61	4	4.91	3
E6	<1	3	3.52	7	--	--
E7	<1	1	58.20	6	<1	1
E12	--	--	<1	5	--	--
E15	<1	1	--	--	--	--
E21	3.98	4	<1	2	<1	3
E25	<1	1	<1	1	--	--
E30	3.33	3	--	--	<1	1
E31	77.78	1	16.04	1	94.90	3
E47	--	--	<1	1	--	--
E48	--	--	--	--	<1	1
E49	--	--	<1	1	--	--
E50	<1	1	<1	1	--	--
E51	<1	1	--	--	--	--
globular						
G44	--	--	<1	1	--	--

Table 3.5. Oribatid mite morphospecies collected throughout the duration of the experiment and which samples they were extracted from, including the proportion they made up of the overall abundance for that group (% abund) as well as the number of mesocosms it was collected from for that treatment.

ID	**Switchgrass survival**		**Sorghum survival**		**Control**	
adults	% abund	n = **8**	% abund	n = **8**	% abund	n = **6**
O2	72.03	4	79.06	5	2.04	1
O3	<1	3	--	--	--	--
O4	5.77	8	2.83	6	20.41	3
O5	<1	4	1.68	4	--	--
O6	11.77	8	3.14	5	--	--
O10	--	--	2.62	5	--	--
O13	--	--	<1	1	4.08	1
juveniles						
O7	6.12	6	<1	2	63.27	2
O8	<1	6	3.77	4	2.04	1
O12	<1	1	<1	2	8.16	2
O18	<1	4	--	--	--	--
O20	3.23	4	5.97	2	--	--

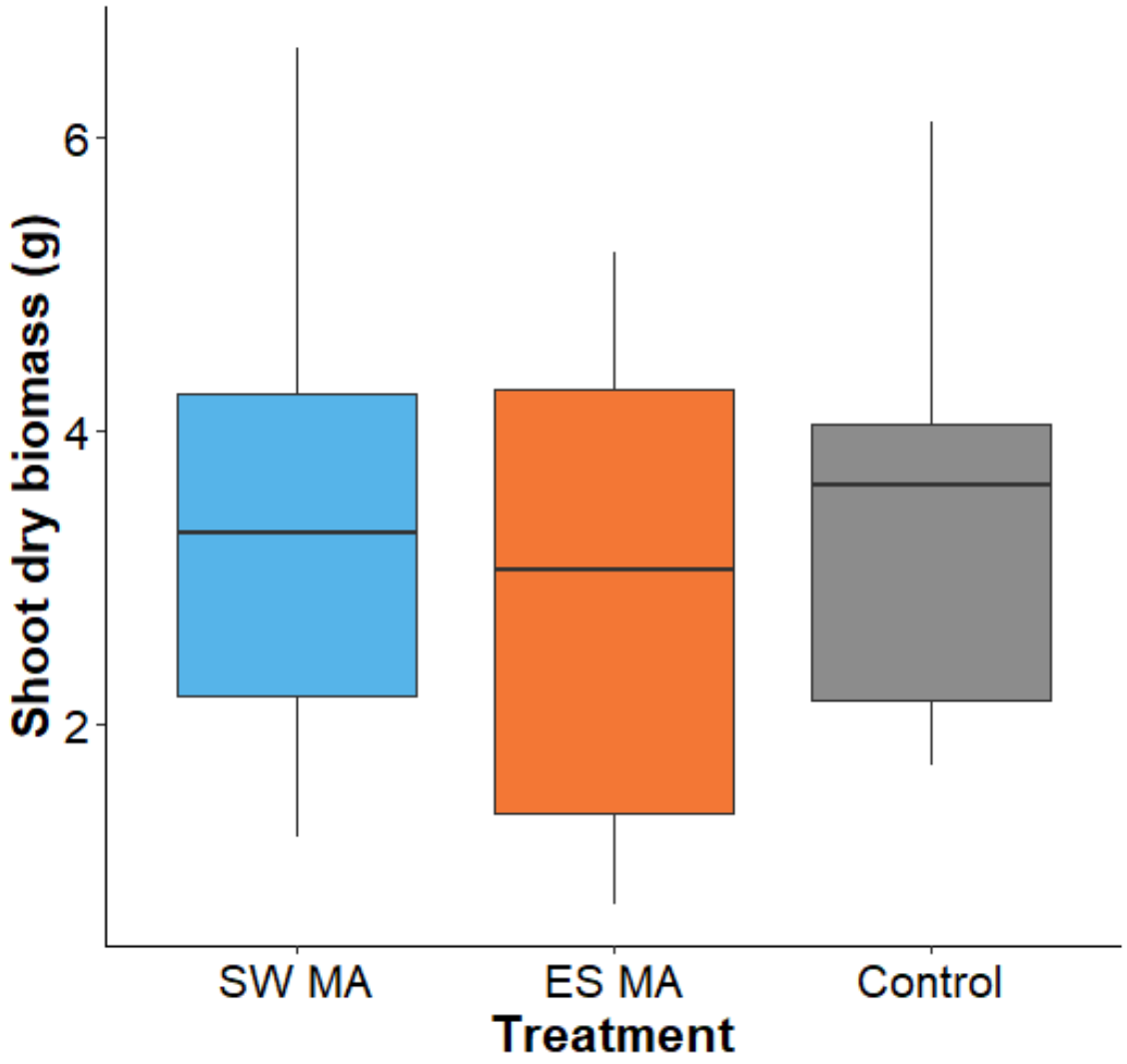

Figure 3.5. Switchgrass shoot dry biomass at the time of harvest between switchgrass microarthropod (SW MA), sorghum microarthropod (ES MA), and control mesocosms. Mean shoot biomass differences between treatments were assessed via linear effects model ($p < 0.05$).

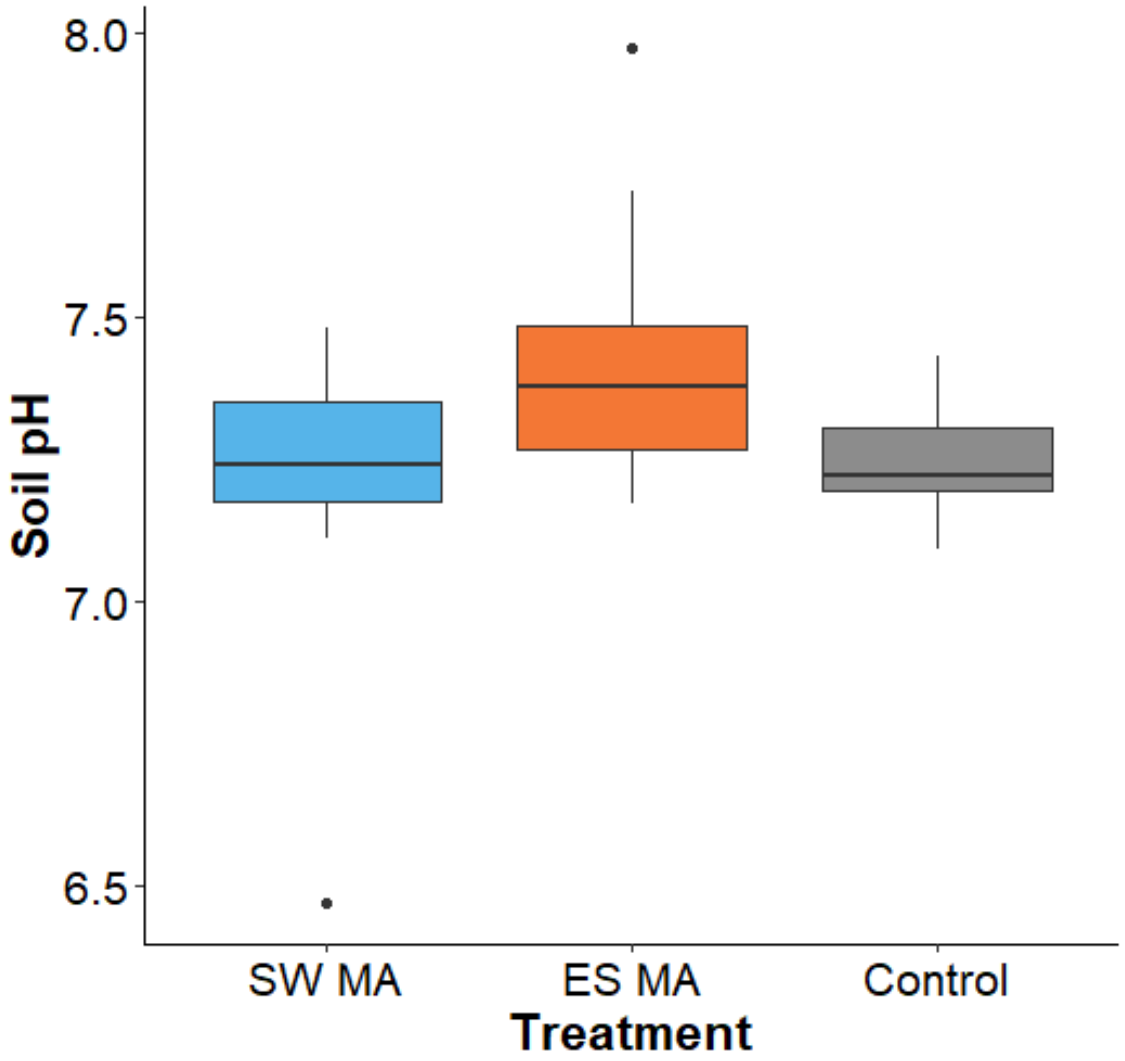

Figure 3.6. Differences in soil pH between microarthropod (SW MA), sorghum microarthropod (ES MA), and control mesocosms. Despite finding a difference between treatments overall, pairwise differences across treatments levels were not statistically significant (emmeans *post hoc*, Tukey method for p value adjustment).

Table 3.6. Average values for microbial activity, biomass, and growth variables measured across treatments ± standard deviation. Italicized variables were directly used in calculating CUE. SW microarth. and ES microarth. are short for switchgrass microarthropod and energy sorghum microarthropod mesocosms, respectively. Treatment-level differences were assessed using linear mixed effects models ($p < 0.05$ for all variables).

	SW microarth.	**ES microarth.**	**Control**
Cumulative respiration (µg C g^{-1} dry soil)	102.01 ± 22.27	107.90 ± 52.37	97.25 ± 33.39
MBC (µg microbial C g^{-1} dry soil)	1218.00 ± 652.40	1059.60 ± 526.77	1079.80 ± 503.84
MBN (µg microbial N g^{-1} dry soil)	12.18 ± 7.63	14.54 ± 9.08	13.30 ± 10.70
Microbial growth (µg C g^{-1} dry soil)	25.17 ± 17.23	21.36 ± 10.27	26.49 ± 14.11
Turnover time (d)	52.87 ± 17.35	53.06 ± 22.66	47.98 ± 18.66

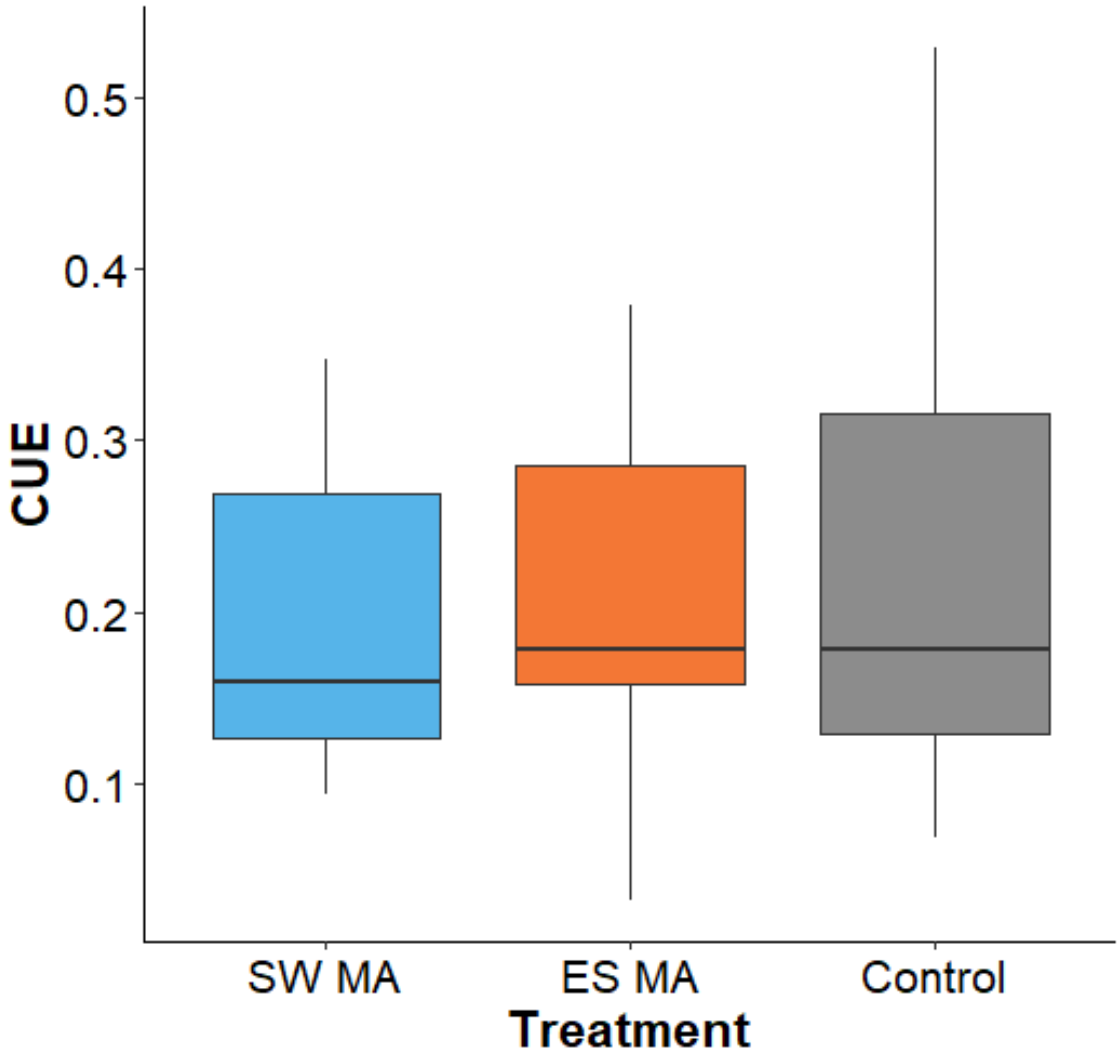

Figure 3.7. CUE estimates for switchgrass microarthropod (SW MA), sorghum microarthropod (ES MA), and control mesocosms. Treatment-level differences assessed via linear mixed effects modeling ($p < 0.05$).

Table 3.7. Results of two-way comparisons performed between only the treatments in which microarthropods were intentionally introduced at the start of the experiment. The variable assessed is given as well as the statistical test utilized and associated output including p value.

CUE (unitless)	Student's t-test	t = 0.169, df = 18, p = 0.868
Cumulative respiration ($\mu g\ C\ g^{-1}$ dry soil)	Wilcoxon test	n = 24, W = 67, p = 0.799
MBC (μg microbial $C\ g^{-1}$ dry soil)	Student's t-test	t = -0.597, df = 18, p = 0.558
MBN (μg microbial $N\ g^{-1}$ dry soil)	Student's t-test	t = 0.601 , df = 16, p = 0.556
Growth ($\mu g\ C\ g^{-1}$ dry soil)	Wilcoxon test	n = 20, W = 51, p = 0.971
Turnover time (d)	Student's t-test	t = 0.022, 18 = 22, p = 0.983

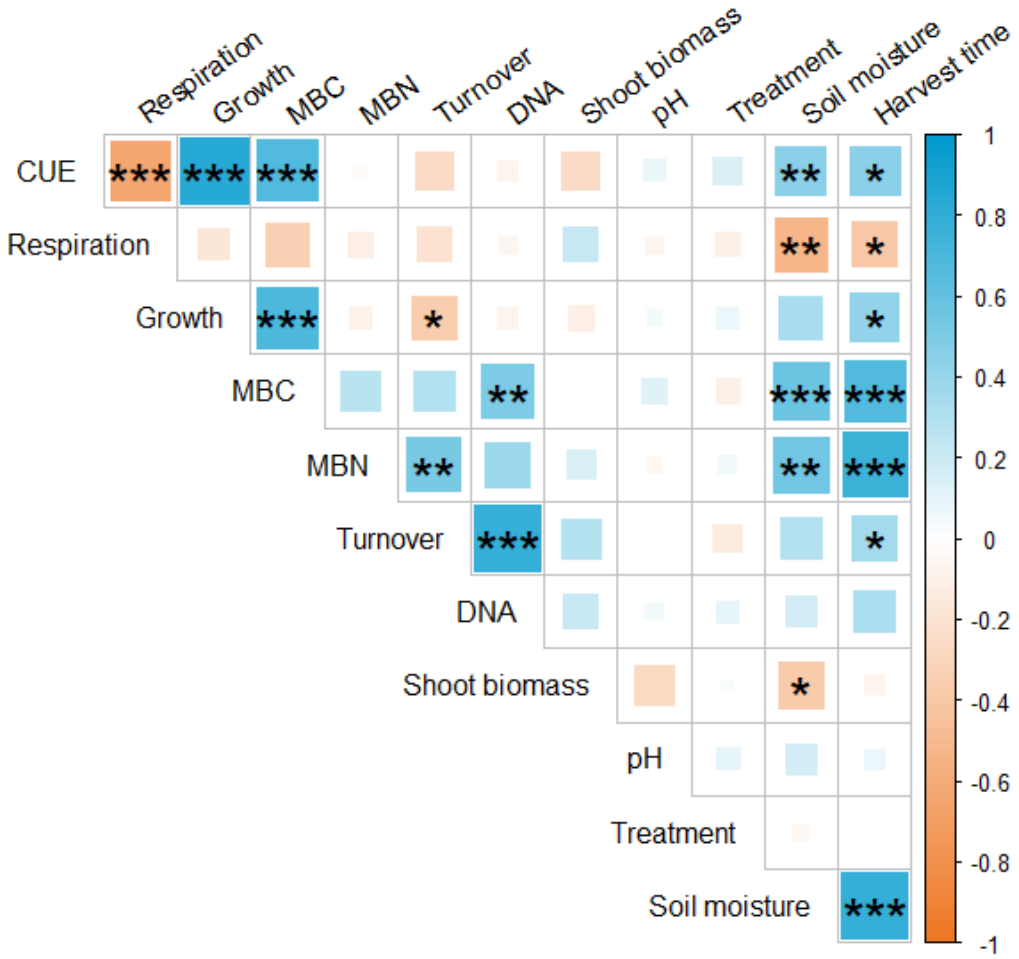

Figure 3.8. Correlation analysis for soil, microbial, and switchgrass properties. While treatment did not significantly affect any of the response variables, there were multiple significant associations between these variables. Positive correlations (Pearson) are shaded blue with negative correlations shaded orange. Significant correlations are indicated with asterisks (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). Growth: microbial growth; Turnover: microbial turnover time; DNA: amount of DNA present per g soil.

Table 3.8. Full and partial correlation analysis results for the variables found to be significantly correlated with soil moisture. Partial correlations (Pearson) indicate the correlation between the response variable to soil moisture given harvest week, with insignificant partial correlations signifying that soil moisture has no independent association with the response variable. Correlations which remain significant independent of the influence of soil moisture and harvest time have been bolded.

	Correlation		**Partial correlation**	
	estimate	p value	estimate	p value
CUE	0.457	9.717^{-3}	0.197	0.366
Respiration	-0.520	1.143^{-3}	-0.222	0.308
MBC	0.577	6.795^{-4}	0.071	0.747
MBN	0.559	2.427^{-3}	-0.143	0.514
Shoot biomass	-0.380	2.233^{-3}	-0.335	0.118

Table 3.9. Full and partial correlation results of significant correlations identified in the correlation matrix (**Fig. 3.7**). Partial correlations (Pearson) indicate the correlation between the response variables given harvest week and soil moisture. Correlations which remain significant independent of the influence of soil moisture and harvest time have been bolded. Growth: microbial growth; Turnover: microbial turnover time; DNA: amount of DNA present per g soil.

	Correlation		**Partial correlation**	
	estimate	p value	estimate	p value
CUE x Respiration	-0.637	1.149^{-4}	**-0.659**	8.576^{-4}
CUE x Growth	0.841	3.085^{-9}	**0.796**	9.406^{-6}
CUE x MBC	0.680	2.577^{-5}	**0.535**	0.010
Growth x MBC	0.696	1.357^{-5}	**0.624**	0.002
Turnover x Growth	-0.358	0.048	**-0.790**	1.222^{-5}
DNA x MBC	0.504	3.861^{-3}	0.319	0.148
MBN x Turnover	0.528	8.071^{-3}	**0.470**	0.027

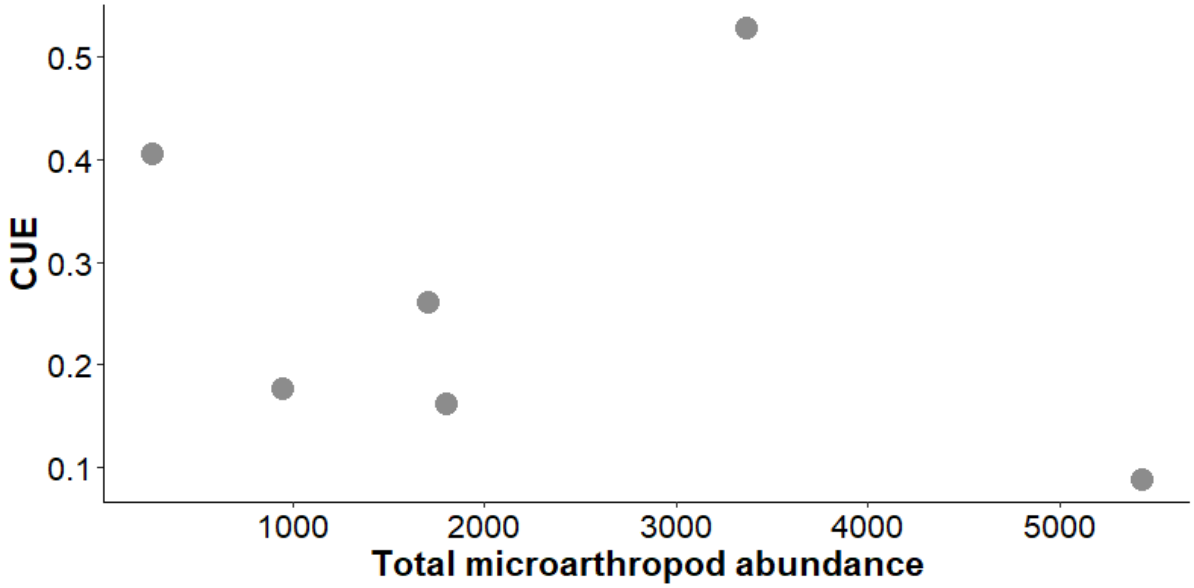

Figure 3.9. Relationship between total microarthropod abundance and CUE from the subset of control mesocosms (n = 6) in which both CUE and microarthropod abundance were measured.

CHAPTER 4: SOIL FAUNA COMMUNITIES IN SWITCHGRASS BIOENERGY SYSTEMS: IMPACTS ON NITROGEN DYNAMICS

ABSTRACT

Due to the interconnected nature of carbon and nitrogen cycles in soils, factors that influence nitrogen availability can have strong implications on carbon dynamics. Soil nitrogen dynamics are particularly important to consider in the context of bioenergy cropping systems, as bioenergy crop production is typically relegated to low nutrient soils to avoid a food-fuel conflict with food crops over arable land. Not only are bioenergy crop yields dependent upon these crops meeting their nitrogen needs in these often nitrogen-limiting soils, but crop-microbe competition for nitrogen can accelerate organic matter decomposition, impacting carbon stability and storage. The activities of soil fauna have long been known to make important contributions to total nitrogen mineralization in soils, but quantifying the strength and direction of their effects, particularly as they relate to soil organic carbon dynamics, remains challenging. This is in part due to the diverse mechanisms and multitrophic interactions operating across scales. Additionally, the influence of different faunal functional groups, which can promote nitrogen mineralization in widely differing ways, on the activity of microbes and with each other further complicates this picture. To address this, I conducted a greenhouse mesocosm experiment to evaluate the effects of microarthropods and nematodes to promote nitrogen mineralization. The primary mechanisms underlying their effects on nitrogen mineralization are generally distinct. Nematodes stimulate rapid microbial turnover via high rates of microbivory, whereas microarthropods have more indirect influence over nitrogen mineralization by influencing the rate and trajectory of organic matter decomposition. Therefore, I evaluated the relative impacts of these groups to see if both mechanisms working in tandem would have a stronger effect on nitrogen availability than either would alone. Microarthropod and nematode communities were collected from switchgrass cropping system fields and introduced, both individually and in combination, to mesocosms containing switchgrass-adapted soil microbe communities to best capture the potentially important fauna-microbe interactions promoting nitrogen mineralization in this system. Further, switchgrass seedlings were planted in mesocosms to determine if faunal effects on nitrogen availability

would correspond with increased productivity. Isotopically enriched litter was added to mesocosms to be their primary source of nitrogen, allowing me to use stable isotope tracer methods to track the movement of nitrogen across distinct pools. From this experiment, I found that fauna, especially nematodes, significantly affected the nitrogen enrichment of switchgrass roots. Litter-derived nitrogen enrichment in root biomass was greatest with both microarthropods and nematodes in combination, intermediate with nematodes only, and similarly low with microarthropods only or no added fauna. Despite this, there was no effect of fauna on overall nitrogen content and biomass of switchgrass, suggesting that switchgrass productivity was unaffected by the presence of either faunal group alone or in combination. These findings illuminate the complicated, often overlapping interactions between microbes, plants, and soil fauna, and highlights the importance of accounting for the activities and interactions of entire soil food webs in understanding soil nutrient dynamics.

INTRODUCTION

Nitrogen (N) is a major component of critical cellular macromolecules (i.e., DNA, RNA) and hence is one of the most essential elements necessary for living organisms along with carbon (C) and oxygen. Aside from a limited proportion of microbes capable of biological N fixation, organisms must obtain N from external sources, either via direct uptake from the environment or by ingesting the biomass other organisms, to meet their nutrient requirements. Advances in our understanding of terrestrial N dynamics have thus been fundamental for developing highly productive agroecosystems capable of supporting the growing global population. Crop productivity is strongly regulated by N availability, with modern conventional agriculture often relying on inorganic N fertilizers to increase the amount of N, in soluble plant-available forms such as ammonium and nitrate, present in soil. While this practice has vastly increased crop yields, widespread fertilizer usage over the last century has created a global N imbalance (Lu & Tian, 2017). This imbalance in the N cycle has resulted in increasingly rapid losses of plant-available N from soils (Vitousek et al., 1997; Vitousek & Howarth, 1991), with substantial consequences for crop yield, human health, and the environment (Penuelas et al., 2020). Furthermore, as terrestrial N and C cycles are closely interconnected, unsustainable N management in agroecosystems has had a substantial contribution on rising C emissions

worldwide (Macdonald et al., 2018). As evidence of the severe consequences of the global N balance continues to mount, it will be increasingly critical to design and manage agroecosystems that balance crop yields with sustainable N management to improve N cycling efficiencies and reduce N loss in soils.

Unlike in many types of agroecosystems, sustainable N use has been an important consideration for bioenergy cropping systems since their initial development. N-limited or otherwise unproductive soils have been specifically targeted for bioenergy crop production to avoid food-fuel conflicts over arable land (Robertson et al., 2017). Maximizing N efficiency and retention while simultaneously minimizing N loss from soils will therefore be increasingly important to balance high-yielding crop production with promoting soil organic C (SOC) accrual and stabilization. Beyond its importance for crop health and productivity, N availability has important implications for soil organic matter (SOM) dynamics. SOC loss and destabilization occurs under N-limiting conditions as heightened competition with plants for N drives microbes to mine N from increasingly recalcitrant, less-efficiently utilized substrates to meet their N requirements (Chen et al., 2013; Cotrufo et al., 2013; Craine et al., 2007). With both adequate crop yields and enhanced SOC accrual necessary for the success of bioenergy as an emissions and climate change mitigation strategy, it will be increasingly essential to better understand N dynamics in the context of bioenergy cropping systems and predict how the balance between N excess and limitation effects bioenergy crop yield as well as SOM and SOC dynamics.

As with SOC, microbial dynamics have immense influence over N availability in soils. Microbial biomass N (MBN) has been found to make up between 2-8% of the total N present in soils (Xu et al., 2013; Friedel & Scheller, 2002), with their necromass likely contributing an even greater proportion (Deng & Liang, 2022; Liang et al., 2019; Simpson et al., 2007). Microbes tend to immobilize N due to their high N requirements relative to C and nutrient assimilation efficiencies, with microbial biomass found to be a significant driver of gross soil N immobilization rates globally (Li et al., 2020). Soil fauna overall biomass, nutrient assimilation efficiencies, and N to C requirements compared to microbes and thus make relatively small direct contributions to the total N pool (Andrén et al., 1990). Despite this, they play an important, albeit less well understood, role in maintaining N availability in soils by promoting N

mineralization, predominately via their interactions with microbes and SOM (Osler & Sommerkorn, 2007; Verhoef & Brussaard, 1990). Microbivorous fauna promote N mineralization both by exerting top-down control over microbial turnover, growth, and community dynamics. Decomposer fauna also strongly influence N mobilization by altering the quality, quantity, and accessibility of SOM, thereby affecting the rate at which these residues are broken down as well as the degree to which the nutrients they contain are recycled, stabilized, or lost from soils (Filser et al., 2016; Soong & Nielsen, 2016). Taken together, soil fauna are believed to contribute an estimated ~30% of the total N mineralized in soils, highlighting their fundamental role in maintaining N availability (Verhoef & Brussaard, 1990). These contributions are expected to be especially important in soils where N is limiting and may help to alleviate the competition for N between plants and microbes, including soils optimal for bioenergy crop growth production.

By stimulating N mineralization via their interactions with microbes and involvement in SOM decomposition, soil fauna are responsible for a considerable proportion of the total N mineralization in soils. These effects on N mobilization can cause cascading effects on plant productivity and microbial activity, with important implications for SOC dynamics as well. Despite this, current attempts to predict soil nutrient cycles, including both theoretical and biogeochemical models, often fail to explicitly account for the contributions of soil fauna (Filser et al., 2016; Grandy et al., 2016; Osler & Sommerkorn, 2007). This is in part because much remains unknown about the soil fauna communities in soils, including their community structure, their relative contribution to N mineralization, and the degree to which their direct and indirect interactions with microbes and plants may be influencing SOC dynamics such as by affecting plant productivity or microbial activity. This includes nematodes and microarthropods, two groups long known to promote N mineralization albeit by generally distinct mechanisms. Nematodes, particularly microbivorous groups, are assumed to have the greatest impact on N mineralization rates via their role in the “microbial loop”, in which their major effects on N arise via their high rates of microbivory stimulating the release of N formerly bound to microbial biomass as well as their ability to regulate microbial community dynamics and activity (Bardgett & Chan, 1999; Andrén et al., 1990). While many microarthropod groups are microbivorous,

particularly fungivorous, to some degree as well, their effect on microbial turnover and subsequent N release is less than that of nematodes (Osler & Sommerkorn, 2007; Verhoef & Brussaard, 1990). Instead, microarthropods are generally expected to have greater influence on N availability via their involvement in SOM decomposition, though they exert a range of indirect effects on microbes as well that may influence N mineralization (Carrillo et al., 2011; Lussenhop, 1992). However, the relative strengths of these two faunal groups to stimulate N mineralization, as well as the potential implications of these effects on plant productivity, in the context of bioenergy cropping systems remain poorly known.

To address this, I conducted a greenhouse mesocosm study to quantify the relative effects of two important groups of soil fauna, microarthropods and microbivorous nematodes, on N mineralization and subsequent partitioning across N pools. Microarthropod and nematode communities were collected from switchgrass (*Panicum virgatum*) bioenergy cropping system field plots and introduced into mesocosms, both individually or in combination, containing ^{15}N-enriched plant litter as their primary N source. This allowed me to quantify the partitioning of litter-derived N following its mineralization in the presence or absence of these faunal groups. To also assess the potential for fauna-mediated impacts on N and microbial dynamics to affect plant productivity or residue quality, mesocosms were planted with switchgrass seedlings in order to quantify plant biomass growth and N content. As their typically positive impacts on N mineralization have been well-documented across soil systems, I hypothesized that both microarthropods and nematodes would promote N availability, with greater ^{15}N enrichment in switchgrass grown in mesocosms which received either faunal group as opposed to controls which did not receive fauna. I further hypothesized that this predicted positive effect on fauna on N mineralization would have a beneficial impact on primary productivity, with switchgrass grown in the presence of one or both faunal groups having more biomass than when fauna were absent. Since microarthropods and nematodes primarily affect N mineralization via distinct mechanisms, by affecting SOC decomposition and stimulating microbial turnover, respectively, I also predicted that these fauna would have the strongest positive effect on N mineralization and switchgrass productivity when they occurred together rather than individually.

METHODS

Mesocosm & experimental design

I constructed mesocosms that enabled me to track the stable isotope ^{15}N following its release from an enriched litter source in the presence of microarthropods and/or nematodes to examine the effects of these faunal groups on N dynamics as compared to when only microbes are present to mobilize litter-bound N. Individual switchgrass (var. Cave-in-Rock) seedlings were grown in mesocosms (24 °C, 18:6 light-dark photo period), allowing me to examine if the potential faunal effects on N mineralization would also influence plant productivity. To investigate the effects of faunal groups specifically via their feeding on litter and litter-associated microbes, I used a split-mesocosm design to minimize the potential influence of direct interactions between fauna with switchgrass roots (i.e., grazing) as well as indirect effects unrelated to those to do with N mineralization (i.e., faunal effects on soil structure). Mesocosms were assembled by subdividing polypropylene nursery pots with 3D-printed polylactic dividers to create two distinct interior sections: one which received an individual switchgrass seedling and the other which received ^{15}N-enriched litter, henceforth "Plant-side" and "Litter-side", respectively (**Fig. 4.1A & 4.1B**). Cut ~7 cm diameter circles of hardware cloth (~8 mm openings) were hot glued to one side of the 6.75 cm hole located at the center of the dividers, over which a cut ~7 cm diameter circle of stainless steel 200 mesh screen (74 µm openings) was secured with hot glue as well as to the opposite side (**Fig. 4.1C**). This mesh-covered hole in dividers allowed fine roots and root-associated AMF hyphae to pass through and access soluble ^{15}N from the Litter-side while preventing fauna from passing to the Plant-side. This is because microarthropods are too small to pass through the mesh while nematodes would be impeded by the 1-2 mm air gap maintained between the two mesh circles due to the hardware cloth (~6 mm openings). This design allowed me to keep the bulk root zone and associated rhizosphere physically separated from introduced fauna, though fauna were still able to access fine roots and AMF hyphae which passed through the dividers into the Litter-side of mesocosms. To measure the amount of ^{15}N lost via leaching, ~2.6 cm diameter holes were cut into the bottom of a subset of mesocosms (n = 32), one hole under the Plant-side and one under the Litter-side. Scintillation vial caps which had their tops removed and replaced with a

cut circle of 200 mesh were then secured with sealant underneath the holes, allowing me to screw on and off 20 mL scintillation vials to periodically collect soil leachates.

Additional modifications were made to the exterior of mesocosms to prevent faunal, mainly microarthropod, movement into the Plant-side of mesocosms as well as between mesocosms. Cut ~14.5 cm^2 squares of 200 mesh were hot glued over pot drainage holes to prevent faunal movement out the bottom of mesocosms. To prevent movement from the tops of mesocosms, shields of 1 mm thick clear acrylic that extended 15 cm above the mesocosm substrate were affixed to top of dividers as well as the sides of pots with silicone sealant. Additionally, 5 cm cut strips of plastic film were secured around the top circumference of the mesocosms with tape. Strips of double-sided mounting tape were placed to either side of where the strips of film met the shield as well as around the circumference of pots approximately midway down from the top to trap fauna crawling on the mesocosm exterior. Baited fly traps and yellow sticky traps were employed to reduce flying insect pest densities in the greenhouse, of which fungus gnats (family Sciaridae) were found to be the most frequently encountered greenhouse pest.

Soil used in mesocosms was collected from the Great Lakes Bioenergy Research Center's (GLBRC) Switchgrass Variety Experiment site which is located immediately adjacent to the west of the BCSE site and is of the same soil type (**Table 4.1**). Approximately 18,927 cm^3 soil was collected from ~28 cm diameter and ~25 cm deep holes dug in the fertilized portion of each of 4 replicate switchgrass (var. Cave-in-Rock) field plots (for more details see ref). Soil was kept refrigerated at ~6 °C before undergoing an initial sieving (sieves with 6.3-8 mm openings used). Once sieved, soils underwent three freeze-thaw defaunation cycles (-10 °C for 24 h, room temperature for 24 h). From here, the defaunated soil was re-sieved (2 mm openings), homogenized, and mixed with triple-washed sand in a 1:1 ratio by volume. This soil-sand substrate was then used to fill both sides of mesocosms to a depth of ~11 cm.

Switchgrass plants (var. Cave-in-Rock) were grown from seeds which were first surface-sterilized with 1% bleach solution and rinsed repeatedly. Following substrate addition, mesocosms were placed in the greenhouse (24 °C, 18:6 light-dark photo period) and switchgrass seedlings were planted in the Plant-side of mesocosms. Seedlings that visually

appeared smaller than average or showed other abnormalities were not used in the experiment.

To track the partitioning of N across different pools via stable isotope tracing, I added a shallow layer of enriched litter to mesocosms. For this, rye grass (*Secale cereale*) was grown from seed in the greenhouse (24 °C, 18:6 light-dark photo period). Rye biomass was isotopically labeled by fertilizing the growing seedlings with ≥98 at% ammonium-^{15}N chloride (Sigma-Aldrich; St. Louis, MO USA). Once oven-dried, the litter was ground into 2 mm pieces. Analyses performed on litter subsamples at the GLBRC's Isotope Analytics Facility at Michigan State University indicated that this enriched litter had an average N content of 1.4% by mass and ^{15}N-enrichment of 23 at%.

In total, 82 mesocosms were deployed in the greenhouse. Mesocosms were randomly separated into four types: ^{15}N-Divided (n = 40), ^{15}N-Undivided (n = 12), ^{15}N-Baseline (n = 15), and Survival (n = 15). ^{15}N-Divided and ^{15}N-Undivided mesocosms received 21 g enriched litter, while ^{15}N-Baseline mesocosms did not receive enriched litter and excluding one which also had a divider. ^{15}N-Divided mesocosms were used to address the primary objective of the study and were evaluated to determine the effects of faunal treatment on N dynamics and switchgrass productivity. While also capturing faunal effects, ^{15}N-Undivided mesocosms were primarily used to evaluate potential effects imposed by mesocosm dividers such as those which could modify the direction or strength of faunal effects observed in ^{15}N-Divided mesocosms. ^{15}N-Baseline mesocosms were used to produce baseline isotopic values for mesocosm contents in the absence of a labeled substrate, as well as evaluate the effectiveness of mesocosm modifications at preventing unintentionally introduced arthropods from entering mesocosms. Survival mesocosms were used to assess faunal abundance and composition at the end of the experiment. From here, mesocosms were randomly assigned one of the following fauna treatments: Microarthropods Only, Nematodes Only, Microarthropods & Nematodes, and No Fauna. The numbers of replicates for each type and treatment cross are given in **Table 4.2**. Watering occurred 3 d per week throughout the experiment with 50 mL added to each side of mesocosms for the first month before increasing the volume to 100 mL per side. In addition to routine watering, weeds were removed from mesocosms when necessary, leachate vials were

periodically removed and replaced, and pest insect traps were replaced as needed.

Microarthropod and nematode collection

Microarthropods and nematodes were collected from switchgrass field plots at the BCSE site (for more details see ref). This field site is located immediately east of the Switchgrass Variety Experiment site and is of the same soil type. Switchgrass field plots in both sites have experiences similar management (**Table 4.1**) such that the faunal communities between sites can be expected to experience similar abiotic and biotic conditions. Field-collected microarthropod and nematode assemblages were used in faunal treatments to capture the net effects of multi-species assemblages in the context of bioenergy cropping systems. Switchgrass-associated communities were collected due to these communities having been surveyed previously (Chapter 2) and switchgrass's status as a model bioenergy crop in the US Midwest (Wright & Turhollow, 2010).

Microarthropods were collected from soil and litter samples taken from each of the 5 replicate switchgrass field plots on December 2, 2021. A cone-shaped soil corer was used to collect 3 cm diameter soil cores to a depth of 15 cm (Chapter 2: **Fig. 2.1**). To collect sufficient numbers of soil-dwelling microarthropods, I collected 21 soil cores per replicate plot with nine cores taken at each of three designated sampling locations within plots. To account for the spatial heterogeneity of microarthropods (Santos et al, 1978), three soil cores (one per sampling location) were composited. Litter was sampled at each sampling location (n = 3 per replicate plot) by collecting the surface litter falling within 625 cm^2 square PVC quadrats. Microarthropods were extracted from soil and litter samples via Tullgren funnel extraction (Chapter 2: **Fig. 2.2**), with soil and litter samples taking 6 and 4 d, respectively, to complete extraction. Excluding a subset of samples that were extracted into 70% propylene glycol to quantify microarthropod abundance and community composition at the time of sampling, soil- and litter-dwelling microarthropods were extracted alive into cups filled with ~50 mL water which were replaced every 18-24 h. Collection cup contents were then visually inspected with any non-acarine, non-collembolan fauna removed using tweezers. Other microarthropods (i.e., diplurans, proturans, etc.) were removed due to having very low abundance. Following this, the contents of the cups were placed in the Litter-side of mesocosms receiving microarthropods

singly or with nematodes. Whenever microarthropods were added to these mesocosms, I added 50 mL water to the Litter-side of all mesocosms not receiving microarthropods such that all mesocosms received water at the same time. Due to lower-than-expected collection densities, mesocosms were supplemented with microarthropods collected from litter on December 16, 2021. As with microarthropods, nematodes were collected from soil samples taken from all replicate switchgrass field plots. Nematode extraction was performed using Baermann funnels. Bacterial- and fungal-feeding nematodes were subsequently isolated by placing extracted nematodes on nematode growth media with bacteria and fungi inoculums.

^{15}N-tracer study for ^{15}N-Divided, ^{15}N-Undivided, and ^{15}N-Baseline mesocosms

After 4 mo in the greenhouse, mesocosms were destructively harvested over a period of 7 d. To quantify fungus gnat prevalence at the time of harvest, I placed yellow sticky traps on either side of ^{15}N-Divided mesocosms 1 d before harvesting began. Traps were placed ~10 cm away from mesocosms at the height of the pot opening and were collected at the time of harvesting. Switchgrass height was recorded for all mesocosms prior to harvest. Switchgrass above- and belowground tissues were harvested and oven dried for 48-72 h at 65 °C to assess shoot and root biomass, respectively. Dividers were largely effective at keeping larger roots separated from the Litter-side of mesocosms, though ~20% of mesocosms were noted as having some larger roots present on the Litter-side, predominately from pushing through the silicone sealant securing dividers at the bottom of pots. Enriched litter was carefully removed from the soil surface, though advanced decomposition of the lower litter layer made complete separation from soil impossible. A 0.5 g litter subsample was collected and oven dried as with shoots and roots. Soils were removed from both sides of pots and gently mixed with a metal spatula to create a homogenized soil representative of the entire mesocosm. Two 10 g soil subsamples were weighed, oven dried, and re-weighed to calculate gravimetric soil moisture. For mesocosms that received microarthropods alone or with nematodes, the remaining litter layer as well as 800 mL soil subsamples were placed into Tullgren funnels to recover microarthropods. Additional soil subsamples were taken from mesocosms not receiving microarthropods to check for non-introduced microarthropod activity. For further details on the different substrates harvested and the analyses performed for each mesocosm type and

faunal treatment cross, see **Fig. 4.2**.

To test the degree to which microarthropods and nematodes, both alone and in combination, influence litter-bound ^{15}N mineralization and its subsequent fate in mesocosms, I quantified ^{15}N-enrichment from N pools: enriched litter, switchgrass shoots and roots, soil, nematode biomass, and microarthropod biomass. Litter, shoot, root, and soil samples were prepared for analysis by placing oven dried 0.5-2 g aliquots into 20 mL scintillation vials along with metal grinders. Sample vials were then placed on a vial roller until the contents had been sufficiently pulverized into a fine powder. The pulverized materials were then weighed into 5x9 mm tin capsules (Costech Analytical Technologies; Valencia, CA USA) which were then shipped to the University of California Davis's Stable Isotope Facility for analysis. Two-pool mixing models were used to calculate the N content and ^{15}N enrichment of these N pools in which the two possible sources of N were the added ^{15}N-labeled litter (source$_1$) and field-collected soil (source$_2$). The differences in enrichment between samples from ^{15}N-Divided and ^{15}N-Undivided mesocosms with those from ^{15}N-Baseline mesocosms was used to calculate ^{15}N atom percent (at‰) excess:

Equation 1: $$^{15}N\ at‰\ \text{excess}_{sample} = {}^{15}\text{N at‰}_{sample} - \text{AVERAGE}({}^{15}\text{N at‰}_{CTRL})$$

^{15}N at‰$_{CTRL}$ is the enrichment of samples from ^{15}N-Baseline mesocosms, which should give the natural abundance of ^{15}N for that sample type in the absence of labeled litter. The at‰ excess is then used to calculate the proportion of sample enrichment contributed by ^{15}N-labeled litter (f_1):

Equation 2: $$f_1 = \frac{^{15}N\ at‰\ \text{excess}_{sample} - \delta source_2}{\delta source_1 - \delta source_2}$$

In this equation, δsource$_1$ and δsource$_2$ are the averaged ^{15}N at‰ values of enriched litter and ^{15}N-Baseline soil samples, respectively. Calculating the amount of sample masses that is N from the stable isotope facility results, this N content value was multiplied by f_1 to yield the amount of litter-derived N in samples scaled to the whole pool size (e.g., total shoot biomass):

Equation 3: $$sample\ N_{litter} = \left(\frac{N\%\ of\ sample\ mass}{100}\right) * total\ mass\ of\ sample$$

Microarthropod biomass samples for ^{15}N-enrichment analysis were obtained using Tullgren funnels to extract microarthropods from remaining litter and up to 800 mL soil subsamples

taken from mesocosms. Extracted microarthropods were collected in 70% propylene glycol and transferred to vials of 95% ethanol prior to sorting. Microarthropods were sorted into major groups with collembola and oribatid mites further separated into morphospecies, rinsed with 95% ethanol, oven dried, manually pulverized, and placed into tin capsules. Due to the extremely low biomasses of the majority of microarthropod samples after oven drying, tin capsules were spiked with 0.3 mg ultrapure salmon sperm DNA prior to sample encapsulation to ensure that the tins would contain enough C and N to provide reliable enrichment measurements. These samples were subsequently analyzed by the Michigan State University's Isotope Analytics Facility.

Microarthropod survival and composition

Survival mesocosms were destructively harvested 2-3 wk after harvesting the other mesocosms. I separated litter from the soil surface as before with all litter then being placed into Tullgren funnels for microarthropod extraction. Next, 800 mL soil subsamples were taken from both the Plant-side and Litter-side of mesocosms, which allowed me to check the effectiveness of the divider at excluding microarthropods in addition to assessing microarthropod colonization in mesocosms after ~4 mo. While I did not take litter or Litter-side soil samples from Survival x Nematodes Only mesocosms, I did take 800 mL soil from the Plant-side of these mesocosms as an additional check for unintentional microarthropod colonization. As with litter, these 800 mL soil subsamples were placed in Tullgren funnels. Extracted microarthropods were collected, sorted, and counted as with those recovered for ^{15}N-enrichment analysis. Count and community composition data from these mesocosms were then used to compare starting and final microarthropod communities as well as evaluate if quantifying the surviving community from only a subset of mesocosms is sufficient to capture the patterns in variability between replicates.

Statistical analysis

To assess changes in microarthropod community treatments over the duration of the experiment, microarthropod abundance and community composition were compared between microarthropods collected from the BCSE switchgrass field plots at the start of the experiment and those extracted from ^{15}N-Divided and Survival mesocosms at the time of harvest.

Differences in microarthropod communities in the presence and absence of nematodes were assessed between Microarthropods Only and Microarthropods & Nematodes mesocosms. Additionally, Survival mesocosm count and composition data was compared to that from ^{15}N-Divided mesocosms to determine if collecting microarthropod community composition for a subset of mesocosms can adequately capture within-treatment variation in microarthropod communities across replicates. As microarthropod abundance data was non-normally distributed, differences in means between treatments and sample types were assessed using the nonparametric Kruskal-Wallis test. Pairwise comparisons were evaluated using Dunn's *post hoc* test (R package: *FSA*). Differences in microarthropod community composition were visualized using NMDS and assessed using PERMANOVA.

Differences in N dynamics and switchgrass productivity in the presence or absence of microarthropods and nematodes were examined for ^{15}N-Divided mesocosms (n = 40). Due to heteroscedasticity in the data, Welch's ANOVA was used via the function *anovaOneW()* (R package: *jmv*) as this test does not require that the assumption of equal variances be met (Selker et al., 2022). Welch's ANOVA was used to assess treatment-level differences for the following variables: switchgrass height, total biomass N content and ^{15}N%, shoot biomass N content, and root biomass ^{15}N%. The Shapiro-Wilk normality test was used to verify that the assumption of normality in the data was met; in cases where this assumption wasn't met, means comparisons were instead assessed using the Kruskal-Wallis test. The Kruskal-Wallis test was used on the following non-normally distributed variables: switchgrass total biomass ^{15}N content and N%; shoot biomass, ^{15}N content, N%, and ^{15}N%; switchgrass root biomass, N content, N%, and ^{15}N content; gravimetric soil moisture; soil N% and ^{15}N%; and litter N%. Pairwise comparisons were performed *post-hoc* using Tukey's test in the case of Welch's ANOVA or Dunn's testing the case of the Kruskal-Wallis test.

RESULTS

Microarthropod community composition

Microarthropods Only and Microarthropods & Nematodes treatment mesocosms received an average of 1127 total microarthropods (**Table 4.3**). In agreement with previously conducted sampling in the BCSE switchgrass cropping system (Chapter 2), the baseline

community was mite dominated: astigmatid, oribatid, prostigmatid, and mesostigmatid mites made up 51.2%, 20.7%, 17.6%, and 4.9% of overall abundance as compared to collembola which comprised only 5.6%. Morphospecies richness was greater for collembola than oribatid mites. However, most of the 16 collembola morphospecies were collected at low abundance, with three collembola morphospecies comprising 77.7% of collembola abundance (**Table 4.4**).

At the time of harvest, the structure of the microarthropod communities which had colonized ^{15}N-Divided mesocosms had diverged from that of the baseline community (PERMANOVA: $F_{[2,24]} = 2.860$, $p = 0.003$). There was substantial overlap in microarthropod communities recovered from mesocosms, while both communities differed strongly in composition compared to the baseline community (**Fig. 4.3**). Mesocosms comprised a subset of the total number of morphospecies found in the field with a smaller subset of morphospecies being numerically dominant (**Table 4.4**). The baseline community had the greatest number of collembola and oribatid morphospecies, with Microarthropods Only and Microarthropods & Nematodes mesocosm having similarly low morphospecies richness (**Table 4.3**). In addition to having reduced morphospecies richness, mesocosm-recovered microarthropod communities exhibited lower oribatid ($\chi^2 = 10.442$, $p = 0.005$) and higher prostigmatid mite abundances ($\chi^2 = 8.142$, $p = 0.017$) on average compared to the baseline community, though total abundance was similar across all three communities ($\chi^2 = 1.241$, $p = 0.538$). While average total microarthropod abundance was similar, mesocosm communities were much more variable: baseline samples ranged from 752 to 1381 total microarthropods whereas microarthropod recovery from Microarthropods Only and Microarthropods & Nematodes ranged from 130 to 4174 and 298 to 7347 microarthropods, respectively. Despite the relatively high within-treatment variability in mesocosm-recovered microarthropod communities, there were no significant differences in microarthropod abundance or community structure between ^{15}N-Divided and Survival mesocosms, suggesting that microarthropod communities extracted from switchgrass field plots followed a generally similar trajectory following mesocosm establishment.

From Tullgren funnel extractions performed on soil taken from the Plant-side of Survival mesocosms which had received microarthropods alone or in combination with nematodes, I

confirmed that dividers were relatively efficient at keeping microarthropods contained to the Litter-side of mesocosms. Soil collected from the Plant-side of these mesocosms contained an average of 46 total microarthropods., Microarthropod abundance in the Plant-side made up an average of 15% and 13% of Litter-side abundance for Survival mesocosms which received microarthropods only and both microarthropods and nematodes, respectively. From mesocosms which received no fauna, 69 microarthropods in total were found to be present. Astigmatid mites made up and average of 60-64% of the total number of unintentionally introduced microarthropods found in both the Plant-side of mesocosms which received microarthropods and in mesocosms where fauna were not introduced.

Faunal effects on switchgrass productivity and N dynamics

At the time of harvest, the majority of switchgrass plants were in the vegetative stage with the remaining plants in the flowering stage. While switchgrass grown in Nematodes Only mesocosms tended to be taller and those with Microarthropods Only slightly shorter, these differences were not statistically significant ($F_{[3,19]} = 1.948$, $p = 0.156$, **Fig. 4.4**). Switchgrass biomass was also similar across faunal treatments ($F_{[3,19]} = 2.839$, $p = 0.066$, **Fig. 4.5**). After approximately 4 mo in the greenhouse, average switchgrass biomass ranged from 6475 to 4962 mg in Nematodes Only and No Fauna mesocosms, respectively. Across treatments, most of the total biomass came from roots, with root biomass tending to be approximately twice that of shoots on average as well as exhibiting greater variability. As for total biomass, neither switchgrass root nor shoot biomass were significantly affected by faunal treatment (roots: $\chi^2 = 4.818$, $p = 0.186$; shoots: $\chi^2 = 3.897$, $p = 0.273$).

Total N content was quantified to evaluate the relative sizes of the major N pools in mesocosms in the presence or absence of microarthropods and/or nematodes. In addition, the level of ^{15}N enrichment was used to assess the amount of N originating from ^{15}N-labeled litter, henceforth referred to as the litter-derived N content. The proportion of soil mass coming from N was consistent across treatments for both soil and litter (soil: $\chi^2 = 0.183$, $p = 0.980$; litter: $\chi^2 = 5.522$, $p = 0.137$). Approximately 0.03% of mesocosm soil was N by mass (**Fig. 4.6A**), which was similar across treatments as well as between mesocosm types. The litter-derived N content of soil was even lower, averaging less than a tenth of a percent of soil by mass, and was also

similar across treatments (χ^2 = 1.440, p = 0.696). Litter had a higher total N content, averaging about 1% by mass (**Fig. 4.6B**). While there was no significant evidence of a treatment-level effect, there was substantial variability in total litter N content within the Nematodes Only treatment.

The percent N of switchgrass biomass was intermediate relative to that of soil and litter and generally ranged between 0.5-1% of the total biomass (**Fig. 4.7A & 4.7C**). The proportion to which N contributed to biomass was generally similar between roots and shoots, though roots contained more N overall due to their greater biomass. There was no evidence that fauna affected total N content for switchgrass, neither by total amount of N (total: $F_{[3,17]}$ = 0.541, p = 0.661; root: χ^2 =3.143, p = 0.370; shoot: $F_{[3,17]}$ = 0.701, p = 0.564) nor its proportional contribution to mass (total: χ^2 = 5.025, p = 0.170; root: χ^2 = 5.807, p = 0.121; shoot: χ^2 = 2.916, p = 0.405).

Litter-derived N generally composed 0.1% or less of the mass overall, which was similar between roots and shoots. In contrast to total N content, fauna significantly influenced root biomass enrichment as indicated by the proportion of litter-derived N contributing to root biomass across treatments ($F_{[3,18]}$ = 4.438, p = 0.017). Despite total N content of roots being similar between treatments, the amount of N specifically originating from litter was highest for switchgrass plants grown in the presence of both microarthropods and nematodes (χ^2 = 10.036, p = 0.018, **Fig. 4.7B & 4.7D**). Compared to when both faunal groups were present, the litter-derived N content of roots was intermediate in mesocosms containing only nematodes, with the lowest litter-derived N content in the roots of switchgrass plants grown with only microarthropods or no added fauna. In contrast, both the proportion (χ^2 = 2.346, p = 0.504) and mass (χ^2 = 2.294, p = 0.514) of litter-derived N in shoots were unaffected by fauna. While there was a trend for total switchgrass biomass from Microarthropods & Nematodes mesocosms to be more composed of litter-derived N, it was only marginally significant ($F_{[3,16]}$ = 3.094, p = 0.056).

While the difference in replication restricted my ability to directly compare results across mesocosm types, several key distinctions were examined between mesocosms with and without dividers (^{15}N-Divided and ^{15}N-Undivided mesocosms, respectively). Switchgrass height,

root biomass, and shoot biomass all tended to be higher on average in ^{15}N-Undivided mesocosms (**Fig. 4.8**). Across all of these metrics, the greatest difference between mesocosms with and without dividers was found for the Nematodes Only treatment. No Fauna controls were intermediate, while ^{15}N-Undivided mesocosms which received microarthropods either alone or in combination with nematodes had values closer to those from ^{15}N-Divided mesocosms. Further, both total and litter-derived N content similarly tended to be greater on average in mesocosms without dividers, regardless of faunal treatment, for both switchgrass roots (**Fig. 4.9A & 4.9B**) and shoots (**Fig. 4.9C & 4.9D**). Soil total N content tended to be similar across mesocosm type x treatment crosses (**Fig. 4.10A**), and litter-derived N was as high or slightly higher when dividers were absent (**Fig. 4.10B**). A different trend was observed for the N content of remaining litter, with litter from ^{15}N-Divided mesocosms found to have greater N than ^{15}N-Undivided mesocosms with the same faunal treatment (**Fig. 4.10C**).

DISCUSSION

While there have been major advances in our ability to model nutrient cycling in soils as well as greater attempts to account for the factors regulating the plant and microbial processes driving the majority of soil N and C transformations, the contribution of soil fauna remain largely absent from these predictions. This is in spite of the fact that as a whole, soil fauna are assumed to contribute an estimated ~30% of the total N mineralization in soils across ecosystems (Verhoef & Brussaard, 1990), therefore having significant potential to increase N availability with potentially strong implications for plant productivity as well as SOC accrual and storage potential. Nematodes and microarthropods enhance N mineralization rates primarily via their interactions with microbes, with microbivorous nematodes stimulating microbial turnover whereas microarthropods influence microbes by enhancing SOM decomposition rates in addition to feeding on microbes directly (Chapter 1). These fauna-microbe-SOM interactions, while ubiquitous across most soils, are likely to have the greatest impact in N-limited soils like those targeted for bioenergy crop production. As these systems also face the additional challenges of needing to maximize crop yield, often with reduced or no inorganic fertilization, as well as promote SOC accrual and stabilization, quantifying the relative impact of these fauna will be necessary to predict long-term nutrient dynamics in bioenergy cropping systems. In this

study, I sought to address this in the context of a switchgrass bioenergy cropping system, as switchgrass has been selected as the US Department of Energy's model bioenergy crop (Wright & Turhollow, 2010). Field-collected microarthropod and microbivorous nematode communities introduced to mesocosms to evaluate the effects of these fauna, both separately and in combination, on N mineralization and subsequent uptake by switchgrass. Adding ^{15}N-enriched litter to be the primary source of N available on the litter-side of mesocosms by physically separating this section of mesocosms from the switchgrass rhizosphere, I was able to track the transformation and portioning of N from litter biomass into switchgrass and soil. Contrary to my initial hypothesis, I did not find evidence that the presence of microarthropods and/or nematodes promoted increased switchgrass productivity. Similarly, neither the presence nor identity of introduced faunal group were found to affect the total N content of switchgrass, nor the N contents of soil and remaining litter. However, faunal treatment did significantly impact the amount of litter-derived N assimilated into root biomass. In particular, switchgrass plants grown in mesocosms containing both microarthropods and nematodes produced roots containing significantly more litter-derived N in their biomass. Even as work to produce full N budgets for mesocosms is ongoing, these findings suggest that microarthropods and nematodes together enhanced the uptake and assimilation of N mineralized from litter even though the overall amount of N in root biomass was unchanged.

Accounting for both the total N content and litter-derived N content across N pools allowed me to assess differences in the relative sizes of these pools as well as the proportion of total N that originated from litter. This allowed me to broadly assess N transformations between pools in the presence or absence of both faunal groups. As microbes in the soil or fauna decomposed the litter in mesocosms, the organic N immobilized in litter biomass could be assimilated into microbial and/or faunal biomass or excreted as inorganic N. Litter N content is temporally dynamic throughout the decomposition, with N content initially decreasing as more labile, N-rich compounds are preferentially utilized by microbes followed by a subsequent increase in N content as the remaining litter becomes increasingly composed of more recalcitrant compounds (Ågren et al., 2001; Teuben, 1991). After ~4 mo in the greenhouse, litter N content was similar across faunal treatments, albeit qualitatively lower and more

variable across replicates for Nematodes Only mesocosms. This suggests that litter decomposition was following a similar trajectory across faunal treatments, though it remains to be seen if faunal treatment affected overall decomposition rate. If not assimilated by microbes and/or fauna and recycled through the soil food web, mineralized litter-derived N present in the soil was available for plant uptake if it was not volatilized or lost via leaching. Across treatments, N presence in soil was considerably low, with total N content typically less than 0.05% with an even smaller proportion of that derived from litter. As the N content of soil also included that present in microbial biomass as well, which can make up as much as 2-6% N present in soil (Brookes et al., 1985), the total amount of N present in soil alone can be assumed to be even lower. This indicates that much of the inorganic N released does not remain in soils but was taken in by switchgrass or lost via leaching, volatilization, or denitrification.

The greatest uptake of litter-derived N occurred in mesocosms containing nematodes, particularly in those with both nematodes and microarthropods together. This finding was not surprising, as microbivory by nematodes and other microfauna have long been known to have considerable positive effects on N mineralization. The contributions of microbivorous nematodes to total N mineralization were estimated to be between 0.3-2.7% in food crop production systems (Andrén et al., 1990; Didden et al., 1994), though Hunt et al. (1987) estimated their contribution to be as high as 17% in a shortgrass prairie system. While nematodes, especially bacterivores, more strongly stimulate microbial turnover by direct predation, microarthropods have been found to enhance N mineralization via their interactions with microbes. Microarthropods have been found to influence N mineralization through a variety of mechanisms, including direct microbivory, especially fungivory (Beare et al., 1992) as well as affecting microbial activity via their effects on SOM decomposition (Blair et al., 1988), and fecal pellet production (Verhoef & Brussaard, 1990). In a microcosm study with the grass *Nardus stricta*, collembola and nematodes in combination were similarly found to have the greatest impact on N mineralization rather than when either group was individually present, with this elevated N mineralization corresponding to higher microbial biomass (Bardgett & Chan, 1999). In this study, collembola were found to be largely responsible for the observed

faunal effects on N mineralization and plant N uptake. In contrast, significant effects of faunal treatment on N in my mesocosm study were driven by nematodes, with both Microarthropods Only mesocosms having the lowest litter-derived N content in roots along with No Fauna controls. In another ^{15}N-labeling experiment, Schon et al. (2011) found the effects of nematodes and mesofauna, largely microarthropods, in combination on N mineralization and subsequent plant uptake were strongly mediated by soil bulk density and fertility, indicating the context-dependency of faunal interactions and their effects on N dynamics. In the current experiment, initial soil bulk density and N content was kept consistent for all mesocosm and gravimetric soil moisture at the time of harvest was similar across faunal treatments. Still, these studies highlight the challenge of generalizing results across diverse soil contexts, particularly when the exact mechanisms underlying faunal effects on N and plant productivity and their relative strengths remain uncertain.

While nematodes, alone and in combination with microarthropods, increased the assimilation of litter-derived N in switchgrass roots, this did not correspond to increased switchgrass productivity in treatments where nematodes were present. Though there was a slight trend of increased switchgrass height and biomass, particularly from the roots, in the presence of nematodes, I did not find strong evidence that either faunal group promoted switchgrass productivity relative to controls with no fauna introductions. While No Fauna mesocosms were not completely impervious to microarthropods and/or nematodes, numbers of these non-intentionally introduced were comparatively low and therefore were not anticipated to have had a meaningful effect on my results. The vast majority of these were astigmatid mite hypopi, a heteromorphic juvenile instar (deutonymph) of astigmatid mites which is adapted for dispersal via phoresy (Walter & Proctor, 2013). As stated earlier, fungus gnats were commonly present within the greenhouse throughout the duration of the experiment. Other unintentionally introduced arthropods, largely rove beetles (family Staphylinidae), were occasionally extracted in low numbers from mesocosms along with microarthropods. While it cannot be confirmed in this study, associations between hypopi and similar insect taxa suggest that it is possible that a substantial proportion of the unintentionally introduced microarthropods recovered were introduced by greenhouse pest arthropods as

opposed to microarthropods which had migrated through the divider. Similarly, fungus gnat pressure, while doubtlessly contributing to N mineralization to some degree, was consistent across treatments and thus should not have impacted my ability to interpret treatment-level differences in N dynamics. Instead, it can be surmised that the lack of faunal effects on switchgrass productivity in spite of elevated litter-derived N uptake in the presence of nematodes may be due to the ability of switchgrass to grow in N limited conditions. As previously mentioned, total N content of switchgrass was similar across treatments, indicating that switchgrass plants were able to obtain their N requirements for growth regardless of faunal effects on litter-N mineralization. This is supported by the fact that switchgrass plants grown in ^{15}N-Baseline mesocosms, which received no litter and therefore only could subsist off of N already present in the soil or fixed by specialized bacteria, had similar switchgrass height, biomass, and total N contents compared to switchgrass grown in ^{15}N-Divided mesocosms. Indeed, one of the major advantages of switchgrass as a model bioenergy crop is its ability to grow in low-N soils (Vogel et al., 2002). Therefore, switchgrass may be less reliant on faunal contributions to N mineralization, at least in the short-term.

Though not the main focus of the current study, I observed interesting effects of mesocosm design, particularly the presence of mesocosm dividers. In particular, all metrics of switchgrass productivity (i.e., height, root biomass, shoot biomass) were found to be greater for plants grown in ^{15}N-Undivided mesocosms compared to those from ^{15}N-Divided mesocosms. While this trend was broadly consistent across all faunal treatments, the greatest difference in switchgrass productivity between mesocosms with and without dividers was observed when only nematodes were present. Additionally, both total and litter-derived N content for switchgrass roots and shoots were also greater in ^{15}N-Undivided mesocosms, whereas the N content of remaining litter was greatest in ^{15}N-Divided mesocosms. Taken together, these findings, while qualitative, seem to indicate that mesocosm dividers were having a significant impact on switchgrass productivity as well as litter-derived N mineralization and subsequent uptake. Particularly, results from ^{15}N-Undivided mesocosms exhibited the same overall pattern as those with dividers but greater, and these positive effects may be amplified in the presence of nematodes only relative to other faunal treatments.

There are multiple likely explanations for these observations. As previously mentioned, mesocosm dividers were implemented in part to mitigate the potentially confounding effects of faunal activity in the rhizosphere, especially that of nematodes. Soil food webs are often conceptualized as two distinct yet overlapping energy channels, a fast cycling bacterial-based channel and slow cycling fungal-based channel, which have broadly generalizable differences in the functional and trophic groups composing them, scales in which they operate, and direction of their responses to C and N availability (Hedlund et al., 2004; Hunt et al., 1987; Moore et al., 2003). Plant rhizospheres predominately support bacterial food webs and are therefore associated with more rapid nutrient cycling and biotic turnover, whereas the food webs in bulk soils are largely fueled by more recalcitrant SOM inputs (i.e., litter) and hence are increasingly dominated by fungi and fungivores. Even though fine roots and symbiotic mycorrhizal fungi were able to pass through the mesh in dividers, switchgrass in ^{15}N-Undivided mesocosms were able to grow throughout the entire volume of soil unimpeded, giving their roots expanded room to grow and greater opportunities to seek out N from the heterogenous soil matrix. Additionally, fauna had unrestricted access to both the litter and switchgrass rhizosphere in the absence of dividers, expanding the potential trophic interactions occurring within mesocosms as fauna could serve as consumers in both litter-based fungal and root exudate-based bacterial energy channels. The availability of labile, C-rich compounds (i.e., root exudates) is generally high and temporally consistent in the rhizosphere, resulting in increased microbial activity and bacterial dominance relative to bulk soil (Jones et al., 2009). In natural soils, this has been linked to greater biomass and activity of microbivorous nematodes, especially bacterivores, in rhizospheres (Moore et al., 2003). With access to the rhizosphere in ^{15}N-Undivided mesocosms, it is likely that nematodes preferentially fed upon microbes in rhizosphere, stimulating more rapid microbial turnover and subsequent N mineralization than when they were largely restricted to feeding upon the saprotrophic microbes associated with more recalcitrant SOM. It is possible that similarly high productivity was not seen in ^{15}N-Undivided mesocosms were not when microarthropods and nematodes occurred together because microarthropods were regulating nematode activity, either by predation, influencing the microbial community, or some other mechanism. While still regarded as predominately fungivorous, recent insights

indicate that microarthropod feeding ecology is highly nuanced and may include a greater degree of nematode predation in certain groups (i.e., oribatid mites) than was traditionally believed (Potapov et al., 2022). Indeed, oribatid mites in the family Galumnidae, which made up 10.6-31.3% of the oribatid mites recovered from mesocosms, have been found to incorporate nematodes into their diet (Schneider et al., 2004). Further investigation will be necessary to better understand the mechanisms underpinning plant-fauna-microbe interactions in both the rhizosphere and detritosphere, including how and when these interactions regulate soil N availability and N mineralization rates, in the context of bioenergy cropping systems.

In conclusion, I found partial support for my initial hypothesis that microarthropods and nematodes would positively influence N availability by promoting the mineralization of litter-immobilized N. This positive effect was seen in mesocosms which received nematodes, with litter-derived N assimilation into root biomass greatest when nematodes and microarthropods co-occurred, intermediate with nematodes only, and similarly low when nematodes were absent (Microarthropods Only and No Fauna treatments). However, this increased availability of litter-derived N in the presence of nematodes with or without microarthropods had no effect on the total N content, biomass, or height of switchgrass. This lack of faunal treatment effect on switchgrass productivity, coupled with its known ability to grow in low-N conditions, indicates that, at least in the short term, switchgrass is relatively insensitive to faunal-mediated changes in N mineralization. That said, qualitative observations of higher overall N mineralization and switchgrass productivity when fauna were not spatially separated from the rhizosphere may suggest that fauna may have significant impacts on switchgrass productivity when both rhizosphere and litter-based food web interactions are considered in tandem, though further investigation on this is needed. This study joins the growing body of evidence to underscore the important roles of soil fauna, particularly as regulators of plant and microbial activity, in terrestrial nutrient cycles. Furthermore, it highlights the need for further research into the key trophic interactions impacting N and C dynamics, including their underlying mechanisms, taxonomic or functional groups involved, the spatial and temporal scales in which they operate, and the contexts in their contributions are predicted to have the most potential impact on critical ecosystem processes such as SOC accrual and storage.

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REFERENCES

Ågren, G. I., Bosatta, E., & Magill, A. H. (2001). Combining theory and experiment to understand effects of inorganic nitrogen on litter decomposition. *Oecologia*, *128*, 94-98.

Andrén, O., Lindberg, T., Boström, U., Clarholm, M., Hansson, A. C., Johansson, G., Lagerlöf, J., Paustian, K., Persson, J., Pettersson, T., Schnürer, J., Sohlenius, B., & Wivstad, M. (1990). Organic carbon and nitrogen flows. *Ecological Bulletins*, *40*, 85-126.

Bardgett, R. D., & Chan, K. F. (1999). Experimental evidence that soil fauna enhance nutrient mineralization and plant nutrient uptake in montane grassland ecosystems. *Soil Biology & Biochemistry*, *31*(7), 1007-1014.

Beare, M. H., Parmelee, R. W., Hendrix, P. F., Cheng, W., Coleman, D. C., & Crossley Jr, D. A. (1992). Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. *Ecological Monographs*, *62*(4), 569-591.

Brookes, P. C., Landman, A., Pruden, G., & Jenkinson, D. S. (1985). Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology & Biochemistry*, *17*(6), 837-842.

Didden, W. A. M., Marinissen, J. C. Y., Vreeken-Buijs, M. J., Burgers, S. L. G. E., de Fluiter, R., Geurs, M., & Brussaard, L. (1994). Soil meso-and macrofauna in two agricultural systems: Factors affecting population dynamics and evaluation of their role in carbon and nitrogen dynamics. *Agriculture, Ecosystems & Environment*, *51*(1-2), 171-186.

Filser, J., Faber, J. H., Tiunov, A. V., Brussaard, L., Frouz, J., de Deyn, G., Uvarov, A. V., Berg, M. P., Lavelle, P., Loreau, M., Wall, D. H., Querner, P., Eijsackers, H., & Jiménez, J. J. (2016). Soil fauna: key to new carbon models. *Soil*, *2*(4), 565-582.

Grandy, A. S., Wieder, W. R., Wickings, K., & Kyker-Snowman, E. (2016). Beyond microbes: Are fauna the next frontier in soil biogeochemical models?. *Soil Biology & Biochemistry*, *102*, 40-44.

Hedlund, K., Griffiths, B., Christensen, S., Scheu, S., Setälä, H., Tscharntke, T., & Verhoef, H. (2004). Trophic interactions in changing landscapes: Responses of soil food webs. *Basic and Applied Ecology*, *5*(6), 495-503.

Hunt, H. W., Coleman, D. C., Ingham, E. R., Ingham, R. E., Elliott, E. T., Moore, J. C., Rose, S. L., Reid, C. P. P., & Morley, C. R. (1987). The detrital food web in a shortgrass prairie. *Biology and Fertility of Soils*, *3*, 57-68.

Jones, D. L., Nguyen, C., & Finlay, R. D. (2009) Carbon flow in the rhizosphere: Carbon trading at the soil-root interface. *Plant and Soil*, *321*, 5-33.

Lu, C., & Tian, H. (2017). Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: Shifted hot spots and nutrient imbalance. *Earth System Science Data*, *9*(1), 181-192.

Lussenhop, J. (1992). Mechanisms of microarthropod-microbial interactions in soil. In M. Begon

& A. H. Fitter (Eds.). *Advances in ecological research* (Vol. 23, pp. 1-33). Academic Press.

Macdonald, C. A., Delgado-Baquerizo, M., Reay, D. S., Hicks, L. C., & Singh, B. K. (2018). Soil nutrients and soil carbon storage: Modulators and mechanisms. In B. K. Singh (Ed.), *Soil carbon storage* (pp. 167-205). Academic Press.

Moore, J. C., McCann, K., Setälä, H., & De Ruiter, P. C. (2003). Top-down is bottom-up: Does predation in the rhizosphere regulate aboveground dynamics?. *Ecology*, *84*(4), 846-857.

Penuelas, J., Janssens, I. A., Ciais, P., Obersteiner, M., & Sardans, J. (2020). Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. *Global Change Biology*, *26*(4), 1962-1985.

Potapov, A. M., Beaulieu, F., Birkhofer, K., Bluhm, S. L., Degtyarev, M. I., Devetter, M., Goncharov, A. A., Klarner, B., Korobushkin, D. I., Liebke, D. F., Maraun, M., McDonnell, R. J., Pollierer, M. M., Schaefer, I., Shrubovych, J., Semenyuk, I. I., Sendra, A., Tuma, J., Tůmová, M., … Scheu, S. (2022). Feeding habits and multifunctional classification of soil-associated consumers from protists to vertebrates. *Biological Reviews*, *97*(3), 1057-1117.

Robertson, G. P., Hamilton, S. K., Barham, B. L., Dale, B. E., Izaurralde, R. C., Jackson, R. D., Landis, D. A., Swinton, S. M., Thelen, K. D., & Tiedje, J. M. (2017). Cellulosic biofuel contributions to a sustainable energy future: Choices and outcomes. *Science*, *356*(6345), eaal2324.

Schneider, K., Migge, S., Norton, R. A., Scheu, S., Langel, R., Reineking, A., & Maraun, M. (2004). Trophic niche differentiation in soil microarthropods (Oribatida, Acari): Evidence from stable isotope ratios ($^{15}N/^{14}N$). *Soil Biology & Biochemistry*, *36*(11), 1769-1774.

Schon, N. L., Mackay, A. D., Hedley, M. J., & Minor, M. A. (2011). Influence of soil faunal communities on nitrogen dynamics in legume-based mesocosms. *Soil Research*, *49*(2), 190-201.

Selker, R., Love, J., Dropmann, D., & Moreno, V. (2022). Jmv: The 'jamovi' analyses. R package version 2.3.4.

Soong, J. L., & Nielsen, U. N. (2016). The role of microarthropods in emerging models of soil organic matter. *Soil Biology & Biochemistry*, *102*, 37-39.

Teuben, A. (1991). Nutrient availability and interactions between soil arthropods and microorganisms during decomposition of coniferous litter: A mesocosm study. *Biology and Fertility of Soils*, *10*, 256-266.

Verhoef, H. A., & Brussaard, L. (1990). Decomposition and nitrogen mineralization in natural and agroecosystems: The contribution of soil animals. *Biogeochemistry*, *11*, 175-211.

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H., & Tilman, D. G. (1997). Human alteration of the global nitrogen cycle:

Sources and consequences. *Ecological Applications*, *7*(3), 737-750.

Vitousek, P. M., & Howarth, R. W. (1991). Nitrogen limitation on land and in the sea: how can it occur?. *Biogeochemistry*, *13*, 87-115.

Vogel, K. P., Brejda, J. J., Walters, D. T., & Buxton, D. R. (2002). Switchgrass biomass production in the Midwest USA: Harvest and nitrogen management. *Agronomy Journal*, *94*(3), 413-420.

Walter, D. E., & Proctor, H. C. (2013). *Mites: Ecology, evolution & behaviour: Life at a microscale* (2nd ed.). Springer.

Wright, L., & Turhollow, A. (2010). Switchgrass selection as a "model" bioenergy crop: A history of the process. *Biomass & Bioenergy*, *34*(6), 851-868.

APPENDIX

CHAPTER 4 FIGURES & TABLES

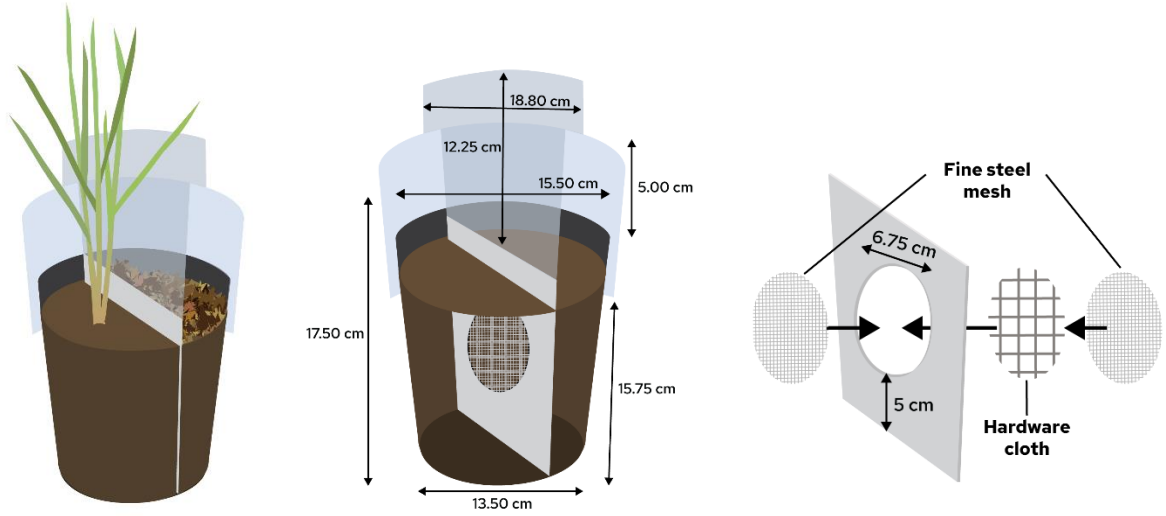

Figure 4.1. Mesocosm diagrams with nursery pots left transparent to view internal components. **A)** Mesocosms were partitioned by a divider into two halves: a plant side to which switchgrass was planted and a litter side containing ^{15}N-enriched rye litter and receiving fauna treatment. A plastic shield and parameter were attached to the top of the divider and around the circumference of the pot, respectively, to mitigate fauna movement. Gaps between the sides of the pot and the shield were filled with silicone sealant. **B)** Mesocosm dimensions, soil left transparent to view the divider. **C)** Mesocosm divider diagram showing how steel mesh and hardware cloth were attached to dividers to create a faunal-excluding barrier that would still allow fine root and fungal hyphae to move between mesocosm halves.

Table 4.1. Agronomic details for the GLBRC's Switchgrass Variety Experiment (SWV) and BCSE switchgrass (Cave-in-rock var.) treatments. Fertilization and pest control details are specific to the 2021 growing season.

Site	Crop details	Fertilization regime	Pest control regime
SVE	Established in 2009, discontinued in 2021	127.8 kg ha^{-1} urea	0.2 L ha^{-1} Quinclorac 75 DF 1.7 L ha^{-1} 2,4-D LV4 Ester 0.4% v/v ha^{-1} crop oil concentrate
BCSE	Established in 2008	40.4 kg ha^{-1} potash 161.8 L ha^{-1} 28% UAN	2.3 L ha^{-1} GlyStar Plus 1.2 L ha^{-1} Atrazine 4L 0.4 L ha^{-1} Quninclorac 75 DF 0.1 L ha^{-1} Scepter 70 DF 0.4% v/v ha^{-1} crop oil concentrate 4.7 L ha^{-1} Crossbow

Table 4.2. Mesocosm faunal treatments as assigned for each mesocosm type.

	^{15}N-Divided	^{15}N-Undivided	^{15}N-Baseline	Survival
Microarthropods only	n = 10	n = 3	n = 2	n = 5
Nematodes only	n = 10*	n = 3	n = 2	n = 5
Microarthropods & Nematodes	n = 10	n = 3	n = 2	n = 5
No Fauna Added	n = 10	n = 3	n = 7	--

* One replicate was affected by a leak in the greenhouse roof which negatively impacted switchgrass growth. This replicate was harvested but excluded from final analyses.

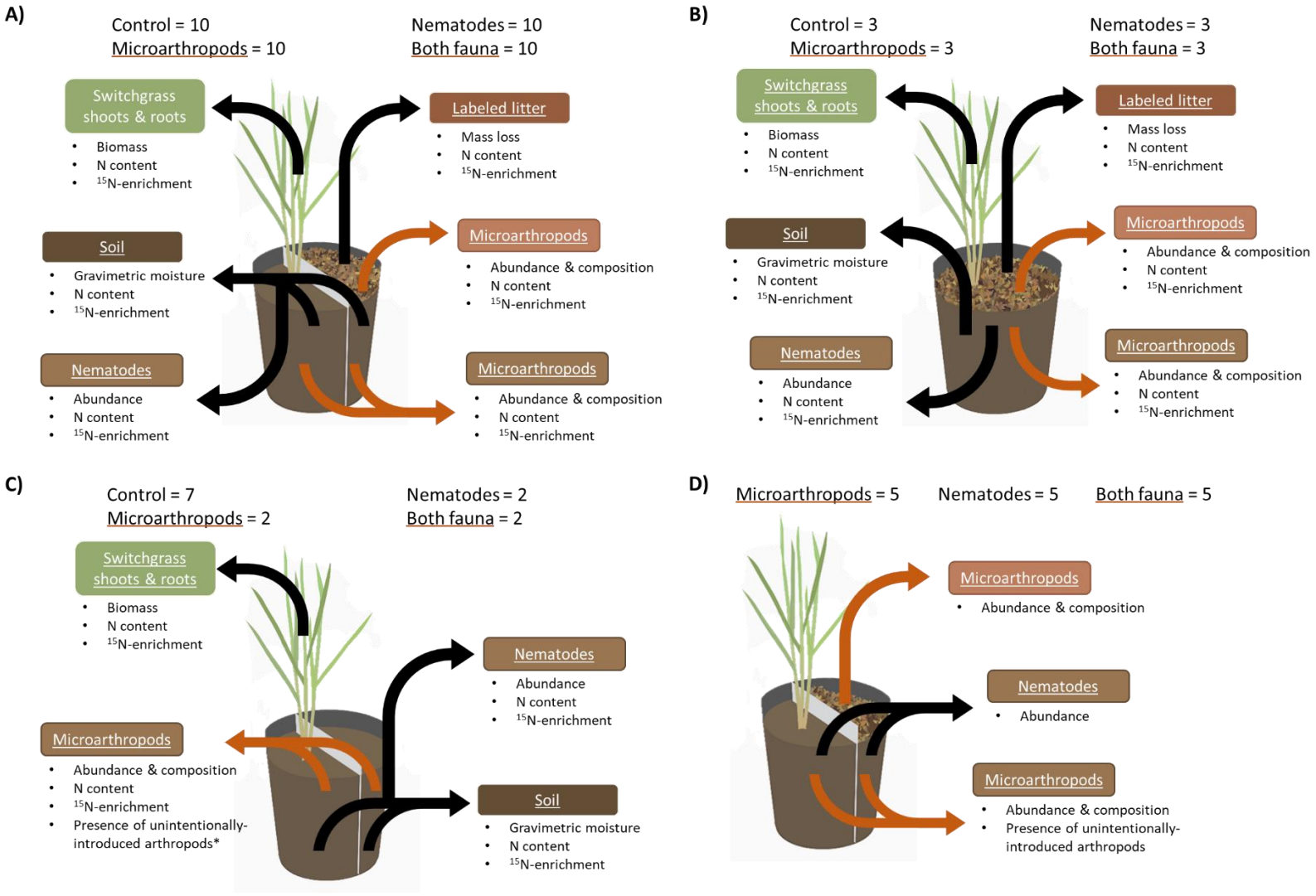

Figure 4.2. Diagram of the substrates harvested from and analyses performed on **A)** ^{15}N-Divided, **B)** ^{15}N-Undivided, **C)** ^{15}N-Baseline, and **D)** Survival mesocosms. Orange arrows and underlines indicate microarthropod extractions which were only performed on mesocosms in which microarthropods were intentionally added (Microarthropods Only and Microarthropods & Nematodes).

Table 4.3. Mean abundance, mean morphospecies richness, and total morphospecies richness of Baseline, Microarthropods Only (MA Only), and Microarthropods & Nematodes communities (MA & N). Morphospecies richness was only calculated for collembola and oribatid mites. Letters indicate significant differences of means as determined by Kruskal-Wallis test ($p < 0.05$, Dunn's *post hoc* test).

		Total	Collembola	Oribatid mites	Mesostigmatid mites	Astigmatid mites	Prostigmatid mites
Mean abundance ± s.d.	Baseline	1127 ± 241	63 ± 49	234 ± 76 **a**	55 ± 43	577 ± 148	198 ± 122 **b**
	MA Only	1275 ± 1424	504 ± 1131	58 ± 33 **b**	90 ± 141	415 ± 436	208 ± 652 **a**
	MA & N	1921 ± 2366	453 ± 1114	97 ± 93 **b**	39 ± 36	1190 ± 1489	142 ± 319 **a**
Mean morph. richness	Baseline	15.6 **a**	6.8 **a**	8.8 **a**	--	--	--
	MA Only	6.2 **b**	1.9 **b**	4.3 **b**	--	--	--
	MA & N	5.6 **b**	1.5 **b**	4.1 **b**	--	--	--

Table 4.4. Collembola and oribatid mite morphospecies collected throughout the duration of the experiment, including where they were collected from, the proportion they made up of the overall abundance for that group (% abund), and the number of mesocosms it was collected from within that sample set. Juvenile oribatid mites are indicated by (j).

ID	Baseline		MA Only		MA & N	
collembola	% abund	n = **5**	% abund	n = **10**	% abund	n = **10**
E2	2.24	1	--	--	--	--
E4	5.75	4	--	--	--	--
E5	23.00	5	<1	6	<1	7
E6	6.71	1	11.12	2	--	--
E7	19.17	3	87.65	3	98.94	4
E14	1.92	2	--	--	--	--
E15	35.46	5	--	--	--	--
E21	--	--	--	--	--	--
E25	<1	2	--	--	--	--
E31	<1	1	--	--	--	--
E33	<1	1	--	--	--	--
G5	<1	1	<1	1	--	--
G13	--	--	<1	2	<1	1
G16	1.28	3	--	--	--	--
G17	1.60	2	--	--	--	--
G21	<1	1	<1	5	<1	3
G61	<1	1	--	--	--	--
G62	<1	1	--	--	--	--
oribatid mites						
O1	5.94	3	--	--	--	--
O2	11.08	5	1.22	2	--	--
O3	4.17	5	--	--	--	--
O4	21.81	5	32.70	10	18.43	10
O5	<1	3	<1	1	<1	2

Table 4.4 (cont'd)

O6	29.43	5	10.78	7	29.76	8
O13	2.84	4	<1	1	--	--
O7 (j)	13.48	5	41.04	10	45.62	10
O8 (j)	<1	4	1.04	4	<1	3
O12 (j)	9.93	4	11.46	5	<1	2
O14 (j)	<1	1	--	--	--	--
O18 (j)	--	--	1.22	3	4.74	6

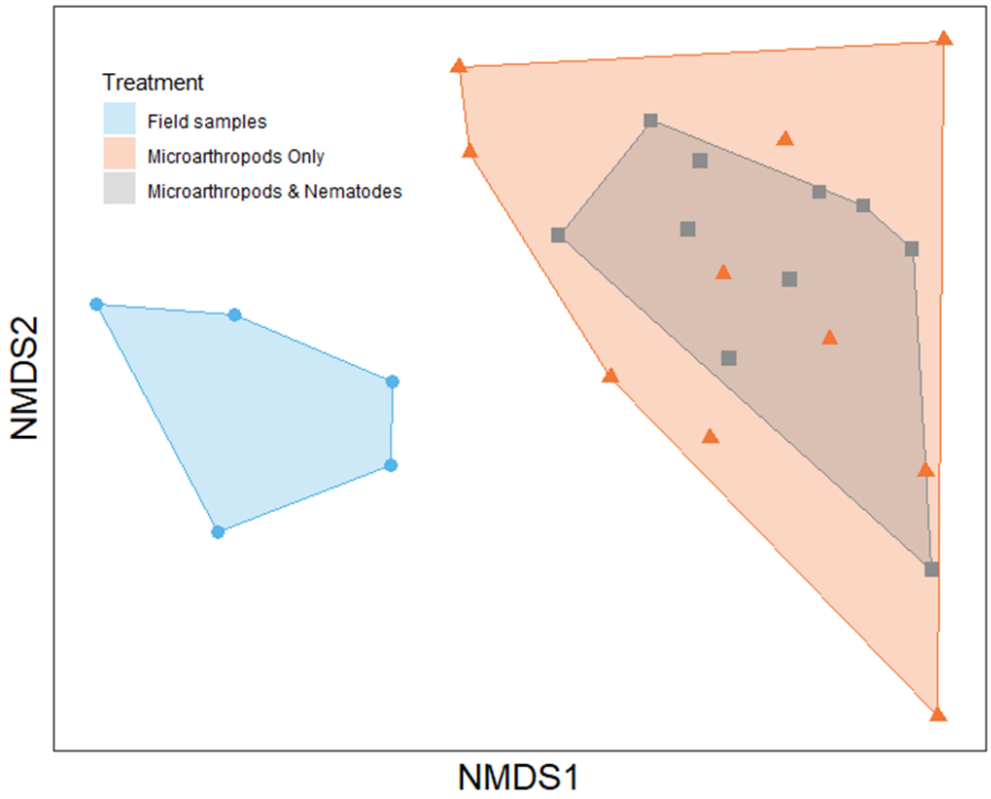

Figure 4.3. Community structure differences between microarthropods collected from switchgrass field plots at the start of the experiment (baseline microarthropod community) and those recovered from Microarthropods Only and Microarthropods & Nematodes mesocosms (^{15}N-Divided) at the time of destructive harvesting visualized using NMDS (k = 3, stress = 0.120).

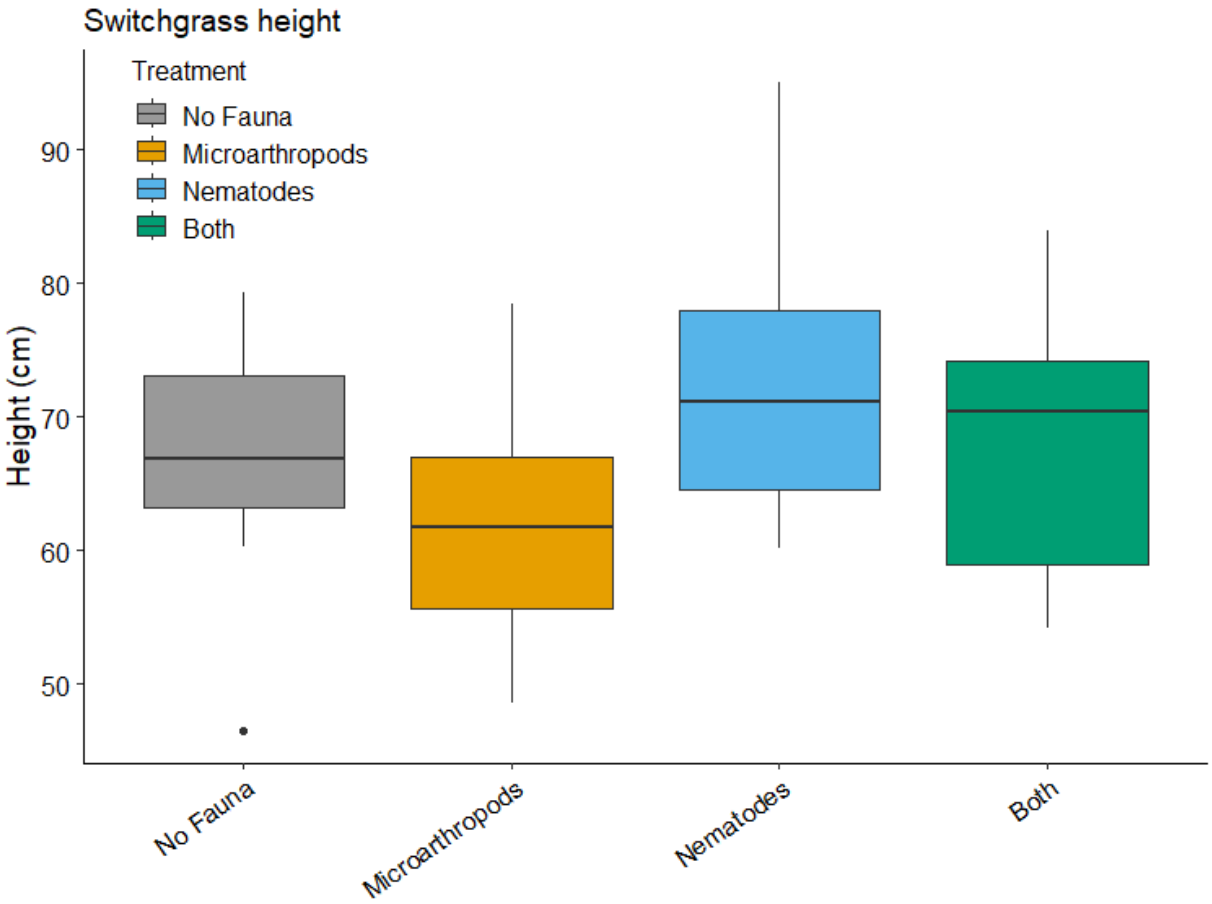

Figure 4.4. Switchgrass height at the time of destructive mesocosm harvesting by faunal treatment.

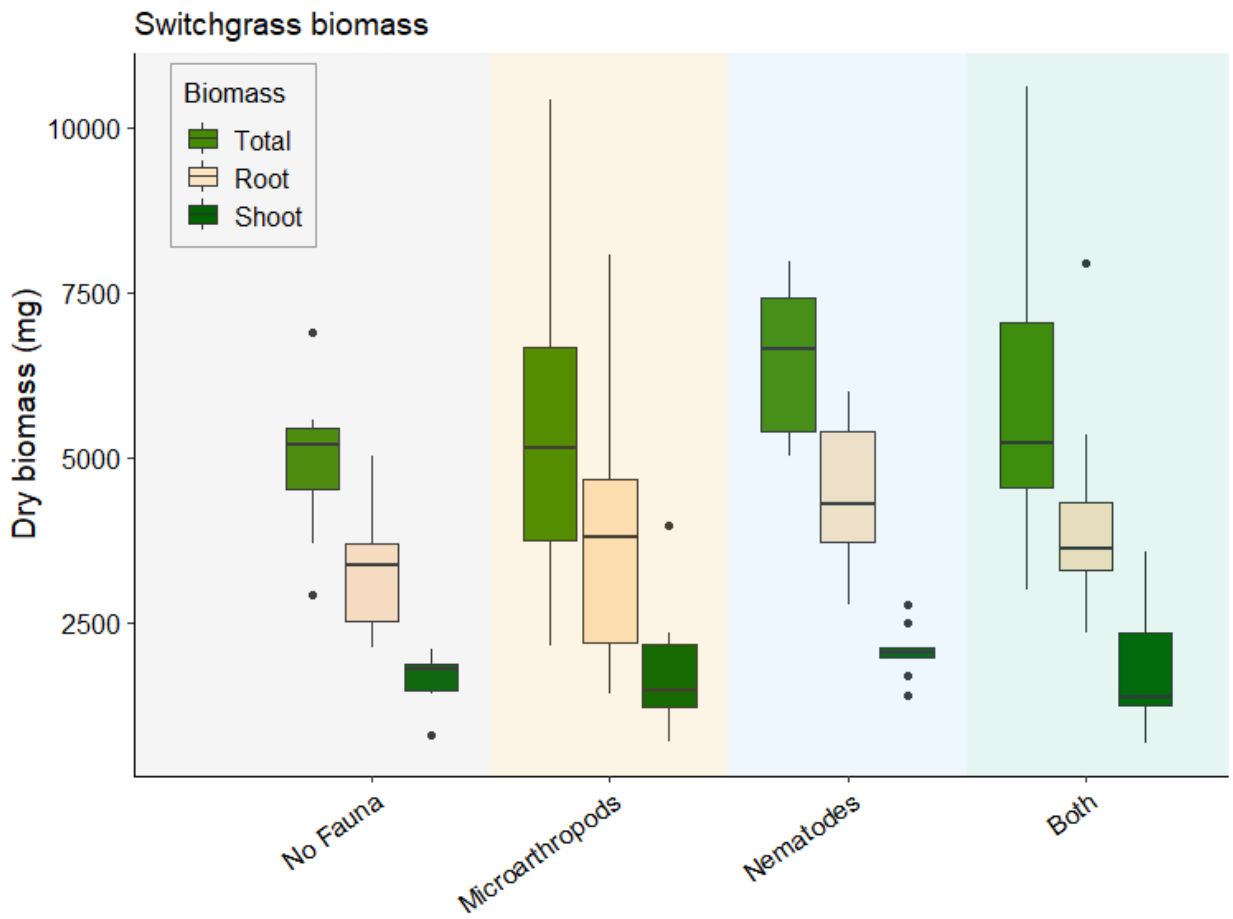

Figure 4.5. Differences in switchgrass total, root, and shoot biomass across faunal treatments.

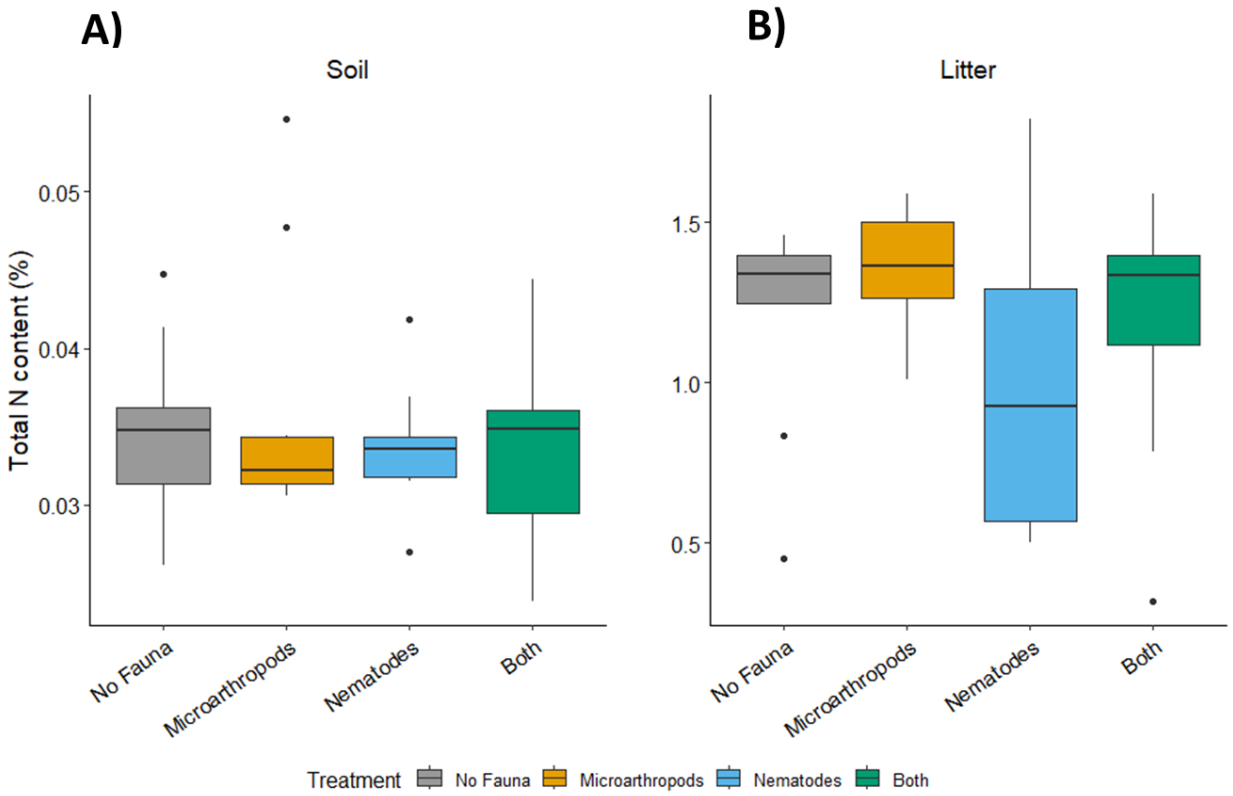

Figure 4.6. **A)** Total N content of soil and **B)** litter by treatment, proportional by mass.

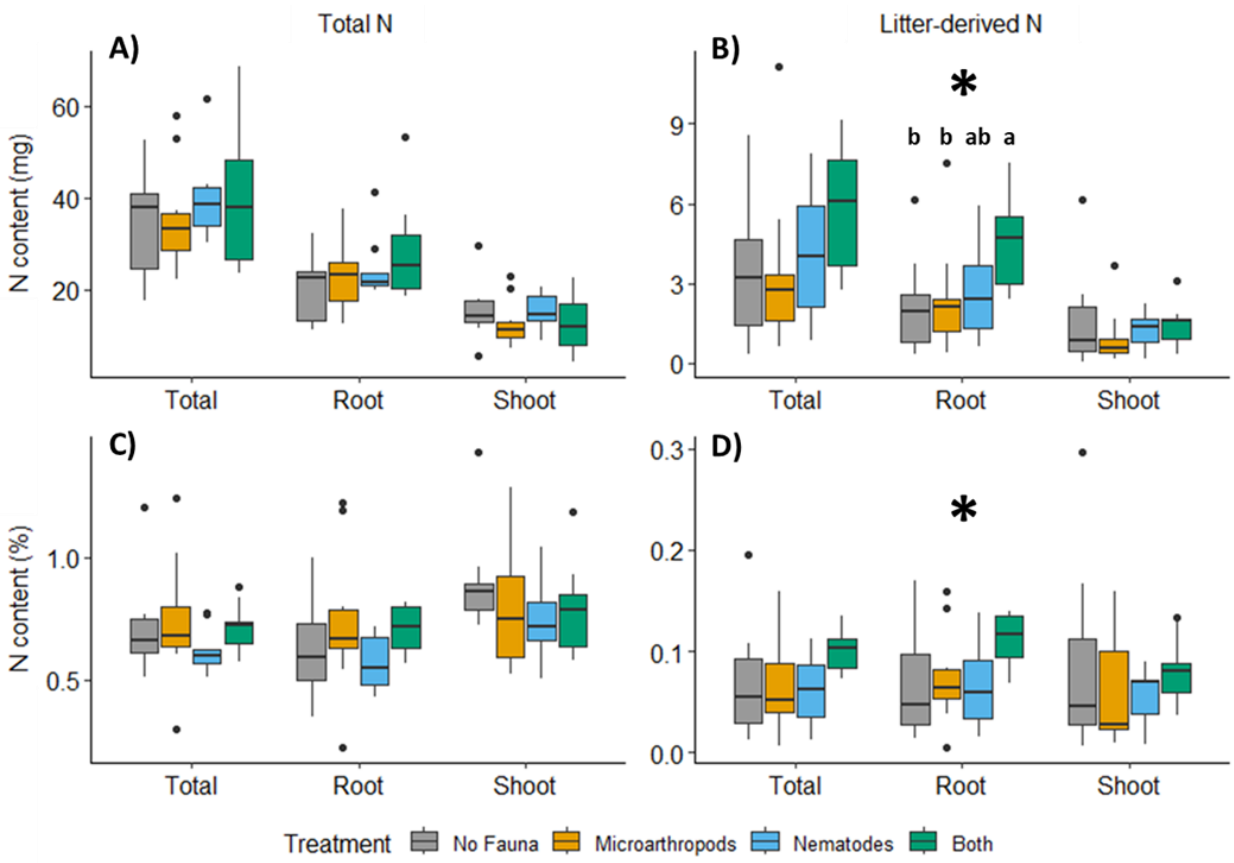

Figure 4.7. Nitrogen content in switchgrass total, root, and shoot biomass. **A)** Mass of total N and **B)** litter-derived N present in switchgrass biomass (mg). **C)** Proportion of total N and **D)** litter-derived N **D)** in switchgrass by mass (%). Significant differences ($p < 0.05$) are denoted by an asterisk with letters indicating pairwise differences (6C: Kruskal-Wallis, Dunn's *post hoc*; 6D: Welch's ANOVA, Tukey's *post hoc*).

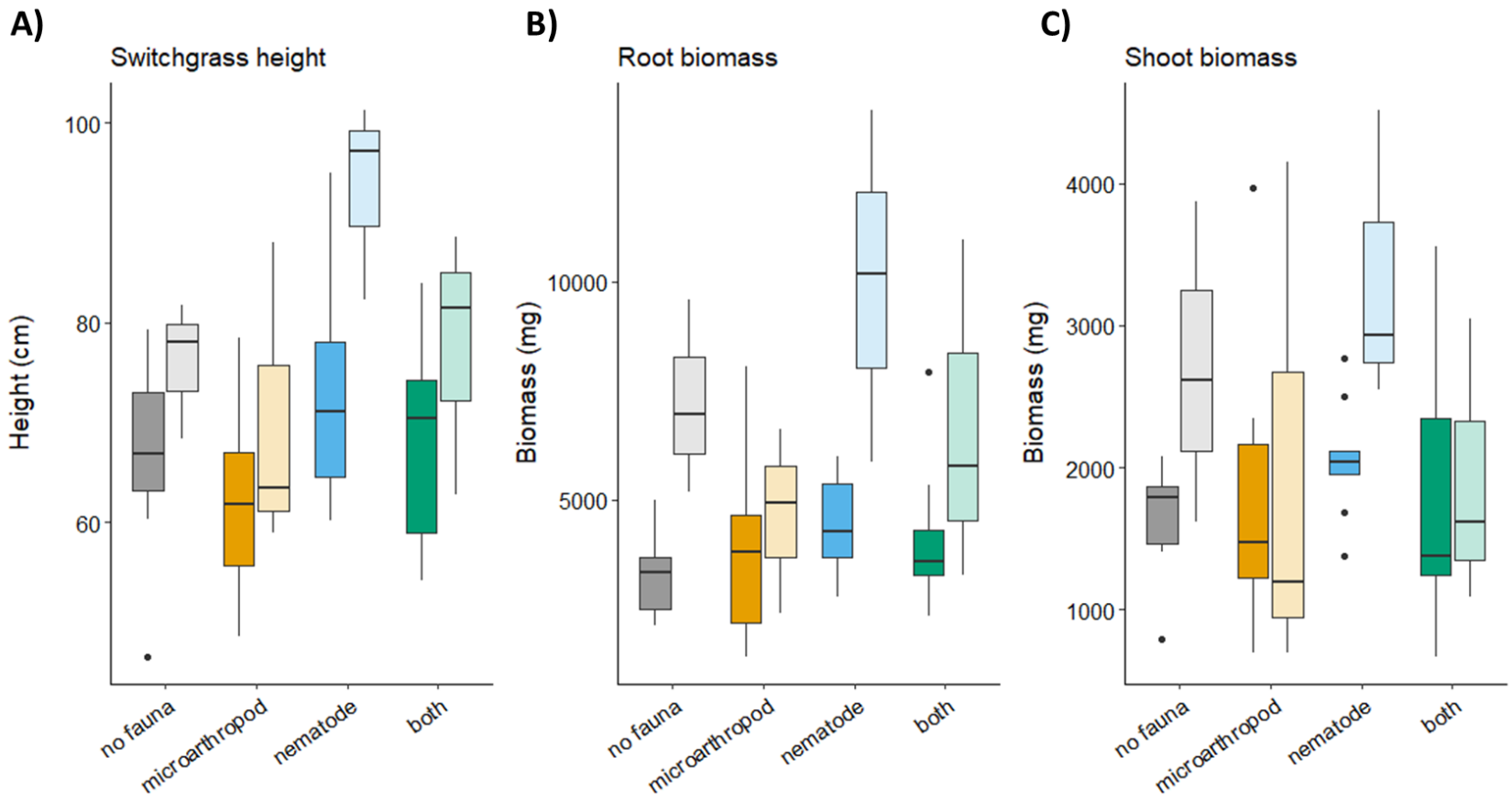

Figure 4.8. Differences in **A)** switchgrass height, **B)** root biomass, and **C)** shoot biomass across treatments between ^{15}N-Divided and ^{15}N-Undivided mesocosms (n = 10* and 3 per treatment, respectively). ^{15}N-Undivided mesocosm bars are lighter in color than ^{15}N-Divided bars.

* n = 9 for ^{15}N-Divided Nematodes Only mesocosms due to one replicate being affected by a leak in the greenhouse roof.

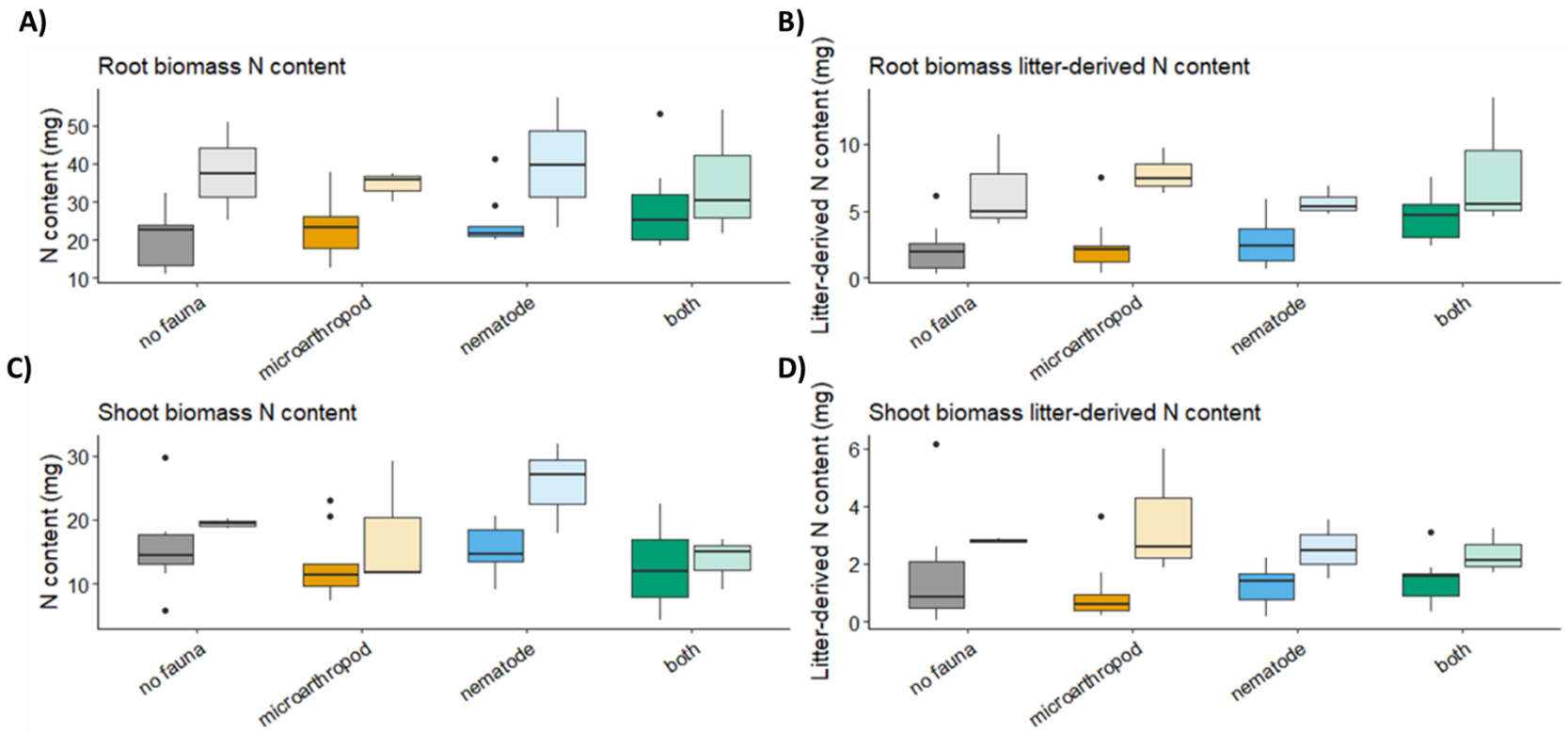

Figure 4.9. Differences in nitrogen content in switchgrass across treatments between ^{15}N-Divided and ^{15}N-Undivided mesocosms (n = 10* and 3 per treatment, respectively). **A)** Root biomass total and **B)** litter-derived N content (mg). **C)** Shoot biomass total and **D)** litter-derived N content (mg). ^{15}N-Undivided mesocosm bars are lighter in color than ^{15}N-Divided bars.
* n = 9 for ^{15}N-Divided Nematodes Only mesocosms due to one replicate being affected by a leak in the greenhouse roof.

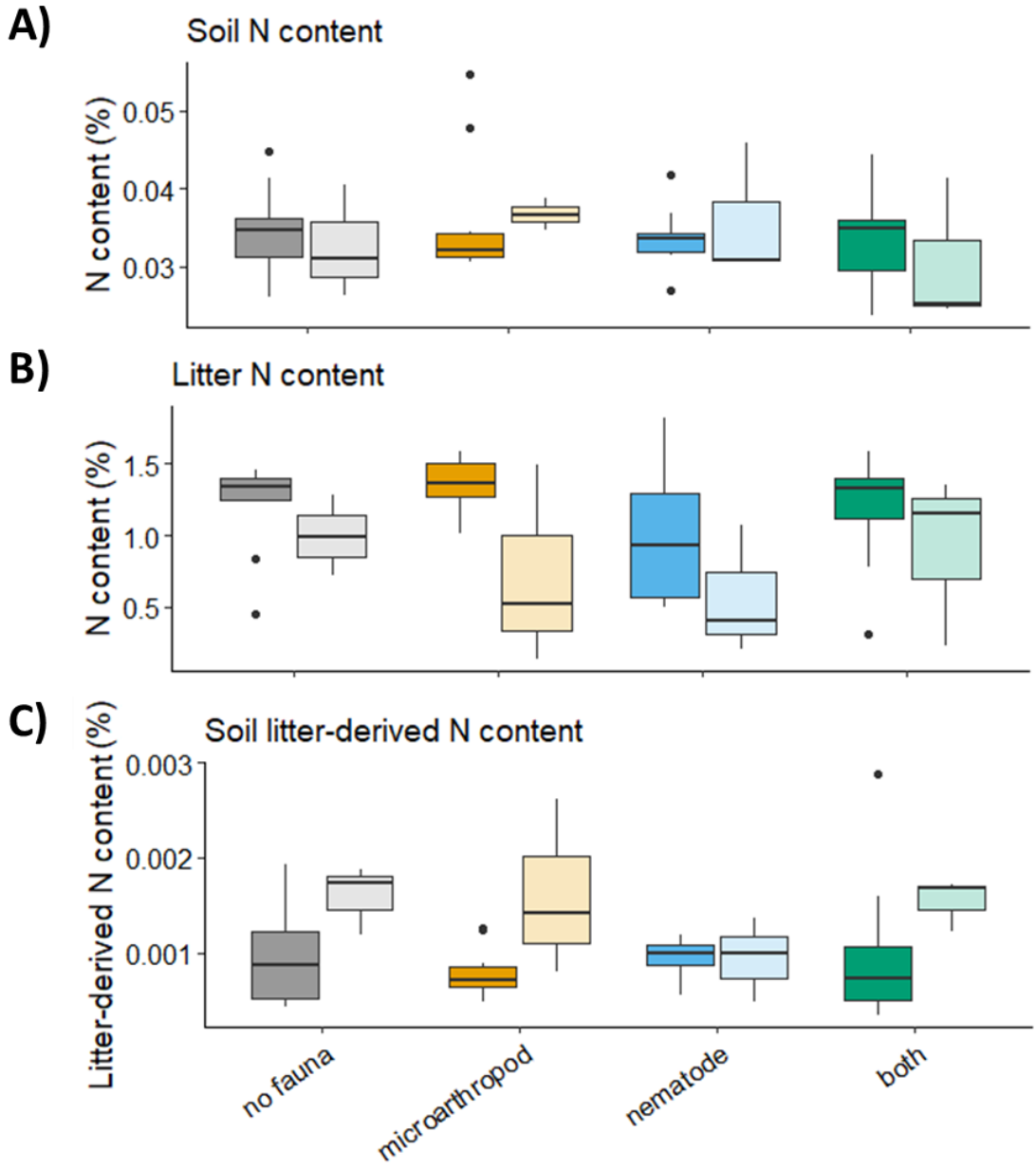

Figure 4.10. Differences in nitrogen content in soil – **A)** total N and **B)** litter-derived N – and **C)** litter across treatments between ^{15}N-Divided and ^{15}N-Undivided mesocosms (n = 10* and 3 per treatment, respectively). ^{15}N-Undivided mesocosm bars are lighter in color than ^{15}N-Divided bars.

* n = 9 for ^{15}N-Divided Nematodes Only mesocosms due to one replicate being affected by a leak in the greenhouse roof.

CHAPTER 5: CONCLUSIONS & FUTURE DIRECTIONS

Microarthropods, particularly mites and collembola, are ubiquitous soil dwellers across ecosystems and are the most numerically dominant arthropods in most soils (Bardgett, 2005; Dindal, 1990; Rusek, 1998). While microarthropods fill a diverse range of niches, their primary functional importance is as decomposers of soil organic matter (SOM). Via numerous direct and indirect effects on soil microbes, microarthropods have long been known to enhance SOM decomposition and subsequent carbon (C) and nitrogen (N) mineralization rates. Despite this, the implications of microarthropod effects, especially by regulating microbes, on organic C (SOC) accrual in soils remain largely unknown. In this dissertation, I address this broad yet increasingly relevant knowledge gap in the context of bioenergy cropping systems, as greater understanding of the key regulators of SOC accrual in these systems is vital to their successful implementation in emissions mitigation strategies.

Microarthropods in Bioenergy Cropping Systems

In Chapter 1, I reviewed the effects of soil fauna, including microarthropods, on SOC dynamics in the context of perennial grass bioenergy cropping systems (PGCS), highlighting their indirect effects via their interactions with microbes as having the greatest potential to meaningfully impact SOC accrual. As soil fauna are strongly regulated by aboveground land use, I also discuss the mediating effects of PGCS attributes, such as bioenergy crop traits and management, on soil fauna community structure and function. I conclude my review by identifying the following knowledge gaps precluding the incorporation of soil fauna in SOC cycling efforts: **1)** many faunal communities remain poorly understood due to a lack of basic bioecological information (Briones, 2014; André et al., 1994), **2)** uncertainty in which fauna-microbe interactions are most likely to affect long-term SOC dynamics, as well as the taxa or functional groups involved and mechanisms underlying these interactions, and **3)** high context dependency in soil and land use effects on soil communities hinders efforts to quantify the strength, direction, and potential variability of faunal effects on SOC and generalize them across ecosystems (Grandy et al., 2016). These key uncertainties served as the basis for my dissertation research detailed in Chapters 2-4, the objectives of which were to address each of these gaps through investigations of microarthropod-microbe interactions in the context of

bioenergy cropping systems.

Microarthropod Community Structure in Annual & Perennial Bioenergy Crop Systems

To investigate the role of microarthropod-microbe interactions on SOC dynamics in bioenergy cropping systems, it was first necessary to characterize the microarthropod communities in these crops under actual field conditions. Further, it was important to investigate how attributes of these systems (i.e., crop type, diversity, management) influence microarthropod abundance and community structure. I address this in Chapter 2, in which I conducted microarthropod surveys to assess microarthropod abundance and community composition across three distinct bioenergy cropping systems: and annual monoculture (energy sorghum), perennial monoculture (switchgrass), and perennial polyculture (restored prairie). Despite seasonal and annual variability, the two perennial cropping systems consistently supported higher microarthropod densities compared to the annual monoculture. Furthermore, the microarthropod communities in perennial systems were more similar and mite-dominated, suggesting that perenniality rather than crop diversity is an important factor influencing microarthropod community structure.

Perennial cropping systems are generally associated with greater SOC accrual potential than annual systems, in part due to favorable interactions between plants, microbes, and the soil matrix promoting enhanced SOC stabilization (Chen et al., 2022; Tiemann & Grandy, 2015). I found strong evidence that perennial bioenergy cropping systems support more abundant, mite-dominated microarthropod communities compared to annual cropping systems. However, the potential functional consequences of these differences in microarthropod community structure between perennial and annual cropping systems, and if they contribute to the greater SOC accrual potential of perennial systems, were unknown.

Microarthropods and Carbon Use Efficiency

In Chapter 3, I conducted a greenhouse mesocosm experiment to evaluate the potential for microarthropod communities to influence microbial carbon use efficiency (CUE), an important predictor of SOC accrual (Tao et al., 2023; Cotrufo et al., 2013). For this, I utilized field-collected microarthropod communities from either a perennial (switchgrass) or annual (energy sorghum) bioenergy cropping systems, allowing me to also assess the degree to which

potential effects on CUE are influenced by microarthropod community structure. Contrary to my initial hypotheses, I did not find evidence that microarthropods affect CUE, though methodological challenges and high within-treatment variability likely played a role in this inconclusive result. One result that was clear from this research, while not the primary focus of this project, was the finding that microarthropod communities undergo substantial changes upon mesocosm colonization. Microarthropod communities recovered from mesocosms exhibited reduced diversity compared to field samples, thus representing the fraction of the whole community best able to recover following field-collection, extraction, and mesocosm introduction. While highly intuitive, this finding highlights an important methodological consideration in working with field-collected microarthropod communities, one which was subsequently addressed in my final research chapter.

Microarthropods and Nitrogen Mineralization

Terrestrial C and N cycles are closely interconnected, with changes in the availability of one of these essential nutrients having important implications on that of the other. N conservation is of special concern in bioenergy cropping systems, both to promote bioenergy crop yields in typically unproductive soils while simultaneously mitigating environmental pollution and greenhouse gas emissions (Robertson et al., 2011). Collectively, the activities of soil fauna are responsible for a significant proportion of the total N mineralization in soils. Microarthropods can stimulate N mineralization by altering the quantity, quality, and accessibility of SOM as well as via their direct and/or indirect effects on microbes. By helping to maintain the balance between N mineralization and immobilization in low N soils, microarthropods could theoretically influence both crop yield and SOC accrual potential within bioenergy cropping systems. In Chapter 4, I conducted a stable isotope tracer study to investigate the potential effects of microarthropods, both alone and in combination with microbivorous nematodes, on N availability and subsequent impacts on switchgrass N uptake and productivity. Compared to microarthropods, nematodes typically have a greater overall impact on total N mineralization, though their contribution comes largely by stimulating microbial turnover via the microbial loop. Including nematodes in the experiment therefore allowed me to evaluate the relative strength of microarthropod effects on N dynamics

compared to nematodes as well as assess if the combined action of both faunal groups would result in greater N mobilization from ^{15}N-labeled litter than either group alone. Following a ~4 mo duration in the greenhouse, switchgrass roots were significantly more enriched in litter-derived N when nematodes were present, with the greatest root enrichment found when both microarthropods and nematodes were present. This result suggests that microarthropods were affecting the N mineralization and uptake by switchgrass even though root enrichment in mesocosms with microarthropods alone did not differ from controls. Despite this positive effect of microarthropods and nematodes in combination, switchgrass height, biomass, and total N content was unaffected by either faunal group. As switchgrass is relatively well adapted to grow in low N conditions (Lemus et al., 2008), it is possible that the contribution of these fauna may only become important for switchgrass in cases where soil N is chronically or severely limiting.

As was seen in Chapter 3, only a subset of the microarthropods collected from the switchgrass cropping system were able to survive introduction into and colonize the soil in mesocosms, which had a substantial influence on microarthropod community structure. Furthermore, the abundance of microarthropods recovered from mesocosms was found to be highly variable. Microarthropod communities were not manipulated prior to mesocosm introduction to account for the spatial heterogeneity of microarthropod communities observed in natural soils. This likely played a role in the high variability in microarthropod communities across replicates in addition to different developmental rates of the surviving microarthropod taxa. Again, these findings are not ecologically surprising. Instead, they are evident of a major limitation that, while long recognized, continues to present substantial challenges in the study of soil food webs and their impacts on nutrient cycles. Compared to other arthropod groups, microarthropods remain relatively understudied. Bioecological information, including physiological constraints, dietary requirements, developmental times, and tolerance to disturbance, is limited or absent for many species. Due to the “black box” nature of soils, soil fauna research often necessitates removing organisms from the soil to directly observe and study them or imposing substantial modifications to natural soils to study them *in situ*. Together, these difficulties create a “double-edged sword” in which ecological studies require directly manipulating soil fauna or their natural habitats to some degree but ensuring that they

remain alive to be studied or are behaving as they would naturally following manipulation is often impeded by lacking basic life history information. This is a particular issue for meso- and macrocosm studies, in which an underlying assumption of these methods is the increased ecological relevance over microcosms studies due to their ability to better account for community-level processes, interactions, and variability more closely. As I have seen in my research, even the best attempts to reduce disturbance and recreate assumed optimal conditions can still have large impacts on the communities being studied. In the face of these challenges and as soil ecology research continues to move away from highly simplified microcosm studies, it will continue to be important that studies on microarthropods and other faunal groups account for the effects of experimental design decisions on community structure and function. This will be especially important for interpreting experimental results and assessing generalizability of findings across studies.

Overall Conclusions

The microarthropod communities supported by the diverse range of potential bioenergy cropping systems and their contributions remain poorly understood. As research continues to shed light onto the critical roles of microbes in SOC accrual, it will be increasingly necessary to further understand both the bottom-up and top-down controls regulating microbial activity and community dynamics. Through their involvement in SOM decomposition, especially in the early stages, microarthropods influence soil nutrient availability and the interactions between microbes, SOM, and the surrounding soil matrix, both of which affect SOC stability and storage. Additionally, many microarthropods are partially or completely microbivorous (Potapov et al., 2022), with microarthropod grazing found to influence microbial composition, growth rate, and activity. Clarifying the relative contributions of microarthropods on decomposition and N mineralization rates, their potential to regulate microbial community structure and function, and the mechanisms driving microarthropod-microbe interactions in bioenergy cropping systems is therefore likely to be necessary to fully understand the potential for these systems to accrue SOC.

In this dissertation, I have begun to address this by surveying microarthropod communities in bioenergy cropping systems ranging from an annual monoculture to a perennial

polyculture (Chapter 2). From here, I conducted two greenhouse experiments investigating the potential effects of field-collected microarthropod communities on key processes and characteristics known to strongly influence SOC dynamics: CUE (Chapter 3), N mineralization and assimilation into different pools, and plant productivity (Chapter 4). While I did not find an effect of microarthropods on CUE, it cannot be conclusively ruled out that this lack of significance was impacted by methodological complications. The finding of increased N mineralization and subsequent assimilation into switchgrass roots in the presence of microarthropods and nematodes in combination, while absent when only microarthropods were present, highlights the importance of considering the multiple food web interactions in tandem. While much uncertainty remains regarding the true potential for microarthropods to influence SOC accrual and stability, further research into the diverse microarthropod-microbe interactions and their functional consequences will be necessary for improving our overall understanding of SOC dynamics in bioenergy cropping systems.

REFERENCES

André, H. M., Noti, M. I., & Lebrun, P. (1994). The soil fauna: The other last biotic frontier. *Biodiversity & Conservation*, *3*, 45-56.

Bardgett, R. (2005). *The biology of soil: A community and ecosystem approach*. Oxford University Press.

Briones, M. J. I. (2014). Soil fauna and soil functions: A jigsaw puzzle. *Frontiers in Environmental Science*, *2*, 7.

Chen, J., Lærke, P. E., & Jørgensen, U. (2022). Land conversion from annual to perennial crops: A win-win strategy for biomass yield and soil organic carbon and total nitrogen sequestration. *Agriculture, Ecosystems & Environment*, *330*, 107907.

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The microbial efficiency-matrix stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter?. *Global Change Biology*, *19*(4), 988-995.

Dindal, D. L. (1990). *Soil biology guide*. Wiley.

Grandy, A. S., Wieder, W. R., Wickings, K., & Kyker-Snowman, E. (2016). Beyond microbes: Are fauna the next frontier in soil biogeochemical models?. *Soil Biology & Biochemistry*, *102*, 40-44.

Lemus, R., Parrish, D. J., & Abaye, O. (2008). Nitrogen-use dynamics in switchgrass grown for biomass. *Bioenergy Research*, *1*, 153-162.

Potapov, A. M., Beaulieu, F., Birkhofer, K., Bluhm, S. L., Degtyarev, M. I., Devetter, M., Goncharov, A. A., Klarner, B., Korobushkin, D. I., Liebke, D. F., Maraun, M., McDonnell, R. J., Pollierer, M. M., Schaefer, I., Shrubovych, J., Semenyuk, I. I., Sendra, A., Tuma, J., Tůmová, M., … Scheu, S. (2022). Feeding habits and multifunctional classification of soil-associated consumers from protists to vertebrates. *Biological Reviews*, *97*(3), 1057-1117.

Robertson, G. P., Hamilton, S. K., Del Grosso, S. J., & Parton, W. J. (2011). The biogeochemistry of bioenergy landscapes: Carbon, nitrogen, and water considerations. *Ecological Applications*, *21*(4), 1055-1067.

Rusek, J. (1998). Biodiversity of Collembola and their functional role in the ecosystem. *Biodiversity and Conservation*, *7*, 1207-1219.

Tao, F., Huang, Y., Hungate, B. A., Manzoni, S., Frey, S. D., Schmidt, M. W., Reichstein, M., Carvalhais, N., Ciais, P., Jiang, L., Lehmann, J., Wang, Y. P., Houlton, B. Z., Ahrens, B., Mishra, U., Hugelius, G., Hocking, T. D., Lu, X., Shi, Z., … Luo, Y. (2023). Microbial carbon use efficiency promotes global soil carbon storage. *Nature*, *618*, 981-985.

Tiemann, L. K., & Grandy, A. S. (2015). Mechanisms of soil carbon accrual and storage in bioenergy cropping systems. *GCB Bioenergy*, *7*(2), 161-174.