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Soil Homogenization: Plant Species Diversity, Ecosystem Properties and Soil Freezing Effects During Tallgrass Prairie Restoration

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“Essentially, all life depends upon the soil. There can be no life without soil and no soil without life; they have evolved together.”

- Charles Kellogg, 1938

Abstract

A legacy of tillage can increase soil uniformity in former agricultural sites. Within plant communities, niche-based species sorting may occur among distinct soil patches (microsites), increasing coexistence and diversity, and the interfaces between microsites (microedges) also may provide unique microsites for increased diversity. However, the influence of soil homogenization and microedges on ecosystem processes and plant responses to stress have not been examined. My PhD thesis assessed if adding microsites and microedges containing sand, woodchips or altered topography (pits and mounds) increased plant species diversity, aboveground productivity, plant litter decomposition and nitrogen retention (^{15}N tracer) and buffered plant responses to soil freezing in the first three years of a tallgrass prairie restoration on former cropland (2015-2017). Soil freezing was investigated by conducting snow removal during the 2015-2016 and 2016-2017 winters and monitoring the effects in subsequent growing seasons. Homogenization decreased diversity in flat topsoil plots relative to topographically heterogeneous plots with pits and in the sand treatment, but increased diversity in the woodchip treatment. Homogenization reduced aboveground productivity and plant ^{15}N retention for the woodchip treatment and increased the rate of litter decomposition. Variation in diversity and ecosystem responses were associated with effects on plant production, suggesting that the influence of soil homogenization may occur indirectly as a result of effects on plant productivity. Elevated levels of plant cover and ^{15}N retention along microedges occurred, indicating microedges may act as unique microsites and small scale ecological transition zones. Soil homogenization increased the sensitivity of total plant cover to soil freezing in the sand treatment and this effect appeared to be driven by greater severity of soil freezing in sand versus topsoil microsites in the heterogeneous treatment. Overall, my results indicate that there is a significant relationship between soil homogenization, plant diversity, ecosystem responses and stress during ecological succession. Human addition of microsites and microedges could be used to benefit plant community diversity and stability in the context of ecological restoration.

Keywords

Community ecology, ecosystem function, edaphology, environmental heterogeneity, niche theory, stability.

Co-Authorship Statement

Excerpts of Chapter 1 were published in the journal *Ecosphere* with Dr. Hugh Henry as a co-author. Dr. Henry funded the research, helped conceive the ideas for the paper and contributed to the writing and editing of the manuscript.

Chapters 2, 3 and 4 are draft manuscripts that will be submitted to peer reviewed journals with Dr. Henry as a co-author. Dr. Henry funded the research, was involved with the study design and contributed to the writing and editing of the works.

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Abbreviations

NH_4^+ = ammonium

$CaCO_3$ = calcium carbonate

CO_2 = carbon dioxide

C = carbon

cm = centimetres

^oC = degrees Celsius

g, G = grams

ha = hectares

h = hours

IPCC = Intergovernmental Panel on Climate Change

L = litres

m = metres

m^2 = metres squared

µg = micrograms

mg = milligrams

ml = millilitres

mm = millimetres

NO_3^- = nitrate

N = nitrogen

^{15}N = ^{15}nitrogen

KCl = potassium chloride

Chapter 1

1 General Introduction[1]

1.1 Soil heterogeneity and plant species diversity

A universal property of soils is that they are extremely variable over time and space. Soil heterogeneity is driven by variation among microsites, which are patches of soil with contrasting composition compared to their surrounding conditions (e.g. nutrient concentrations, topography, texture) (Killham 1994). Poorly developed soils are characterized by high spatial heterogeneity, because they often overlie bedrock with irregular structure, which results in high variability in soil depth across space (Lundholm and Larson 2003). Heterogeneity can be 'environmentally-induced' by geological processes such as weathering and soil formation (Ricklefs 1977) and heterogeneity can be increased by plants through biotic processes such as colonization, root activity, and decomposition, a process referred to as 'plant-induced heterogeneity' (Gibson 1988, Jackson and Caldwell 1993).

Other than the very early stages of pedogenesis, which involve parent material, and possible later geologic processes such as deposition, the remaining pedogenic processes result from interactions with living organisms and non-living material in the soil (Hinsinger 2013). For example, in acid tropical soils in arid environments, calcium builds up at the root surfaces of higher plants. Plants synthesize and discharge oxalate crystals, which prevents internal calcium toxicity, leading to the production of calcrete deposits in soil (Cailleau et al. 2004, 2005). Plants have profound effects on numerous pedogenic processes and dramatically influence several soil parameters, such as pH and clay mineral composition via biological nutrient metabolism (Neumann and Römheld 2012). Soil particles consist of organo-mineral complexes, where organic matter binds to minerals

[1] Some of the content in this chapter was published and is presented here under the terms of the Creative Commons Attribution License. Appendix C: Permission to reproduce published material provides further details. Citation: Stover, H.J. and H.A.L. Henry. 2018. Soil homogenization and microedges: Perspectives on soil-based drivers of plant diversity and ecosystem processes. *Ecosphere*. doi: 10.1002/ecs2.2289.

via cationic bridges (Powlson et al. 2013). Therefore, it can be difficult to distinguish between environmental or plant induced heterogeneity (if any distinctions can really be made at all).

Plants are an important source of biodiversity, providing vital resources to associate species in ecological communities, as well as goods and services critical to society. However, it is currently estimated that one-fifth of the world's botanical species are at risk of becoming extinct in the wild (Pimm and Raven 2017). Understanding the factors that control plant species diversity has been a central goal of plant community ecology, and in the context of global declines in biological diversity it has become increasingly important to refine this knowledge. Environmental heterogeneity has long been recognized as an important driver of plant species diversity (Levin 1974, Ricklefs 1977, Chesson 2000, Stein et al. 2014). Ecological niche theory suggests that a greater number of species can coexist, resulting in increased diversity, in an ecosystem where there is greater environmental heterogeneity (Tilman and Pacala 1993, Laliberté et al. 2013). At the spatial scale of within plant communities, niche based sorting may occur among distinct microsites, roughly the size of an individual rooting zone or the root zone occupied by a small population of a plant species (Day et al. 2003). These patches (i.e. centimetres to metres) are often referred to as microsites by plant ecologists, and they differ from the much smaller scale microsites (i.e. nanometres to micrometres) typically defined by soil scientists.

A large body of research has focused on understanding the influence of heterogeneity in soil and other environmental variables on plant species diversity at the scale of within plant communities (Lundholm 2009). While environmental heterogeneity-plant species diversity relationships are well supported by theoretical work and the majority of observational research, it has been more difficult to successfully demonstrate this relationship experimentally, particularly in soil heterogeneity studies involving nutrient manipulation (Reynolds and Haubensak 2009, Eilts et al. 2011, Tamme et al. 2016). The few experimental studies that have demonstrated increased local scale plant diversity with increased soil heterogeneity have involved the manipulation of vertical layers of soil profiles to create distinct microsites within the upper layer of soil (i.e. replacing patches

of topsoil with patches of lower strata) (Fitter 1982, Williams and Houseman 2014), manipulation of topography in wetland soil (Vivian-Smith 1997) and heterogeneity in disturbance (Wilson and Tilman 2002, Questad and Foster 2008). However, when other soil parameters, such as soil depth to bedrock, physical texture and chemical properties have been altered, negative or null effects on diversity have been observed (Grime et al. 1987, Baer et al. 2004). A better understanding of the mechanisms underlying such discrepancies is essential for clarifying the relationship between plant diversity and soil heterogeneity.

Williams and Houseman (2014) proposed that while positive correlations between soil heterogeneity and plant diversity are often attributed to plant-induced soil heterogeneity, neutral processes may instead be the dominant factor (i.e. plant-derived heterogeneity may be detectable, but it does not contribute significantly to the species diversity patterns). However, the results of experiments on this topic appear to suggest that plant-induced heterogeneity promotes coexistence in a manner consistent with niche theory and invasibility criteria (Hendriks et al. 2015, Burns et al. 2017). The balance between niche and neutral processes likely depends upon the extent of soil heterogeneity present, and neutral processes can dominate when soil heterogeneity at the local scale is low (Williams and Houseman 2014). There are of course many other theories, in addition to niche theory and neutral processes, that have been used to explain plant species coexistence and increased diversity (e.g. pest pressure, allogenic disturbance), but most include at least some component of spatial or temporal variability in plant growth conditions (Wilson 2011).

1.2 Microedges

Causes for ecological variability in the relationship between heterogeneity and diversity may be linked to belowground, small scale processes. For example, microfragmentation (i.e. increased patchiness with increased heterogeneity), was recently discussed as a mechanism whereby increased heterogeneity can decrease diversity as a result of increased fragmentation at a small scale (Tamme et al. 2010, Laanisto et al. 2013). However, research on soil heterogeneity typically has examined its impact as a

summative effect of the component microsites in a plant community without considering the interfaces between them. These microsite edges or 'microedges' may act as small scale ecological transition zones, analogous to the ecotone concept (Clements 1905), but at a smaller scale (the centimetre to metre scale) than is typically considered for plants (i.e. the ecotone concept is typically only considered in the context of adjacent plant communities). Microedges may provide additional niche spaces for increased plant diversity by offering a transitioning blend of the neighboring patches, or by functioning as interfaces with properties distinct from the neighboring patches. Microedges are also analogous to the transition zones between soil horizons described by soil scientists. Vertical strata found within soil profiles do not have distinct boundaries, but zones of overlap between the upper and lower layer, and are named as distinct sublayers in the soil taxonomy hierarchy (Weil and Brady 2016). These two concepts can be combined; most research on soil heterogeneity has examined its impact in two dimensions, either vertically or horizontally, but Liu et al. (2017) demonstrated that soil heterogeneity should be examined in three dimensions.

1.2.1 Microedges and ecosystem properties

Non-additive interactions among patches along microedges also may apply to ecosystem processes. For example, decomposition rates along microedges may be greater than the sum of neighboring patches; such a response would be analogous to the results of litter mixture experiments, where the presence of litter from multiple plant species experiences faster decomposition than the litter from the component species in isolation (Gartner and Cardon 2004). These non-additive responses can result from complementarity among microsites (i.e. soil microbial activity limited by a given nutrient in one microsite may be increased by the higher availability of that nutrient in a neighboring microsite, and vice versa). Small scale processes occurring on a microedge therefore are analogous to larger scale processes that have been observed to be driven by complementarity, such as hot spots and hot moments of elemental cycling at the interface between wetland and upslope areas (McClain et al. 2003).

With respect to soil hydrology and physical properties, there may be important implications of microsites and microedges for soil water movement; the resulting variation in moisture conditions can influence plant community composition and structure. Water movement can be impaired in stratified soils, as can occur with the stratification or layering of microsites in a heterogeneous soil. As water moves between strata or layers (i.e. microsites), it slows down when it reaches the edge of the next distinct layer due to changes in soil composition, especially changes in pore size (Miller and Gardner 1962). The latter results in a longer duration of dry conditions in the adjacent microsite, and a longer duration of wet conditions at the microedge, as water builds up at the boundary. Microedge properties also may be analogous to soil particle size relationships. If soil volume is held constant, as soil microsites decrease in size, their frequency increases and microedge surface area increases. Similar relationships are present for soil particles at smaller spatial scales (i.e. millimetres to nanometres), with colloids, the smallest of soil particles (< 2 μm), exhibiting an extremely high surface area to volume ratio, and imparting unique properties on the colloid particle, such as electrostatic charge for holding nutrients and water (Jones 2012).

1.3 Soil homogenization

Experiments that have explored the relationship between plant diversity and soil heterogeneity have focused predominately on the addition of increased soil heterogeneity to natural soils, above that of the background level of heterogeneity. However, such an approach does not address the full range of environmentally-relevant soil heterogeneity scenarios, because it does not include fully-homogenized treatments (but see Brandt et al. 2014). In addition, knowledge is lacking regarding the role of disturbance in the study of heterogeneity-diversity relationships. While disturbance often is viewed as an undesirable artefact of soil heterogeneity experiments (Lundholm 2009), soil often becomes more homogeneous and uniform specifically as a result of continuous disturbance from activities such as tillage (Anderson and Coleman 1985, Elliott 1986). Resource extraction, agricultural operations and other land uses have modified and disrupted the natural soil profile to the extent that an anthropogenic soil order has been proposed (Naeth et al. 2012). The homogenization of soil properties within a site caused

by disturbance could result in loss of microsites from the ecosystem, decreasing opportunities for plant species coexistence. Thus, soil homogenization via disturbance may be a significant factor limiting the recovery of plant species diversity and composition in early successional environments (Grime 1979, Baer et al. 2004).

Soil homogenization is a subset of variation in soil heterogeneity; it is the process of soil becoming more homogeneous or uniform across space as a result of continuous mixing from activities such as tillage, erosion, compaction, and displacement (Anderson and Coleman 1985, Elliott 1986). The potential impacts of soil homogenization from legacies of tillage in row crop agriculture deserve specific attention, because cropland under tillage represents a significant proportion of land use worldwide (Vitousek et al. 1997). For example, approximately 40 % of Earth's land area is in agricultural use (Foley et al. 2005). The number of old fields – former croplands abandoned from agricultural land use – has dramatically increased over the past century to about 200 million hectares globally (Cramer et al. 2008). Tillage or disturbances with similar effects represent a unique type of soil homogenization, because over time the subsoil layer becomes heavily compacted (e.g. hardpan formation), preventing root penetration. This process effectively eliminates the ability of vertical strata to act as microsites.

Chronic disturbance can homogenize the spatial distribution of soil properties (Robertson et al. 1988, 1993, Röver and Kaiser 1997, Celik 2005), which can lead to decreases in plant diversity and changes in community structure and composition (Grime 1979, Coffin et al. 1996). The addition of environmental (soil) heterogeneity to previously cultivated sites has been attempted as a strategy to increase plant diversity with mixed success (Williams and Houseman 2014, Baer et al. 2016). In recovering grasslands subject to soil mixing, increased heterogeneity in microtopography and soil chemical and physical properties was associated with increased plant species diversity, and more so in older sites (Deák et al. 2015, Conradi et al. 2016). Soil homogenization also has several non-anthropogenic causes such as pedoturbation, the vertical mixing of the soil profile caused by soil-dwelling animals or geological processes (churning clays, cryoturbation, and bioturbation), that occur on a wide variety of spatial scales (Weil and Brady 2016). Other

natural causes of soil homogenization can include erosion on steep slopes and sites with weak soil strength (high silt, low organic matter) that experience heavy precipitation.

1.3.1 Soil homogenization and ecosystem properties

Variation in plant species diversity is frequently associated with variation in ecosystem properties and processes such as nutrient cycling, decomposition and primary production (Tilman 1999, Hooper et al. 2012). It follows that soil heterogeneity can alter ecosystem processes indirectly via its effects on plant species and compositional diversity (e.g. Cardinale et al. 2000, Maestre et al. 2006, Tylianakis et al. 2008) and plant functional traits (García-Palacios et al. 2011, 2013). With respect to soil homogenization, the resulting reduction in plant species diversity could decrease facilitative interactions and niche partitioning among species (decreased overyielding), decreasing nutrient retention and productivity, both above and belowground (Tilman 1999, McKane et al. 2002, Griffin et al. 2009). In addition, loss of functional groups of species due to a reduction in diversity could reduce the complexity of leaf chemistry and phenology, lowering the rate of decomposition (Hector et al. 2000, Zak et al. 2003).

Soil heterogeneity also may influence nutrient cycling directly, as evidenced by the results of split pot experiments, where plant nutrient acquisition differs among individuals grown with homogenous nutrient availability and those grown with the same amount of nutrients available, but distributed heterogeneously (Fitter et al. 2000, Henry and Jefferies 2002, 2003a, 2003b, Holzapfel and Alpert 2003). Beyond the level of plant-soil interactions, animals may potentially interact with soil microsites to alter ecosystem processes. For example, burrowing mammals and nesting birds can show preferential activity in soil microsites with unique topographical or soil structure properties, and altered local disturbance patterns and nutrient deposition (from animal waste) resulting from this activity could alter nutrient cycling locally.

1.3.2 Soil homogenization and plant community responses to stress

In addition to plant community diversity and ecosystem function, soil homogenization may also influence plant community responses to stress. Increased plant species richness

was associated with maintenance of productivity levels during drought (Tilman and Downing 1994) as with a greater number of species present there is a greater likelihood of having more productive species to replace those lost due to drought (Tilman 1999). Therefore, decreases in plant species diversity due to soil homogenization could indirectly influence the maintenance of productivity and other functions in response to drought and other stresses.

Soil homogenization may also influence plant community stability to stress directly by reducing the number of 'safe sites' or 'microrefugia' found in more heterogeneous environments. Fridley et al. (2011) demonstrated in a local plant community that microrefugia to climate warming can be provided by microsites differing in soil depth. Plant species were lost from shallow microsites that experienced warming treatments, while the deep microsites gained species from shallow microsites. Dryland plant communities also showed resistance to a nine-year drought due to temporal and spatial heterogeneity (Tielbörger et al. 2014). Microtopographic variation and variability in aspect along mountain sides result in a range of temperatures deviant from atmospheric conditions, providing potential microrefugia to alpine species from climate warming (Scherrer and Körner 2010). Variability in microtopographic relief can also have a strong influence on plant diversity and community composition by increasing variability of soil moisture (Vivian-Smith 1997, Økland et al. 2008). In a pot experiment, in response to elevated CO_2, aboveground productivity in soils with nutrient homogeneity increased while that of heterogeneous soils remained constant (Maestre et al. 2005). Under heterogeneous conditions of nutrient supply, the influence of CO_2 (Maestre et al. 2005) and precipitation (Xi et al. 2015) were non-additive, and instead plants responded more strongly and consistently to the effects of soil nutrient heterogeneity despite changes in CO_2 and precipitation. These findings indicate that soil heterogeneity could buffer plant community responses to stress.

Evidence from pot experiments suggests increases in nutrient availability, CO_2 and the presence of earthworms can facilitate or inhibit the influence of soil nutrient heterogeneity on plant productivity (Maestre and Reynolds 2006a, 2006b, 2007, Maestre et al. 2007, García-Palacios et al. 2014). Maestre and Reynolds (2007) found greenhouse

plant assemblages had greater biomass under heterogeneous nutrient supply and the effect was more pronounced as nutrient levels increased and at lower moisture conditions. Environmental context has important implications for the effect of soil heterogeneity, as indicated by evidence of facilitative and suppressed effects along moisture, CO_2, and nutrient gradients (García-Palacios et al. 2012). The explanation for such outcomes is not well understood, but in theory it could occur due to differences in plant community composition between homogeneous and heterogeneous treatments prior to environmental change or due to differences in conditions experienced in homogeneous and heterogeneous treatments during the environmental change.

Despite what is known about interactions between soil heterogeneity and environmental changes/stresses, no sources of heterogeneity have been directly tested or manipulated other than soil nutrient heterogeneity. Studies of soil nutrient heterogeneity restricted to artificial mesocosms tend to favour fewer large, dominant species with similar functional traits (Gazol et al. 2013, Price et al. 2014, Xi et al. 2015, Tamme et al. 2016). Furthermore, no field studies have confirmed trends found in pot experiments by direct manipulation of levels of heterogeneity and stress in a natural ecosystem (but see Arnone 1997).

1.4 Study system

1.4.1 Tallgrass prairie restoration: A relevant system for investigating soil homogenization

Ecological restoration is used as a practice of reinstating valuable habitat for species at risk of extinction and managing an ecosystem so that its species, populations and functions are self-sustaining (Choi et al. 2008). Tallgrass prairie was once a dominant grassland ecosystem in the North American plains and has been reduced to small fragments on the landscape (Sampson and Knopf 1994). Tallgrass prairies are maintained in a treeless state by regular fire cycles and dominated by C_4 bunchgrasses and a diversity of other graminoids, legumes and forbs (Rodger 1998). Tallgrass prairie restoration is helping to recover this important ecosystem but would benefit from knowledge gained from ecological investigations. One of the most effective ways of studying something is

to take it apart and reassemble it, so ecological restoration provides a valuable opportunity to enhance the scientific study of ecology (Jordan et al. 1990). Grasslands are ideal study systems for ecological experiments on soil systems because, after belowground treatments are implemented, herbaceous plant communities can be established in a relatively short term (< 1 year) period.

1.4.2 Soil freezing

Frost exposure is a source of plant stress that is expected to increase in some temperate regions due to climate warming (Groffman et al. 2001, Hardy et al. 2001, Henry 2007, 2008) because of reductions in snow cover (Kapnick and Delworth 2013). During winter, the snowpack has an insulating effect and in the absence of snow cover an increase in soil freezing and freeze-thaw cycles occur (Henry 2008), which damages overwintering plant tissues and negatively impacts plant growth in subsequent growing seasons (Vankoughnett and Henry 2014). Differences in soil characteristics among microsites in heterogeneous soils may lead to variability in intensity of soil freezing; for example, wetter soils are known to have more severe freezing effects (Oztas and Fayetorbay 2003, Wu et al. 2017). Given that soil freezing is predicted to increase in the coming decades in northern temperate regions, it is important to understand how this will impact early successional ecosystems also affected by soil homogenization.

1.5 Thesis overview

For my PhD, I investigated the legacy effects of soil homogenization from cropland tillage on plant species diversity and community composition in a restored tallgrass prairie. I also examined how soil homogenization affects productivity, nitrogen retention, decomposition and plant responses to soil freezing. The connections among soil homogenization, plant diversity, ecosystem properties and stress that I initially set out to explore in my thesis are summarized in Fig. 1.1 which is found after my objectives and hypotheses below. I used a former agricultural field that was last cultivated in 2014 to conduct two experiments. For these experiments, patches of sand and woodchips were added to the soil to construct heterogeneous plots, whereas the same materials were added and then tilled and mixed into the surrounding area to construct homogenized

plots. For the first experiment I also added patches with microtopographic relief (i.e. pits and mounds) for heterogeneous plots and compared them with flat plots that were tilled (homogeneous plots). For the second experiment, I exposed both homogeneous and heterogeneous plots to stress (increased soil freezing via snow removal) and compared them to homogeneous and heterogeneous plots not exposed to stress (i.e. no snow removal, ambient conditions).

1.5.1 Objectives

My PhD thesis had the following objectives:

Objective 1) Explore the effects of soil homogenization on plant community composition and species diversity during early ecosystem succession in a tallgrass prairie restoration and explore plant diversity and species composition along microedges (microsite edges), which represent the interfaces between adjacent soil patches in heterogeneous soil,

Objective 2) Investigate the influence of soil homogenization on productivity, decomposition and nitrogen retention and patterns of these properties along microedges in heterogeneous soils, and

Objective 3) Research the relationship between soil homogenization and soil freezing by exploring the responses of plant community composition, diversity and productivity to soil freezing in homogeneous and heterogeneous soils.

1.5.2 Hypotheses

Chapter 2: Soil homogenization in a tallgrass prairie restoration: Toward resolved understanding of the relationship between soil heterogeneity and plant species diversity (Experiment 1a)

Hypotheses: (1) I hypothesized that soil homogenization decreases plant species diversity and alters community composition by reducing the availability of distinct patches of soil, which would otherwise provide unique ecological niches and thereby increase coexistence and diversity and (2) Microedges act as small scale ecological transition zones, analogous to ecotones, but at a smaller scale (the centimetre to metre scale) than is

typically considered for plants (i.e. the ecotone concept is typically only considered in the context of adjacent plant communities) and provide additional niche spaces for increased plant diversity by offering a transitioning blend of the neighboring patches, or by functioning as interfaces with properties distinct from the neighboring patches.

Chapter 3: Soil homogenization modifies productivity, nitrogen retention and decomposition during grassland restoration (Experiment 1b)

Hypotheses: (1) In addition to direct loss of substrate heterogeneity, homogenization is expected to decrease plant species and functional group diversity and reduce complementarity in resource use. Therefore, I hypothesized that soil homogenization decreases productivity, nitrogen retention and plant litter decomposition. (2) Based on the assumption that complementarity occurs between adjacent microsites, microedges exhibit ecosystem function (i.e. productivity, nitrogen retention and plant litter decomposition) that is not a simple additive effect of the adjacent microsites.

Chapter 4: Interactions between soil heterogeneity and soil freezing: Implications for the diversity and relative abundances of grassland species (Experiment 2)

Hypothesis: Based on the assumptions that soil heterogeneity would increase plant species diversity and availability of microrefugia, I hypothesized that soil heterogeneity would buffer the effects of soil freezing, such that freezing effects on overall plant abundance in heterogeneous substrates would be less severe than in homogeneous substrates.

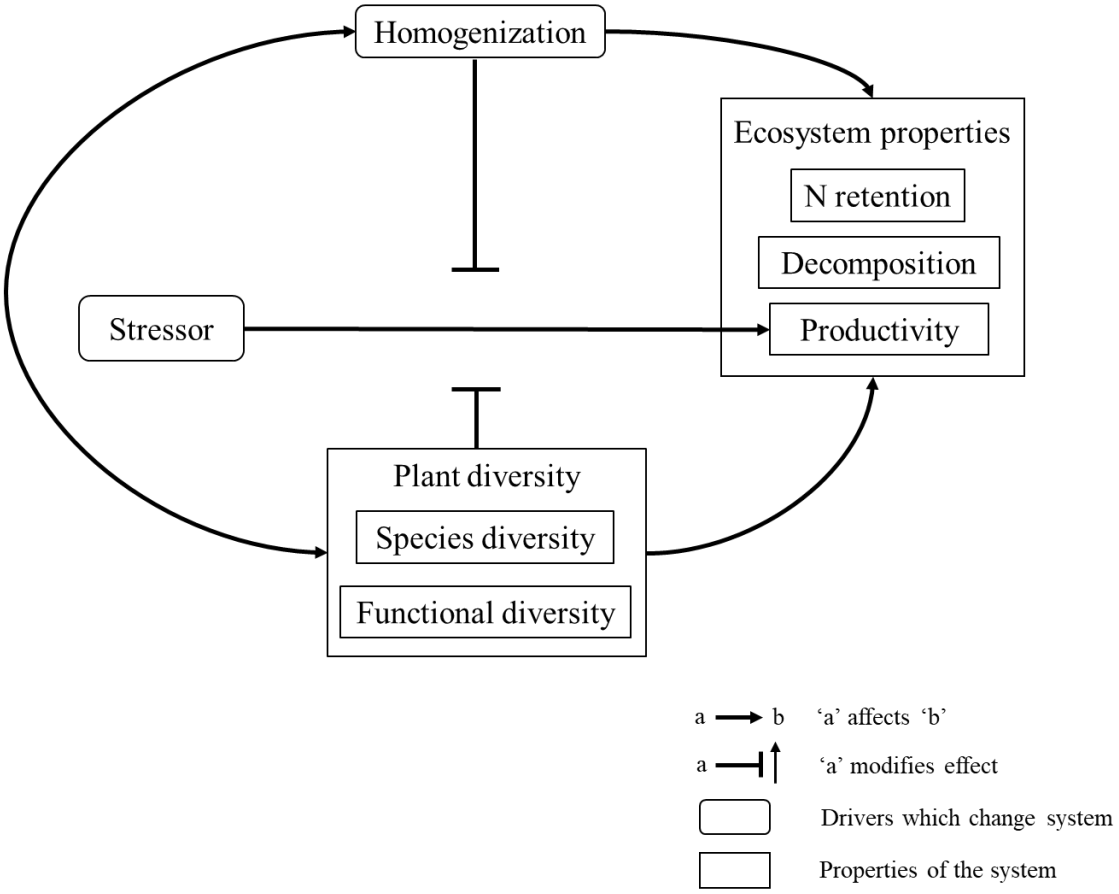

Figure 1.1 Preliminary conceptual model linking the associations among soil homogenization, stress (soil freezing), plant diversity (i.e. species diversity, functional diversity and community composition) and ecosystem properties (e.g. nitrogen retention, decomposition, productivity). Homogenization, the process of soil becoming more spatially uniform, decreases diversity and ecosystem properties due to a lack of diverse microsites and microedges. Homogenization also can decrease ecosystem properties indirectly by decreasing plant diversity. Stress decreases plant productivity. The effect of stress is lessened with increased diversity since stress tolerant species are more likely to be available to compensate for more stress sensitive species. Homogenization increases the effects of stress by reducing the number of safe sites or microrefugia and by decreasing plant diversity. Plant diversity also can decrease homogeneity by increasing 'plant induced' heterogeneity.

1.5.3 Thesis organization

My PhD thesis is organized in integrated article format with three data chapters or manuscripts. Chapter 1: General introduction provides an overview of the thesis, including the context for the research and pertinent literature. Chapter 2: Soil homogenization in a tallgrass prairie restoration: Toward resolved understanding of the relationship between soil heterogeneity and plant species diversity (Experiment 1a) addresses Objective 1. Chapter 3: Soil homogenization modifies productivity, nitrogen retention and decomposition during grassland restoration (Experiment 1b) addresses Objective 2. Chapter 4: Interactions between soil heterogeneity and soil freezing: Implications for the diversity and relative abundances of grassland species (Experiment 2) addresses Objective 3. Chapter 5: General discussion, concludes the thesis with a synthesis of the results from each chapter and concluding remarks, including future research directions.

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Chapter 2

2 Soil homogenization in a tallgrass prairie restoration: Toward resolved understanding of the relationship between soil heterogeneity and plant species diversity

2.1 Introduction

In grassland restoration projects, a diverse range of plant species is typically sown to maximize species diversity (Smith et al. 2010). However, grassland restoration is often conducted on former cropland (Kucharik et al. 2006), where soils are typically more homogeneous as a result of a legacy of tillage, erosion, compaction and mixing (Anderson and Coleman 1985, Elliott 1986). Niche theory suggests that plant species can best coexist in an ecosystem where there are distinct niches available in the soil for plant species to colonize and differentially dominate, resulting in increased diversity (Tilman and Pacala 1993). Loss of soil heterogeneity caused by decades of tillage from crop cultivation therefore could be an important factor that limits plant species and community diversity during subsequent restoration. However, soil homogenization has not been examined explicitly in investigations of soil heterogeneity-plant relationships other than serving as a control treatment (Brandt et al. 2014, Stover and Henry 2018).

In observational studies of undisturbed ecosystems, sites containing numerous soil patches (microsites) with differing characteristics (e.g. soil depth, moisture, microtopography, nutrient concentrations) have been positively correlated with plant diversity (Lundholm 2009). However, despite theoretical and observational support, experimental manipulation of soil heterogeneity has produced inconsistent results, with a range of positive, negative or null effects of soil heterogeneity on plant diversity (as reviewed by Williams and Houseman 2014). Increased within-site plant diversity with increased soil heterogeneity has been demonstrated by studies that have created distinct microsites by replacing patches of topsoil with patches of subsoil (Fitter 1982, Williams and Houseman 2014), variation in microtopography (i.e. pits and mounds) in wetland soils (Vivian-Smith 1997) or disturbance as a source of heterogeneity (Wilson and Tilman 2002, Questad and Foster 2008). However, experimental manipulation of

heterogeneity in soil depth to bedrock, soil texture and nutrient manipulation have resulted in negative or null effects on plant diversity (Grime et al. 1987, Baer et al. 2004). The vast number of different heterogeneity sources available to be tested likely contributes to variability in heterogeneity-diversity relationships, because each source (e.g. topography, soil texture, temperature, etc.) has its own unique effects on plant communities.

The variability in experimental results observed also is likely due to the influence of factors such as plant size, microsite size, competition and productivity. Diversity is likely greatest when microsites are, at minimum, the size of individual plant rooting zones or plants may easily overcome resource differences (Day et al. 2003). In terms of competition, highly productive and competitive species may exploit soil nutrient heterogeneity and dominate a community, decreasing diversity, regardless of the extent of heterogeneity present (Stevens and Carson 2002, Eilts et al. 2011, Stromberg et al. 2011, Baer et al. 2016, Tamme et al. 2016). Liu et al. (2017) found no effect of heterogeneity on species richness when inferior competitors for nitrogen were pooled with superior competitors, but positive effects when only superior competitors were considered. Increasing plant density, or number of individuals, can increase diversity due to stochastic/neutral effects, and this may be the dominant mechanism explaining diversity patterns in homogeneous environments (Williams and Houseman 2014, Walker and Lundholm 2018).

Variability across temporal and spatial scales and environmental conditions can influence soil heterogeneity-diversity relationships, which are typically tested in short-term (< 1 year) experiments. However, soil heterogeneity may be more common in late-successional, mature ecosystems (Williams and Houseman 2014) and would therefore benefit from longer term study (but see Baer et al. 2016). Temporal variation in terms of phenological variability among seasons has not been examined. Moreover, the strength of association between heterogeneity and diversity may be weaker at fine spatial scales within plant communities compared to the landscape level (Gazol et al. 2013). Environmental context has important implications for the effect of soil heterogeneity, as indicated by evidence of facilitative and suppressed effects along moisture, CO_2, and

nutrient gradients (García-Palacios et al. 2012). Further understanding of the factors which cause variability in heterogeneity-diversity relationships is required for better prediction and exploitation of this relationship as a conservation tool.

Research on soil heterogeneity has traditionally considered its impact as a summative effect of microsites within plant communities, without considering the edges between them. These microsite edges (microedges) may have unique properties, providing an additional form of niche space and increasing plant diversity (Stover and Henry 2018). Microedges also may have properties intermediate between the two neighboring patches, acting as small scale ecological transition zones, analogous to the ecotone concept (Clements 1905), but at a much smaller spatial scale (the centimetre to metre scale) than ecotones (i.e. ecotones describe the transition between adjacent plant communities). However, few (if any) studies to date have examined the influence of microedges on plant communities.

My objective was to investigate the influence of soil homogenization on plant species diversity and community composition during early ecosystem succession at the beginning of a tallgrass prairie restoration on former cropland. I also examined plant species diversity and composition along microedges. Two different sources of substrate heterogeneity – sand and woodchips – were added to the topsoil layer of a former agricultural field and compared to homogeneous treatments where the same materials were added but distributed homogeneously. Microtopographic heterogeneity was investigated by comparing pits and mounds with flat soils. Total canopy cover and density (number of individuals) were characterized in the early growing season and late summer and at two different spatial scales, and precipitation patterns were documented, with the goal of furthering understanding of variability in heterogeneity-plant relationships. Based on the assumption that the availability of distinct soil microsites provides unique ecological niches, and thereby increases coexistence and diversity, I hypothesized (1) soil homogenization would decrease plant species diversity and alter community composition and (2) microedges would exhibit properties unique from their adjacent patches, providing additional niche spaces for increased plant diversity.

2.2 Methods

2.2.1 Research site

The study was conducted in a four hectare tallgrass prairie restoration site located in a former agricultural field at the Environmental Sciences Western field station near Ilderton, Ontario, Canada (43º04'29''N, 81º20'18''W). The local area has London Clay Loam soils with well to imperfectly drained silt loam and loam glacial till (Hagerty and Kingston 1992), mean annual precipitation 1012 mm and air temperature 7.9 ºC, with mean January low -5.6 ºC and July high 20.8 ºC for 1981-2010 (Environment and Climate Change Canada 2018). Prior to the experiment and restoration, the field was used for cash cropping under annual rotations of soybean, corn, winter wheat and red clover (cover crop).

2.2.2 Experimental design and treatments

Soil treatment plots were set up in May 2015, prior to the prairie restoration. The experimental area was divided into 28 blocks. Plots within blocks were 100 cm × 50 cm and surrounded by a 2 m wide buffer zone (Fig. 2.1). Plots were prepared manually with shovels and a 100 cm × 50 cm plastic frame, with plastic buckets used to mix the soil components. For the substrate heterogeneity treatments, heterogeneous plots were constructed consisting of two halves: 1) a 50 × 50 cm area and 15 cm deep soil patch containing either a 4:1 mix of sand and topsoil (sand patch) or a 2:1 mix of sugar maple woodchips and topsoil (woodchip patch) and 2) a 50 × 50 cm patch of topsoil with no modification other than being mixed with a shovel down to 15 cm depth to match the disturbance effect in the other half of the plot. The edge in the middle of each plot, between the two patches, was defined as the microedge. The specific ratios of sand and woodchips to topsoil were determined in preliminary greenhouse experiments to produce highly contrasting rates of germination and growth among species compared to topsoil. Sand was obtained from a local quarry in London, Ontario and woodchips were collected from sugar maple trees cut down on the field site property and stored outside in a stockpile for one year prior to the experiment. The topsoil blended to make the sand- and woodchip-enriched patches was collected from each plot when the top 0-15 cm of topsoil

was removed to construct the patch. To explore the effects of homogenization, corresponding plots were prepared as described above, but then mixed with a shovel down to 15 cm depth across the entire 100 cm × 50 cm plot area.

To explore the effects of microtopographic homogenization, three types of plots were constructed using topsoil. The first consisted of a 50 × 50 cm × 15 cm deep pit located next to a level, 50 × 50 cm patch of background topsoil mixed with a shovel down to 15 cm depth. The pits were dug to 30 cm depth and backfilled with 15 cm of topsoil to maintain the appropriate soil depth profile. The second type of plot consisted of a 50 × 50 × 20 cm high mound located next to a level, 50 × 50 cm patch of background topsoil, with 15 cm of subsoil placed below the mound to maintain the soil depth profile. I examined the effect of topographic homogenization by comparing the pit and mound plots to level topsoil plots mixed with a shovel down to 15 cm depth. The seven different plot types in total were replicated once within each of 18 blocks (Fig. 2.1). In connection with a different experiment, 20 additional sand and woodchip homogeneous and heterogeneous plots each were replicated within an added set of blocks, giving n=38 for the substrate heterogeneity treatments, although the substrate treatments were reduced to n=28 in the final two years of the study. Note, none of the Chapter 2 and Chapter 3 plots received snow removal (only Chapter 4 plots).

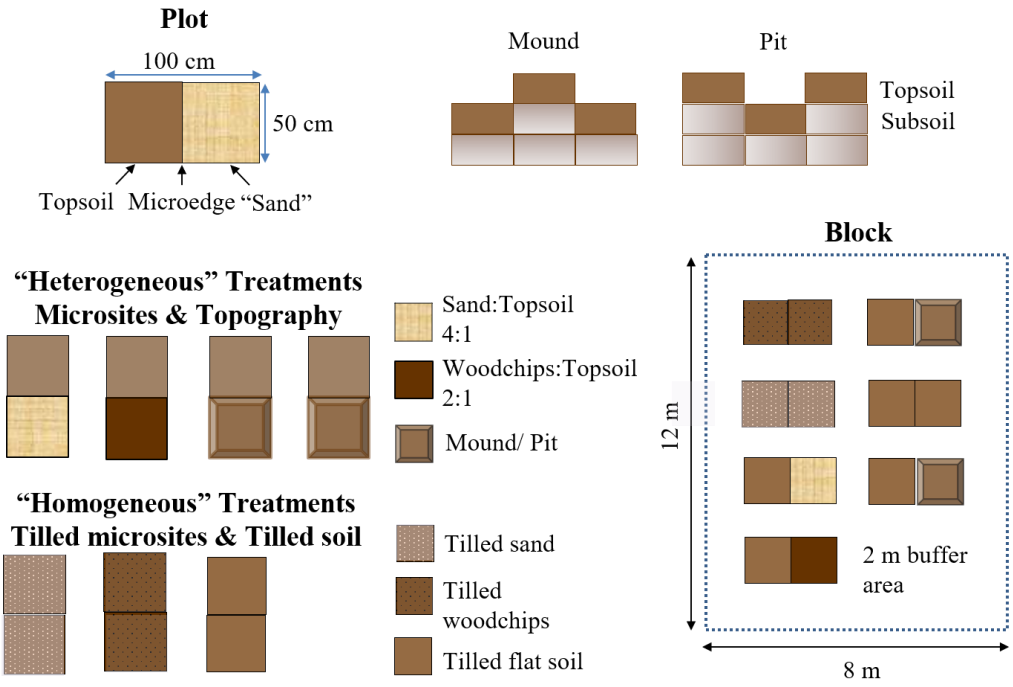

Figure 2.1 Experimental design. Plots were used as replicates of the seven different treatments (displayed left). The upper right depicts a cross section of a pit and mound. Plots were randomly assigned to 28 blocks.

2.2.3 Planting

Following plot establishment, in late May 2015, glyphosate was sprayed over the entire experimental area to kill the resident vegetation. In June 2015, plot seeding took place at the same time that the entire restoration site was being planted with a tractor, with the research blocks seeded separately by hand. The tallgrass prairie species were seeded evenly over each plot, with each plot receiving the same amount of seed per species. Seeding was repeated a second time in May 2016 to increase the available species pool, and a total of 34 forbs and 14 graminoids were seeded (Appendix Table A.1). Species were chosen to represent a diversity of native tallgrass prairie plant functional types (e.g. C_4 and C_3 grasses, leguminous and non-leguminous forbs) and the amount of seed planted per species was determined based on standard rates used in restoration (Smith et al. 2010). Seed of each species was counted out in early April 2015 and mixed with dry sand (grasses and forbs) and moist potting soil (forbs requiring moist stratification) in separate bags for each plot and stored at 4 °C. Each bag (seed mix) was diluted and thoroughly mixed in a bucket with an equal volume of potting soil (4 L) as a filler to increase volume, to allow the seed mix for each plot to be scattered evenly over the entire plot surface. After seeding, each plot was watered with one full watering can (2 L) and covered with a biodegradable erosion control blanket to prevent soil and seed movement. The buffer area around the plots was seeded with a subset of prairie species using the same methods discussed above to provide consistent background vegetation (Table A.1).

Seeds were obtained by wild collection from local sites in southern Ontario or by purchase of regionally similar ecotypes from Ernst Conservation Seeds (Meadville, PA, USA). Percent viable seed was determined either by using certificates obtained at the time of purchase (which listed percent germination) or, for wild collected seed, by sowing a pre-determined amount of seed on potting soil-filled trays in a greenhouse, counting emerging seedlings for 14 days, and dividing the number of emerging seedlings by the number sown and multiplying by 100 to calculate percent emergence (Table A.1). All seeds sown were produced and collected within two years of planting and kept in cold dry storage at 4 °C until sowing.

2.2.4 Soil properties

I assessed the properties of the sand, woodchips and topsoil used to create the homogeneous and heterogeneous treatment plots. Topsoil was sampled by taking a 2 cm diameter by 15 cm deep soil core from a random location within each block just outside the study plots. Sand and woodchips samples were collected randomly from the stockpiles on site. Sand-topsoil and woodchip-topsoil mixtures were prepared for analysis using the same ratios as present in the experimental plots. Soil pH was measured by mixing 10 g of soil from each sample with deionized water in a 1:2 ratio to create a slurry, and analyzing the slurry with a pH meter. Extractable NH_4^+ and NO_3^- were measured by extracting 7 g of each soil sample in 35 ml of KCl for 1 h on a shaker, filtering through pre-leached cellulose filter paper, then analyzing the filtrate colourimetrically (NH_4^+-N: EPA method 353.2; NO_3^--N: EPA method 350.1) using a SmartChem 140 discrete auto-analyzer (Westco Scientific Instruments, Brookfield, CT). Percent sand, silt and clay were determined for a 20 g subsample using a graduated cylinder, and soil organic matter as loss on ignition following drying of 1 g of the sample in a muffle furnace at 500 °C for 24 h. Total carbon and nitrogen data were obtained from soil mass spectrometry analyses performed on soil cores taken from experimental plots (Chapter 3). Soil properties are described in Table A.2 and Table A.3.

2.2.5 Vegetation sampling

Vegetation in all plots was surveyed twice per growing season in late spring and late summer (June-July and September-October) during 2015–2017 to coincide approximately with the timing of peak biomass for the different plant species. Plots were sampled for plant species diversity and community composition by overlaying three 25 × 25 cm (0.0625 m^2) sampling quadrats over the plots. One quadrat was placed in the center of each of the two 50 × 50 cm halves of each plot, and a third quadrat was placed along the edge, between the two halves, in the center of the plot (Fig. 2.2). Homogeneous plots also had three quadrats placed in the same positions to equalize sampling effort. Percent canopy cover of each individual plant species rooted in each quadrat, and ground cover (bare ground, moss, vegetation, litter, rock), was visually estimated. The number of

individual plants of each species also was counted to give an estimate of species density. Nomenclature followed Voss and Reznicek (2012).

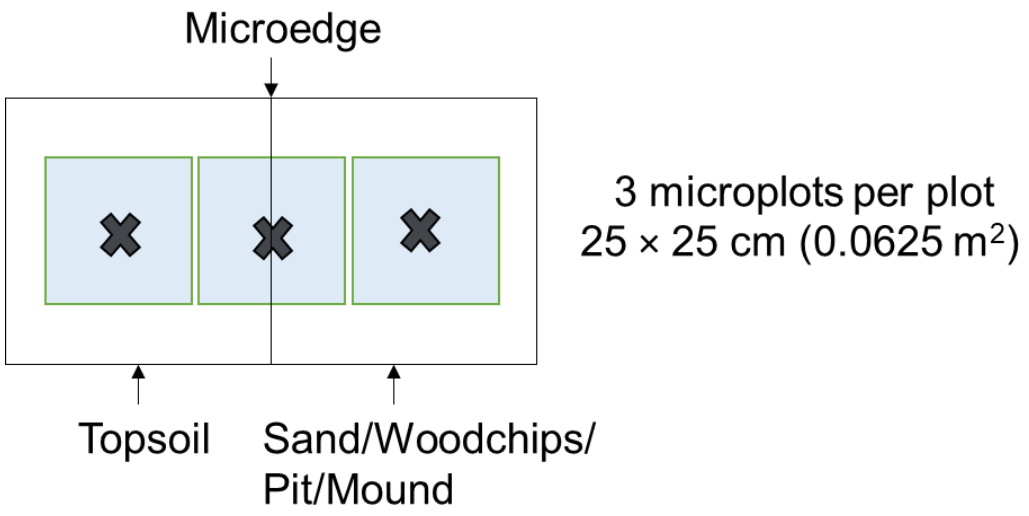

Figure 2.2 Vegetation sampling design. Within each plot three microplots (quadrats) were placed, each 25 × 25 cm (0.0625 m^2). Individual plant species cover and density were measured within each quadrat.

2.2.6 Meteorological conditions

Mean daily temperature, total monthly precipitation and long-term climate normals for the time period of the study were obtained from the Environment and Climate Change Canada National Climate Data and Information Archive (Environment and Climate Change Canada 2018). In addition, at the Environmental Sciences Western field station, the water level on a rain gauge was recorded by Research Technician C. Rasenberg after each rainfall during April 1 to October 31 from 1988 to 2017 to provide a local estimate of total monthly precipitation over time.

Mean monthly temperatures from April to October 2015–2017 were typical of the long-term average (Table A.4). However, precipitation from April to October 2015–2017 was approximately 20 % less than long-term climate normals (Table A.4). The second year (2016) was drier than long term normals during April to June (the early growing season), when plants require the most moisture to establish, whereas 2017 was drier than the climate normals later in the growing season from July to September (Table A.4). During these dry spells, precipitation was at least 50 % lower than the long term normals or lower.

2.2.7 Data analyses

The Shannon index of species diversity and species richness were calculated in R using dplyr, vegan and rich (Rossi 2011, Oksanen et al. 2016, Wickham and Francois 2016). Total canopy cover was calculated by summing the individual species cover values. Species cover data also were pooled into the following vegetation categories for analysis: native (seeded) vs. adventive (non-seeded), with both subdivided into graminoids, leguminous forbs and non-leguminous forbs. All response variables (Shannon index, richness, functional group cover categories and total cover and density) were calculated for each quadrat (subplot) (Fig. 2.2). The effects of homogenization on the response variables were then analyzed using a linear mixed model with block and plot nested within block as random effects and soil treatment (the seven levels of woodchips, sand, topography heterogeneous and homogeneous), year (2015, 2016, 2017) and season (early summer versus late summer) as fixed effects. Quadrat was nested within plot in the error

term and response variables were averaged over the three 0.0625 m^2 sampling quadrats to estimate soil treatment effects. The model quantified the means of responses at the patch level (0.0625 m^2). In addition to the above, for Shannon index and species richness, I ran the same model a second time, but instead of taking individual quadrat values, I modelled the means of diversity responses at the whole plot scale by pooling quadrat data for species occurrences/abundances for each plot (0.0625 $m^2 \times 3 = 0.1875$ m^2). This follow-up analysis provided a second spatial scale of observation for the data. An additional linear mixed model also was used to test for significant differences among subplots and microedges (i.e. the same model described above was used except 'soil treatment' was replaced with the fixed effect 'subplot' consisting of the levels microedge, topsoil patch and microsite – sand, woodchips, pit or mound).

For each response variable having significant soil treatment effects, within each heterogeneity source (sand vs woodchips vs topography), an *a-priori* contrast (assessed with a t-test) was used to compare heterogeneous to the homogeneous mean; the same approach was also used to compare means among the microedge and the two adjacent patches. A $\log_{10}(y+1)$ transformation was used for cases when residuals did not meet assumptions of normality or homogeneity of variance. For response variables with residuals that could not be transformed to meet assumptions Friedman's test and Wilcoxon rank sum tests were used. Statistical analyses were conducted with R v. 3.3.3 (R Core Team 2017). For all statistical tests alpha was 0.05 but marginally significant differences at $p < 0.1$ were also considered biologically important and reported.

2.3 Results

2.3.1 Plant species richness and Shannon diversity index

Soil homogenization significantly altered species richness and Shannon index, and these effects varied over time and among the different sources of heterogeneity. There was a significant year by treatment interaction for species richness ($p < 0.0001$, $F_{12,3077} = 8.8$) and Shannon index ($p < 0.0001$, $F_{12,3067} = 16.3$). For the woodchip treatment, soil homogenization increased richness in the second ($p = 0.010$, $t_{506} = -2.6$) and third year ($p < 0.0001$, $t_{506} = -4.87$), and it increased Shannon index in all years (year 1: $p = 0.001$, t_{378}

= -3.3; year two: $p = 0.015$, $t_{492} = -2.44$; year three: $p < 0.001$, $t_{492} = -4.4$) (Fig. 2.3a-b). Within the woodchip heterogeneous plots, the microedge species richness and diversity ranged among years from being intermediate between topsoil and woodchip patches to more similar to the topsoil patches (Table 2.1). For the sand treatment, soil homogenization decreased species diversity in the first year ($p = 0.070$, $t_{378} = 1.82$) and richness in the final year ($p = 0.085$, $t_{506} = 1.73$) (Fig. 2.3a-b). In the sand heterogeneous plots, the sand patches had greatest diversity and richness in all three years, and there was no significant difference between the topsoil patches and microedges (Table 2.1).

For the microtopography treatments, Shannon index and richness in the pit plots were lower than the flat plots in year one ($p = 0.007$, $t_{378} = 2.71$ and $p = 0.035$, $t_{385} = 2.12$, respectively), whereas Shannon index was higher in the pit plots than in the flat plots in the second year ($p = 0.01$, $t_{389} = -2.57$) and third year ($p = 0.068$, $t_{389} = -1.83$) (Fig. 2.3a-b). Shannon index and richness were equivalent among subplots for the pit treatment (Table 2.1). Mound plot Shannon index and richness were not significantly different from those of flat plots (Fig. 2.3a-b), although in year one the microedges in the mound plots had lower diversity than the adjacent patches (Table 2.1).

When the models for Shannon index and species richness were run a second time to characterize species diversity at the whole plot scale (0.1875 m^2), the same effects as mentioned above were observed for the sand treatment, but were more significant (Fig. A.1). The diversity trends for pit plots and woodchip plots were non-apparent and weaker, respectively (Fig. A.1). Mound plot Shannon index was marginally significantly greater than flat plots in the first year (Fig. A.1).

Table 2.1 Mean species richness and Shannon index (standard error) in microsites present in heterogeneous plots.

		Species richness			Shannon index		
		2015	**2016**	**2017**	**2015**	**2016**	**2017**
Woodchips	Topsoil	3.2 (0.2)a	8.0 (0.3)a	8.0 (0.4)a	0.67 (0.06)a	1.26 (0.06)a	1.40 (0.06)a
	Microedge 2:1	1.8 (0.2)b	6.4 (0.3)b	7.5 (0.3)a	0.40 (0.05)b	1.19 (0.06)ab	1.33 (0.06)a
	Woodchips topsoil patch	0.9 (0.1)c	4.8 (0.3)c	5.7 (0.3)b	0.12 (0.03)c	1.10 (0.07)b	1.18 (0.07)b
Sand	Topsoil	4.2 (0.3)a	8.5 (0.3)a	8.8 (0.4)a	0.93 (0.06)a	1.48 (0.05)a	1.51 (0.05)a
	Microedge	4.6 (0.3)a	9.0 (0.4)a	8.8 (0.4)a	1.05 (0.07)a	1.50 (0.06)a	1.49 (0.05)a
	4:1 Sand topsoil patch	6.5 (0.3)b	11.0 (0.3)b	10.2 (0.4)b	1.48 (0.06)b	1.71 (0.04)b	1.69 (0.04)b
Pit	Topsoil	3.9 (0.4)a	8.3 (0.4)	9.5 (0.4)a	0.89 (0.10)a	1.41 (0.05)	1.60 (0.05)
	Microedge	2.4 (0.3)b	8.3 (0.5)	8.3 (0.4)b	0.54 (0.08)b	1.48 (0.05)	1.52 (0.07)
	Pit	2.1 (0.3)b	8.3 (0.4)	8.5 (0.4)b	0.48 (0.09)b	1.53 (0.06)	1.58 (0.06)
Mound	Topsoil	4.7 (0.4)a	8.7 (0.4)a	9.2 (0.5)	1.08 (0.08)a	1.42 (0.06)	1.55 (0.05)
	Microedge	3.6 (0.4)b	7.8 (0.4)b	9.1 (0.4)	0.77 (0.10)b	1.33 (0.07)	1.61 (0.05)
	Mound	3.4 (0.4)b	7.2 (0.5)b	8.9 (0.5)	0.89 (0.09)ab	1.30 (0.08)	1.48 (0.06)

Within year and heterogeneity source, means with different letters are significantly different ($p < 0.05$).

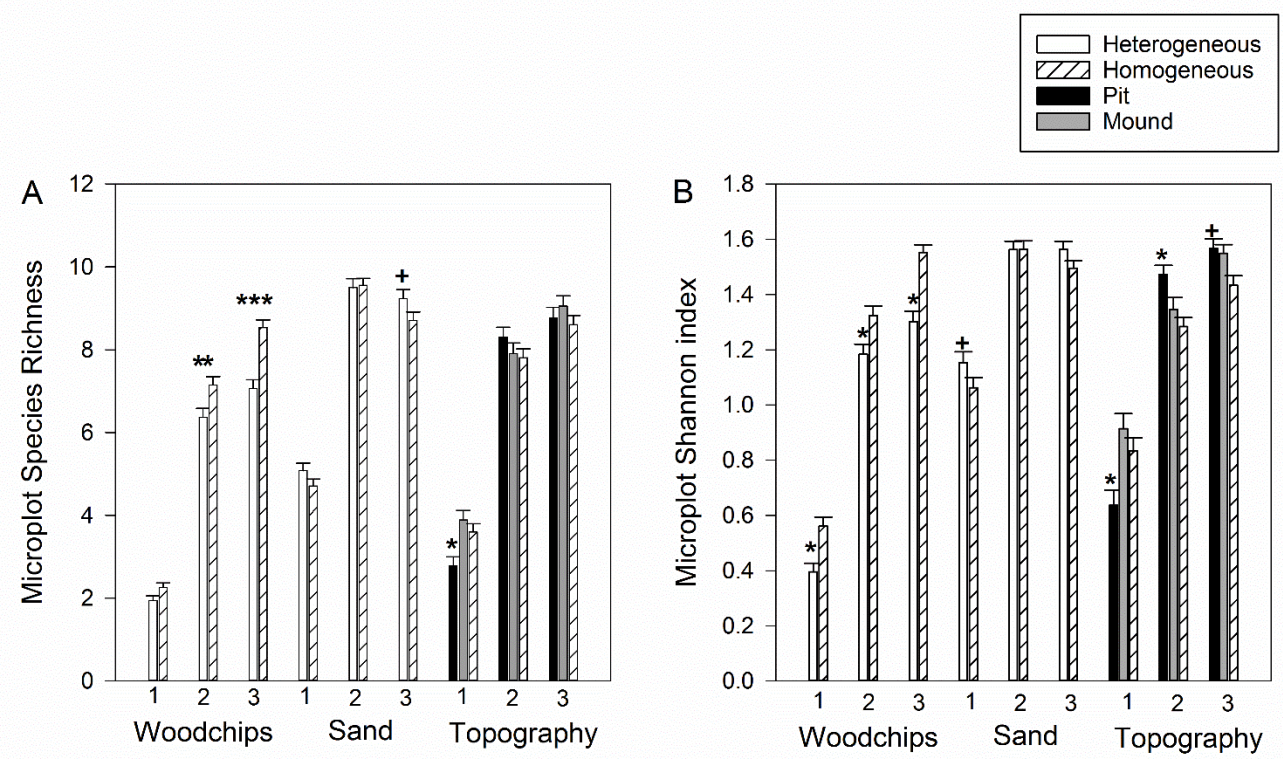

Figure 2.3 Mean species diversity over three years (1 = year 1, 2015; 2 = year 2, 2016; 3 = year 3, 2017) in the three different sources of heterogeneity studied. Part A is species richness and B is Shannon index for mean microplot diversity (spatial scale 0.0625 m^2). Within year and heterogeneity source, heterogeneous treatments followed by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) are significantly different from the homogeneous treatment and + are marginally significantly different ($p < 0.1$).

2.3.2 Total canopy cover and density

Total canopy cover and density were significantly affected by soil homogenization but the effects varied over time and among heterogeneity sources. A significant year by treatment interaction for total canopy cover ($p < 0.0001$, $F_{12,3081} = 6.3$) and density ($p < 0.0001$, $F_{12,3071} = 23.5$) occurred. For total canopy cover, there was a marginally significant decrease with homogenization for the woodchip treatment ($p = 0.09$, $t_{551} = 1.7$) (Fig. 2.4a). Within the woodchip heterogeneous plots, the cover of the microedges was intermediate between the adjacent patches (Table 2.2). For the sand treatment, homogenization decreased total canopy cover in the third year ($p = 0.02$, $t_{677} = 2.3$) (Fig. 2.4a). Within the sand heterogeneous plots, the sand patches had greater cover than the microedges and topsoil patches in the third year (Table 2.2). Flat plots had greater total canopy cover than the pit plots in all three years (e.g. year two $p < 0.001$, $t_{565} = 4.69$) (Fig. 2.4a). Within pit plots, the pits and microedges had lower total canopy cover than the flat topsoil patches in all years (Table 2.2). Flat homogeneous plots had greater cover than the mound plots in years one ($p = 0.098$, $t_{551} = 1.7$) and two ($p = 0.02$, $t_{551} = 2.4$) (Fig. 2.4a). Within mound plots, the mounds and edges had lower total canopy cover than the flat patches in all three years (Table 2.2).

Soil homogenization increased plant density in the woodchip treatment in all three years ($p < 0.001$, $t_{545} = -4.4$) (Fig. 2.4b). For the woodchip heterogeneous plots, the woodchip patches had lower density than the topsoil patches in the second and third year (Table 2.2). Woodchip microedges were most similar to the woodchip patches in year two and to the topsoil patches in year three (Table 2.2). There were no significant effects of homogenization on density in the sand plots (Fig. 2.4b). The flat plots had greater density than the pit plots in year one, ($p = 0.001$, $t_{431} = 3.2$) and lower density in the second year ($p < 0.001$, $t_{443} = -6.37$) (Fig. 2.4b). Within pit heterogeneous plots, pits had the greatest density in years two and three, and the microedges had lower density than the flat patches and pits in the second year (Table 2.2). There were no significant differences in density between the mound and flat plots (Fig. 2.4b).

Table 2.2 Mean total canopy cover and density (standard error) in microsites present in heterogeneous plots.

		Total canopy cover			Density		
		2015	**2016**	**2017**	**2015**	**2016**	**2017**
Woodchips	Topsoil	28.2 (4.0) a	82.8 (7.1) a	87.6 (5.9) a	5.2 (0.4)	67.1 (6.3) a	39.3 (2.8) a
	Microedge 2:1	14.7 (2.6) b	44.6 (5.1) b	65.0 (5.5) b	3.3 (0.3)	45.9 (4.8) b	40.9 (3.4) a
	Woodchips topsoil patch	3.6 (1.4) c	17.9 (3.3) c	32.8 (3.7) c	1.9 (0.2)	40.6 (4.7) b	24.6 (2.4) b
Sand	Topsoil	25.3 (3.2)	72.0 (6.0)	96.5 (6.7) a	7.3 (0.6)	69.5 (7.6) a	43.2 (3.0)
	Microedge	24.8 (2.9)	72.0 (6.2)	91.8 (5.7) a	7.8 (0.5)	61.0 (4.2) a	41.5 (2.7)
	4:1 Sand topsoil patch	26.2 (3.8)	83.6 (6.2)	146.0 (7.6) b	13.0 (0.7)	82.2 (9.0) b	48.3 (2.2)
Pit	Topsoil	29.1 (5.6) a	76.9 (7.7) a	112.3 (9.0) a	5.5 (0.8)	104.6 (16.7) a	48.5 (4.7) ab
	Microedge	21.3 (4.4) ab	50.4 (7.1) b	71.2 (7.2) b	1.8 (0.4)	73.6 (9.4) b	39.3 (3.5) a
	Pit	15.8 (4.0) b	47.2 (5.0) b	63.1 (6.0) b	1.6 (0.5)	159.1 (18.8) c	60.8 (4.8) b
Mound	Topsoil	33.4 (5.3) a	91.2 (8.0) a	100.5 (6.7) a	7.1 (1.0)	54.7 (6.1)	44.2 (3.5)
	Microedge	26.7 (4.9) ab	67.2 (8.4) b	83.9 (8.8) b	4.0 (0.6)	54.6 (7.4)	44.6 (2.6)
	Mound	17.5 (3.8) b	57.1 (7.2) b	86.1 (7.4) ab	4.4 (0.7)	68.4 (11.4)	53.0 (5.9)

Within year and heterogeneity source, microsites with different letters are significantly different ($p < 0.05$).

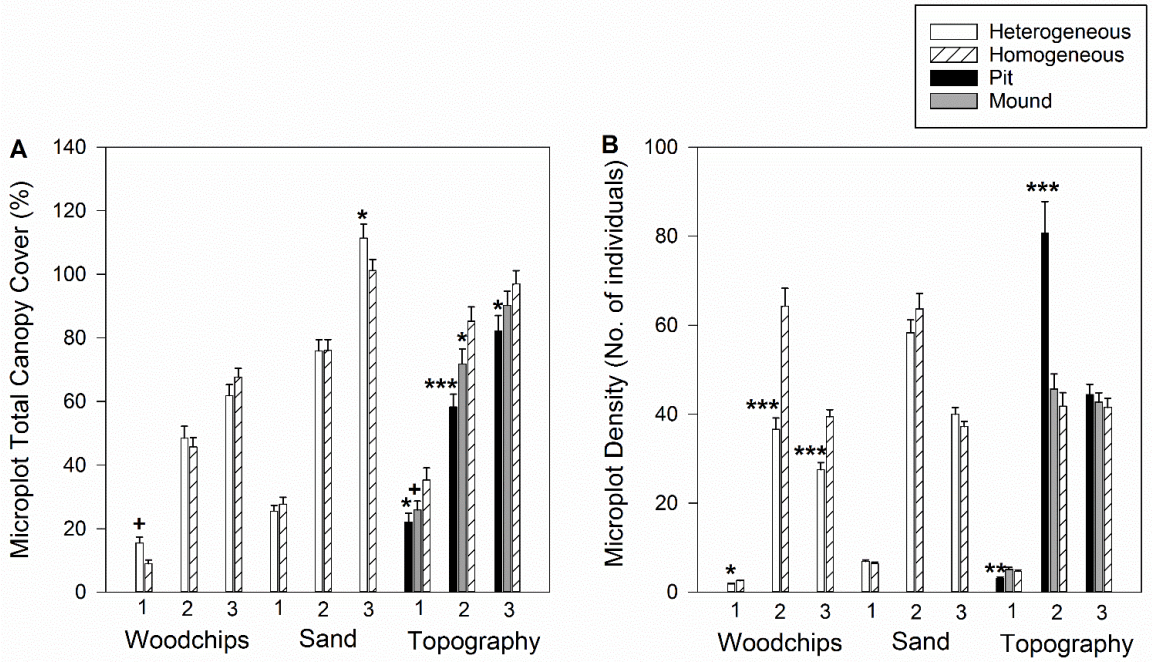

Figure 2.4 Mean plant abundance over three years (1 = year 1, 2015; 2 = year 2, 2016; 3 = year 3, 2017) in the three different sources of heterogeneity studied. Part A is total canopy cover (%) and B is density (number of individuals) for mean microplot abundance (spatial scale 0.0625 m^2). Error bars are standard error. Density was backtransformed from the log scale. Standard error in the positive direction is shown. Within year and heterogeneity source, heterogeneous treatments followed by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) are significantly different from the homogeneous treatment and + are marginally significantly different ($p < 0.1$).

2.3.3 Functional group cover

For seeded grass cover there was a significant year by treatment interaction ($p < 0.0001$, $F_{12,3067} = 3.3$). Seeded grass cover was reduced by soil homogenization in the sand treatment in the second year, but this effect was only marginally significant ($p = 0.08$, $t_{404} = 1.8$) (Fig. 2.5a). There was no effect of homogenization on seeded grass cover in woodchip plots, where there was low seeded grass cover (below 10 %) in all study years (Fig. 2.5a). Soil homogenization increased seeded grass cover in flat plots relative to pits in the second ($p = 0.01$, $t_{311} = 2.6$) and third year ($p = 0.04$, $t_{311} = 2$) and there was a marginally significant effect in mounds compared to flat plots in the third year ($p = 0.088$, $t_{302} = 1.7$) (Fig. 2.5a).

There was a significant year × season × treatment interaction for seeded forbs ($p = 0.025$, $F_{12,2980} = 1.95$). During the early part of the growing season, homogenization increased cover in the woodchip treatment in the third year ($p = 0.01$, $t_{812} = -2.5$) (Fig. 2.5b), but the effect weakened later in the growing season ($p = 0.09$, $t_{812} = -1.7$) (Fig. 2.5c). There were no significant effects of homogenization on seeded forb cover for sand plots (Fig. 2.5b-c). Homogenization significantly increased seeded forb cover in flat plots relative to pit and mound plots by the late growing season in the first (pit: $p < 0.0001$, $t_{591} = 5.7$, mound: $p = 0.0028$, $t_{591} = 3$) and second year (pit: $p < 0.0001$, $t_{611} = 4.3$, mound: $p = 0.019$, $t_{591} = 2.35$) (Fig. 2.5c). However, in the third year, seeded forb cover was similar, with the exception of pit plots early in the growing season ($p = 0.05$, $t_{611} = 1.96$) (Fig. 2.5b). Mound and woodchip microedges had seeded grass and forb cover intermediate between their neighboring microsites (Table A.5). Seeded legume cover was low throughout the three years (mean 2.2 %, standard deviation 2.6 %) and few significant differences were found.

Adventive (non-seeded) grass cover was greatest in the second year and very minimal (below 2 %) in the third year (year-season combinations were analyzed with Friedman's tests). In the woodchips treatment, adventive grass cover increased with soil homogenization in the early growing season of the second year ($p = 0.07$, $F_R = 3.2$) and in the third year in the late growing season ($p = 0.03$, $F_R = 4.5$) (Fig. 2.5d-e). Mound plots

had triple the amount of adventive grasses in the first year than flat plots, which resulted in a marginally significant trend in both seasons (e.g. late season: $p = 0.058$, $F_R = 3.6$) (Fig. 2.5d-e). Pit heterogeneous plots had more adventive grass cover than flat plots in the second (late growing season: $p = 0.046$, $F_R = 4$) and third year (early growing season: $p = 0.0075$, $F_R = 7.1$), although the trend weakened by the third year in the late growing season ($p = 0.6$, $F_R = 0.3$) (Fig. 2.5d-e).

There was a significant year by treatment interaction for adventive forbs ($p < 0.000001$, $F_{12,3078} = 8.9$). Homogenization reduced cover in the first year in the woodchip treatment ($p = 0.075$, $t_{376} = 1.8$) but by the third year homogenization increased adventive forb cover ($p = 0.0017$, $t_{495} = -3.16$) (Fig. 2.5f). Adventive forb cover was greater in pit plots relative to flat plots every year, and most significantly in the second year ($p = 0.03$, $t_{387} = -2.17$) (Fig. 2.5f). Adventive legume cover was very low throughout the three years (mean 1.6 %, standard deviation 1.6 %) and few significant differences were found.

A list of all plant species found in the research plots and their mean cover in each treatment is provided in Tables A.6-A.11.

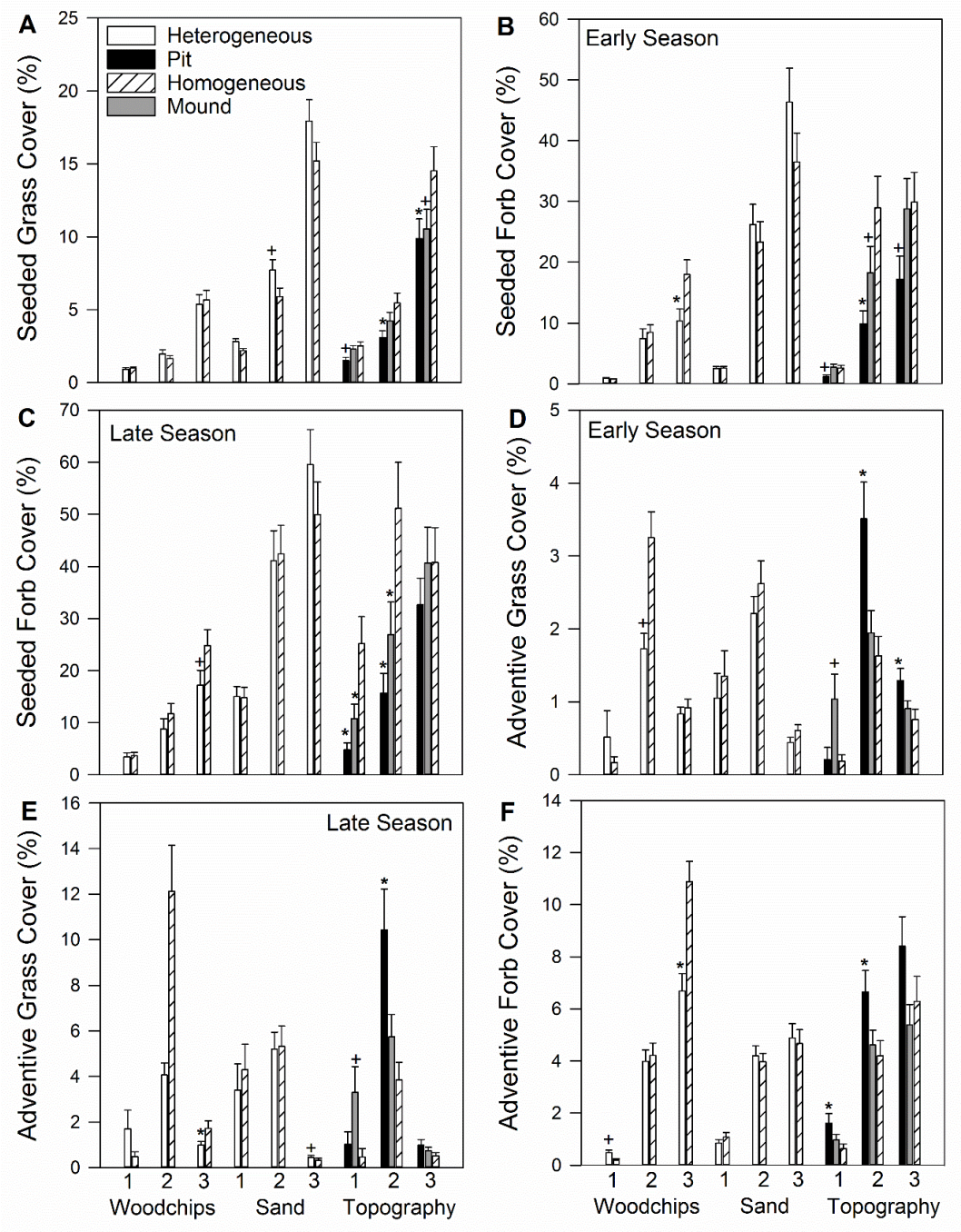

Figure 2.5 Mean functional group cover over three years (1 = year 1, 2015; 2 = year 2, 2016; 3 = year 3, 2017) in the three different sources of heterogeneity studied. Numbers were back-transformed from the log scale $\log_{10}(y+1)$, standard error in the positive direction is shown. Within each year and heterogeneity source, heterogeneous treatments followed by * are significantly different from the homogeneous treatment ($p < 0.05$) and + are marginally significantly different ($p < 0.1$).

2.4 Discussion

This study provides evidence that soil homogenization alters plant species diversity and community composition. In the sand and topographic heterogeneity treatments, soil homogenization decreased species diversity. Functional group cover and diversity differed among the topsoil, microedge and sand, woodchip, pit and mound microsites. Therefore, the microsites in this study likely contributed to diversity patterns by functioning as unique niches and facilitating species sorting due to their unique properties, which supports the importance of microtopographic heterogeneity in influencing plant species composition and diversity patterns in early succession and restoration (Biederman and Whisenant 2011, Deák et al. 2015, Naeth et al. 2018). Soil heterogeneity derived from disturbance (Wilson and Tilman 2002, Questad and Foster 2008), vertical soil horizons (Fitter 1982, Williams and Houseman 2014), and microtopography (Vivian-Smith 1997) was experimentally shown to increase plant diversity, and I demonstrated that heterogeneity in soil particle size also can have a positive influence on plant species diversity. This result contrasts that of greenhouse experiments that used crushed rock to create soil heterogeneity and observed a null effect on plant diversity and productivity (Grime et al. 1987, Xue et al. 2016).

Microedges exhibited properties unique from their adjacent patches, indicating they may provide additional niche space for increased plant diversity. For example, low plant density was observed along pit microedges and low plant diversity along mound microedges. Microedges also had vegetation and soil properties that were intermediate between adjacent patches (e.g. canopy cover for the heterogenous woodchip plots), suggesting they may function as small-scale ecotones. However, I did not observe increased species diversity along microedges in this study. Few studies have considered the role of microedges (Stover and Henry 2018); however, a common theme is the recognition of larger scale ecological processes analogous to small scale processes. For example, microfragmentation (i.e. increased patchiness with increased heterogeneity), may be a mechanism whereby increased heterogeneity can decrease diversity by increasing fragmentation at a small scale (Tamme et al. 2010, Laanisto et al. 2013).

The combination of null, negative and positive effects of soil heterogeneity on plant species diversity that occurred in this study may help clarify understanding of the variability observed in heterogeneity-diversity relationships. This variability can be explained, in part, by the wide variety of sources of heterogeneity (e.g. topography, soil texture, temperature, etc.), with each having its own unique characteristics. However, there are important commonalities among studies that will lead to better prediction of heterogeneity-diversity relationships. Patterns in plant size or productivity were associated with diversity patterns in this study, with the least productive treatments (as measured by total canopy cover) with the greatest plant density often having the greatest plant diversity. This observation suggests that heterogeneity or homogeneity treatments that result in a large number of small plants will likely also have high diversity. Density-driven diversity effects are often attributed to stochastic/neutral processes, and thought to be an independent diversity mechanism that should be controlled for in heterogeneity-diversity studies (Williams and Houseman 2014, Walker and Lundholm 2018). However, my results suggest that varying levels of soil heterogeneity can significantly influence plant size and density, and could be an additional mechanism explaining how heterogeneity influences diversity. Similarly, microhabitat heterogeneity in a limestone alvar pavement increased seedling density and richness in early establishment, and increased density associated with increased richness was attributed to a 'diversity model' for community assembly (Tilman 1994, Richardson et al. 2012). Collectively, these results indicate there may be a potential role of diversity-productivity relationships (e.g. Fraser et al. 2015) in influencing heterogeneity-diversity relationships.

Contrary to my hypothesis, homogenization increased diversity in the woodchip treatment. Woodchip microsites within the heterogeneous plots had lower levels of available nitrogen, which can reduce plant diversity (e.g. as with the heterogeneous conditions of reduced nitrogen supply created by sawdust addition by Baer et al. 2004). The latter contrasted with the topsoil microsites within the woodchip heterogeneous plots, which were dominated by large tallgrass prairie grasses and forbs; both scenarios corresponded with low plant density and low diversity. When plant size exceeds microsite size, diversity may decrease because plants can easily forage and compete for

resources among different patches (Lundholm 2009, Stover and Henry 2018). The homogeneous woodchip plots may have supported greater diversity due to a 'goldilocks' effect, because the even spatial distribution of the woodchips may have had a beneficial effect on seed germination by reducing water loss, moderating soil surface temperatures and reducing erosion (Naeth et al. 2018).

In addition to plant growth patterns, interactions between environmental conditions and soil heterogeneity appeared to be influential in this study. Soil homogenization decreased diversity in the sand and microtopography plots, but in the second year, when an early growing season drought occurred, this effect was suppressed in the sand plots and enhanced in the pit plots. The sand plots may have been more sensitive to drought, with a lower water holding capacity compared to clays and silts, while the pits would have had the greatest capacity to retain soil moisture. The pits may have functioned as small scale refugia with elevated soil moisture during drought. Such effects have been demonstrated previously in climate warming experiments featuring microsites with variation in soil depth (Fridley et al. 2011). Similarly, Maestre and Reynolds (2007) found that increases in productivity in response to soil nutrient heterogeneity were the most pronounced as nutrient levels increased and at low soil moisture. Therefore, environmental conditions, especially soil moisture, are likely highly important in explaining variability in heterogeneity-diversity relationships.

Baer et al. (2016) proposed that environmental heterogeneity may be less important than direct manipulation of processes that reduce dominance in increasing plant diversity during community assembly. However, my results and others suggest that heterogeneity plays a direct role in influencing dominance/competition reducing processes, along with stress reducing processes, that can ultimately increase plant species diversity (Richardson et al. 2012). Given the importance of species interactions in heterogeneity-diversity experiments, it is surprising that 'plant-induced heterogeneity' has not been investigated more deliberately alongside 'environmentally-induced' heterogeneity, particularly because many recent studies have confirmed the importance of plant-soil feedbacks in promoting coexistence (e.g. Wubs and Bezemer 2018).

My results confirm that heterogeneity can drive diversity patterns at spatial scales of less than 1 m^2 (Lundholm 2009, Bergholz et al. 2017). However, heterogeneity-diversity relationships may be weak at fine spatial scales, especially with respect to soil nutrient heterogeneity, which often unintentionally favours competitive dominance of a few species (Gazol et al. 2013). Differences in diversity patterns were observed at very small changes in spatial scale in this study (between the plot 0.1875 m^2 and subplot 0.0625 m^2 scale), highlighting the sensitivity of heterogeneity-diversity relationship to changes in spatial scale, even at resolutions of less than 1 m^2. Diversity and functional group cover also varied temporally during the dynamic stages of early succession, and varied phenologically between the early and late growing seasons. Therefore, seasonal and temporal variation should be further considered in future studies. Although plant communities were monitored for three growing seasons, my study only took place during early succession, so additional long term (> 5 year) studies are needed. Indeed, most experimental studies report the results of heterogeneity diversity relationships during the first 1–3 years of plant establishment. Positive relationships observed may be a short-lived 'honeymoon effect' whereby the introduction of propagules overcomes dispersal limitation (Baer et al. 2016). However, events during early succession can have a lasting influence on plant communities in the long term (Houseman and Gross 2011), and only future long term studies can resolve whether positive relationships between heterogeneity and diversity observed are transient or more permanent features of plant communities and why.

Soil homogenization has not been considered explicitly in experimental investigations of heterogeneity-plant relationships to date, but my study showed it may represent an important outcome of disturbance that should be further researched. My results demonstrated that adding contrasting microsites at the 0.5 m spatial scale may facilitate increased plant species diversity, which is meaningful in the context of ecological grassland restoration. Few (if any) studies have considered microedges to date, so further research on microedges is required to determine their role in both promoting increased plant diversity and functioning as small scale ecotones. Variability in heterogeneity-plant relationships may be influenced by interactions with plant growth patterns and

environmental conditions. Overall, my results build upon a framework that is being developed across experimental studies for understanding variability in heterogeneity-diversity relationships, which will hopefully lead to better prediction and utilization of this ecological phenomenon as a conservation tool.

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Chapter 3

3 Soil homogenization modifies productivity, nitrogen retention and decomposition during grassland restoration

3.1 Introduction

In North America, less than one percent of native tallgrass prairie remains, largely due to conversion to agriculture (Sampson and Knopf 1994). Ecological restoration of tallgrass prairie and other native grassland ecosystems is occurring worldwide, with the goal of restoring ecosystem functions and biodiversity (Neill et al. 2015, Horrocks et al. 2016). For example, productivity, nitrogen retention and decomposition are important components of ecosystem function since they can regulate species composition and carbon storage (de Vries and Bardgett 2016, Zirbel et al. 2017). However, restoration of former cropland must address a legacy of soil disturbance (Krause et al. 2016). In particular, soil homogenization from decades of tillage (mixing of the upper topsoil) leads to more uniform habitat and soil properties in agricultural fields (Anderson and Coleman 1985, Elliott 1986). Greenhouse experiments on temperate grassland mesocosms have demonstrated that heterogeneous soil nutrient supply increases above and belowground productivity, nitrogen use efficiency and nitrogen uptake via root proliferation into nutrient patches (Maestre et al. 2005, 2006, 2007, Maestre and Reynolds 2006a, 2006b, 2007a, 2007b, Liu et al. 2017). While field experiments in restored grasslands have been used to examine the relationship between soil heterogeneity and plant species diversity (Richardson et al. 2012, Williams and Houseman 2014, Baer et al. 2016), they have not examined the influence of soil heterogeneity (or homogenization) on ecosystem responses (García-Palacios et al. 2012). The question therefore arises as to how soil homogenization may alter the ecosystem functioning of restored grassland ecosystems on former agricultural land.

Soil homogenization could directly decrease ecosystem functioning because of loss of substrate diversity and heterogeneity (i.e. a decrease in frequency and spatial variability of distinct soil patches, otherwise known as microsites). Variability in soil moisture

derived from microtopographic heterogeneity (hummocks and hollows) and variability in soil depth to bedrock can drive increased rates of productivity, nitrogen retention and decomposition in specific microsites that lead to overall greater rates than those observed in a more uniform soil. For example, decomposition rates may increase with increasing soil moisture (Zirbel et al. 2017). The interface between two microsites, microsite edges or 'microedges', may exhibit increased ecosystem function by possessing a blend of limiting resources from each neighboring microsite (Stover and Henry 2018). For example, soil microbial activity limited by a nutrient in one patch may be increased by the higher availability of that nutrient in the neighboring patch, and vice versa. Complementarity along microedges could be analogous to landscape level processes such as elemental cycling at wetland-upland transition zones (McClain et al. 2003). Increases in decomposition may occur along microedges analogously to litter mixture experiments where the presence of litter from several species decomposes faster than single species litter (Gartner and Cardon 2004). However, research is required to confirm if loss of microsites and microedges via homogenization decreases ecosystem function.

Loss of soil patches (soil homogenization) also could decrease productivity, ecosystem nitrogen retention and decomposition indirectly as a result of decreased plant species diversity. Niche theory suggests that a larger number of species can coexist in an ecosystem where there is a greater number of distinct niches available for species to colonize and differentially dominate (Tilman and Pacala 1993). Environmental heterogeneity therefore can increase species diversity (Stein et al. 2014) and increased species diversity can in turn benefit ecosystem function (Hooper et al. 2005). Heterogeneity can improve ecosystem function by providing diverse niches for complementarity in resource use to occur (Tylianakis et al. 2008). Conversely, reduced plant diversity could decrease the variability of rooting depths, rooting phenology and forms of nitrogen uptake in the community, decreasing nitrogen retention (McKane et al. 2002). Similarly, the rate of decomposition can decrease with reduced species diversity, because the litter pool lacks structural and chemical diversity, as observed in litter mixture experiments where the litter of individual species decomposes slower than that of multiple species (Gartner and Cardon 2004).

Plant compositional diversity rather than species diversity may benefit ecosystem function in the context of soil heterogeneity. For example, in pot experiments, manipulation of plant species diversity did not facilitate an increase in productivity in response to increased soil nutrient heterogeneity, but the presence of specific plant functional groups did (Maestre et al. 2006). Likewise, while carbon, phosphorus and nitrogen cycling (measured via beta-glucosidase and acid phosphatase enzyme activity, and *in situ* N availability) were not influenced directly by soil nutrient heterogeneity (García-Palacios et al. 2011), specific plant functional groups and traits had large effects on the cycling of these nutrients in response to nutrient heterogeneity (García-Palacios et al. 2013).

I investigated the influence of soil homogenization on productivity, nitrogen retention and decomposition in a grassland restoration field experiment conducted in a former agricultural field. I also investigated levels of productivity, decomposition and nitrogen retention along microedges (the edges between patches in heterogeneous treatments). Patches of sand and woodchips were added to the soil to construct heterogeneous plots, whereas the same materials were added then mixed into the surrounding area to construct homogenized plots. Patches with microtopographic relief (i.e. pits and mounds) also were used to create heterogeneous plots and compared with flat plots that were tilled (homogeneous plots). In addition to direct loss of substrate heterogeneity, homogenization was expected to decrease plant species and functional group diversity and reduce complementarity in resource use. Therefore, I hypothesized that soil homogenization decreases productivity, nitrogen retention and plant litter decomposition. Due to complementarity between adjacent microsites, I hypothesized microedges exhibit ecosystem function (i.e. productivity, nitrogen retention and plant litter decomposition) that is not a simple additive effect of the adjacent microsites.

3.2 Methods

3.2.1 Study site

I conducted this study at the Environmental Sciences Western Field Station, located near Ilderton, Ontario, Canada (43º04’29’’N, 81º20’18’’W). The site had a mean air

temperature of 7.9 ºC and annual precipitation of 1012 mm (1981-2010 Canadian Climate Normals), and the soil was characterized as London clay loam with a mean pH of 7.5 (Hagerty and Kingston 1992, Environment and Climate Change Canada 2018). The region was rural, and the 4 ha research site was situated in a field formerly used for cash cropping under rotations of corn, soybean and winter wheat for decades until 2014.

3.2.2 Experimental design

In May 2015, 18 experimental blocks were established. Each block contained homogeneous and heterogeneous treatment plots that were 50 × 100 cm and spaced 2 m apart. Heterogeneous plots were divided into two distinct halves: a 50 × 50 cm and 15 cm deep patch of tilled topsoil (mixed to a depth of 15 cm with shovels) and an adjacent 50 × 50 cm patch to provide either topographic heterogeneity (i.e. a pit or a mound) or substrate heterogeneity (i.e. a topsoil patch enriched with sand or a topsoil patch enriched with woodchips). The edge between the two in the center of the plot was the microedge investigated in this study. Therefore, heterogeneous plots had three distinct sampling areas (microsites): a topsoil patch, a microedge and a distinct microsite (sand, woodchips, pit or mound). The pits were 15 cm in depth and the mounds were 20 cm in height (the pits were underlain with 15 cm of topsoil and mounds with subsoil to make their substrate equivalent to the other side of the plot). The sand-topsoil patches were a 4:1 mixture of sand and topsoil, and the woodchip-topsoil patches were a 2:1 mixture of woodchips and topsoil. For each heterogeneous plot in a block, there was a corresponding homogenous plot: the topographically heterogeneous plots were compared to a flat, tilled topsoil plot, and the sand and woodchip patch plots were compared to plots with the corresponding ratios of topsoil, sand or woodchips tilled and mixed thoroughly across the entire 50 × 100 cm plot area. After the plots were installed, in early June 2015, an equal amount of tallgrass prairie grass and forb seeds were planted on each plot. The areas outside of the plots were planted separately with the same tallgrass prairie species. See Chapter 2 for further details, including figures for the experimental design, soil characteristics of microsites (e.g. pH, nutrients) and plant species and quantities of seed planted.

3.2.3 Productivity and diversity

Aboveground shoots rooted in the heterogeneous plots were harvested by overlaying three sampling quadrats (25 × 25 cm), with one in the center of each distinct patch and one on the center of the edge between the patches. Aboveground shoots were harvested from homogeneous plots using the same quadrats and positioning. Shoots were sorted into three functional group categories: grass, non-leguminous forb (forb) and leguminous forb (legume). Samples were dried at 50 ºC until their mass became constant (approximately 4 days) and weighed. The total mass of aboveground shoots (grasses, forbs and legumes) was used to estimate aboveground productivity. The relative abundance of grasses, forbs and legumes was used to estimate functional group diversity. See Chapter 2 for information on plant species diversity in each of the treatments.

3.2.4 Nitrogen retention

Growing season (over summer) nitrogen retention was assessed by applying a ^{15}N tracer solution ($^{15}NH_4{}^{15}NO_3$ at a rate of 0.054 g ^{15}N m^{-2}) evenly over each plot on 24 June, 2016. During November 2016, one soil core (2 cm diameter and 20 cm depth) was collected from the center of each homogeneous plot, and three were collected from heterogeneous plots: one in the center of each distinct patch, and one in the center of the edge between the two patches. Aboveground shoots and soil cores were sampled from outside the research blocks to provide a set of non-enriched control samples to establish the natural background level of ^{15}N at the site.

Soil samples were dried at 50 ºC until their mass became constant (approximately 4 days) and weighed. The grass, forb and legume aboveground biomass samples were bulked into a single sample for ^{15}N analyses. Soil and biomass samples were ground and weighed into tin capsules (4 ± 0.5 mg subsamples for plant material and 40 ± 3 mg for soil). Soils were ground using a mortar and pestle and plant samples were ground using a ball mill (SPEX Sample Prep Model 2000 Geno/Grinder, Metuchen, New Jersey, US). The capsules were sent to the University of California Davis Stable Isotope Facility, where ^{15}N and total N (atom%^{15}N and atom%N) were measured with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass

spectrometer (Sercon Ltd., Cheshire, UK). The atom%^{15}N natural abundance was estimated from control samples (0.367012 for plant and 0.36858 % for soil) and subtracted from enriched samples to determine atom% excess ^{15}N (de Vries et al. 2012):

(1) atom% excess ^{15}N = atom%^{15}N enriched – atom%^{15}N natural abundance

Percent sample excess ^{15}N was then calculated as follows:

(2) % sample excess ^{15}N = (atom% excess ^{15}N / 100) × % sample total N

The % sample excess ^{15}N was then expressed as an amount (mass) of excess ^{15}N per unit area:

(3) ^{15}N aboveground pool (g/m^2) = (% sample excess ^{15}N / 100) × total aboveground biomass (g/m^2)

(4) ^{15}N belowground pool (g/m^2) = (% sample excess ^{15}N / 100) × mass of soil (g/m^2)

Percent ^{15}N retained was then calculated using the ^{15}N tracer application rate (0.054 g^{15}N/m^2) as follows:

(5) % ^{15}N retention = (0.054 g^{15}N/m^2 – ^{15}N pool g/m^2) / 0.054 g^{15}N/m^2 × 100

Over summer nitrogen retention was estimated by calculating the percent of ^{15}N tracer added that was retained over the 2016 growing season (% ^{15}N retention) in aboveground and belowground pools.

3.2.5 Decomposition

A decomposition experiment was initiated in the plots in fall 2015. Donor litter was collected from a similar nearby tallgrass prairie restoration site as sufficient litter was not yet available at the recently planted research site, on 3 November, 2015. Big Bluestem (*Andropogon gerardii* Vitman) tussock leaf litter was collected from several plants over a 100 m^2 area to a total of approximately 500 g (dry weight). The litter was collected on a hot, dry, sunny day and spread thinly to air dry for one week. Big Bluestem litter was chosen because it is a dominant species in tallgrass prairie, and it provided a uniform

source of litter. Decomposition was assessed using 10 × 5 cm litterbags constructed using fiberglass window screen mesh sealed with hot glue (mesh hole size: 1 mm to balance exclusion of soil fauna against sample loss). Three hundred milligrams of litter were weighed and placed in each litter bag. Litter bags were positioned on the soil surface of the plots the week of 23 November 2015. Homogeneous plots had one litter bag in the center and heterogeneous plots had three litter bags (one in the center of each distinct patch and one on the center of the edge between the patches). In fall 2016 (one year after placement), the litter bags were collected, oven-dried and reweighed. The final mass of the dry litter (mass remaining) was used to calculate percent mass loss: initial mass – final mass / initial mass × 100. Prior to drying and reweighing, the litter bags were gently rinsed with distilled water to clean off soil and debris.

3.2.6 Statistical analyses

For the response variables, the three measurements collected for each heterogeneous plot were averaged to compare with the measurements from the homogeneous plots. Within each heterogeneous treatment, mean levels of each response variable were compared among the topsoil patch, microedge and microsite (sand, woodchips, pit or mound). Data for each source of heterogeneity (topography, sand and woodchips) were analyzed using a linear mixed model with block as a random effect and soil treatment as a fixed effect. A $\log_{10}(y+1)$ transformation was used for cases when residuals did not meet assumptions of normality or homogeneity of variance. Statistical analyses were conducted with R v. 3.3.3 (R Core Team 2017).

3.3 Results

3.3.1 Productivity

Total aboveground biomass and the biomass of individual plant functional groups significantly varied between homogeneous and heterogeneous plots, but the effect depended upon the type of heterogeneity (topography versus sand versus woodchips). In the woodchip treatment, soil homogenization significantly decreased total aboveground biomass and forb biomass each by approximately 50 % ($p = 0.028$, $t_{34} = 2.3$ and $p = 0.3$,

t_{34} = 0.98, respectively) (Fig. 3.1). The topsoil and microedge in woodchip heterogeneous plots had significantly greater biomass than the woodchip microsite (Table 3.1). Forb productivity was greater along woodchip microedges relative to the neighboring topsoil and woodchip patches (Table 3.1) and greater than the average of the two (p = 0.097, t_{34} = -1.71).

In the topography treatment flat topsoil had 50 and 30 % more total aboveground biomass and 75 and 50 % more forb biomass compared to pit and mound plots, respectively (total p = 0.0006, t_{50} = 3.65 and p = 0.05, t_{50} = 1.98, respectively and forb p = 0.001, t_{50} = 3.49 and p = 0.06, t_{50} = 1.92, respectively) (Fig. 3.1a-b). Pit and mound microsites had less than half the biomass of the topsoil patches present in topographically heterogeneous plots (Table 3.1). Within the mound heterogeneous plots, forb productivity was greater along microedges relative to topsoil and mounds (Table 3.1) and greater than the average of the two (p = 0.067, t_{34} = -1.9).

Soil homogenization decreased grass biomass in the sand treatment by 50 % (p = 0.057, t_{17} = 2.04) (Fig. 3.1c) and homogenization of the sand plots increased mean total and forb aboveground biomass by about 10 % (p = 0.6, t_{34} = -0.56 and p = 0.5, t_{17} = -0.62, respectively) (Fig. 3.1 a-b). Topsoil microsites in sand heterogeneous plots had greatest grass biomass but were not significantly different from microedge and sand patches (Table 3.1). There were no treatment effects on legume biomass. Legume biomass was extremely low (mostly zero) in all samples.

Table 3.1 Mean aboveground biomass (g) (standard error) in microsites present in heterogeneous plots.

		Total aboveground biomass	Forb aboveground biomass	Grass aboveground biomass
Woodchips	Topsoil	17.4 (5.0) a	6.3 (3.4) a	4.1 (1.5) a
	Microedge	12.7 (5.3) a	7.0 (3.9) a	2.4 (0.9) a
	2:1 Woodchips topsoil patch	1.8 (0.8) b	1.0 (0.6) b	0.5 (0.2) b
Sand	Topsoil	27.5 (8.5)	9.2 (5.1)	7.6 (3.0)
	Microedge	21.1 (6.0)	9.8 (4.5)	4.8 (1.8)
	4:1 Sand topsoil patch	24.6 (4.8)	12.9 (4.8)	5.3 (1.4)
Topography				
Pit	Topsoil	19.8 (4.2) a	9.2 (3.1) a	6.5 (1.7) a
	Microedge	9.3 (4.2) b	3.9 (2.5) ab	3.5 (1.0) b
	Pit	7.2 (2.3) b	3.0 (1.4) b	2.9 (0.8) b
Mound	Topsoil	21.3 (3.5) a	10.3 (3.3) ab	4.9 (1.7) a
	Microedge	20.1 (8.9) a	14.2 (7.6) a	2.0 (0.8) b
	Mound	9.3 (2.8) b	4.0 (1.9) b	2.6 (0.8) b

All numbers were back-transformed from the log scale $\log_{10}(y+1)$, standard error in the positive direction is shown.
Within each source of heterogeneity, microsites with different letters are significantly different ($p < 0.05$).

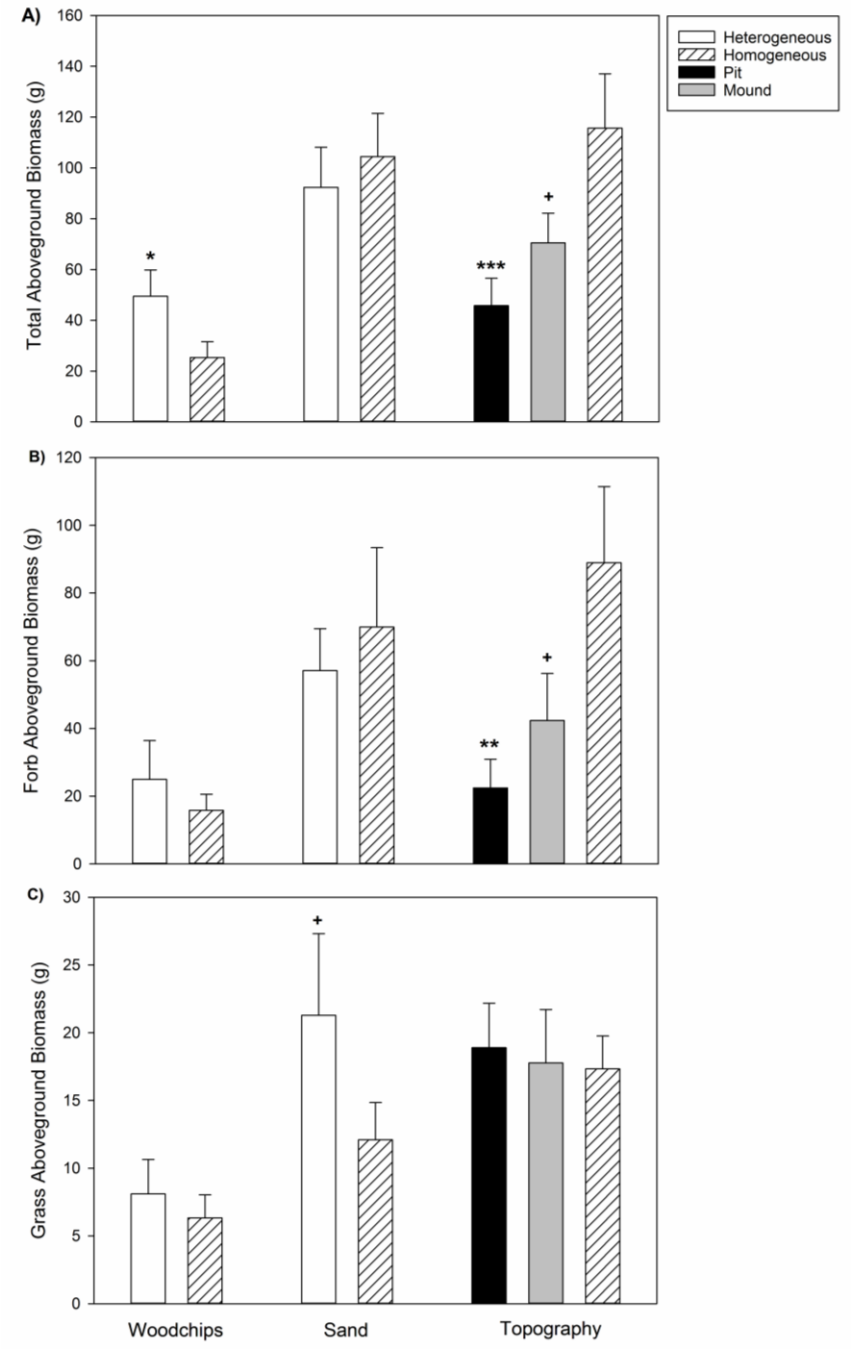

Figure 3.1 Total (a), forb (b) and grass (c) aboveground biomass (g). Means were back-transformed from the log scale $\log_{10}(y+1)$ and standard error in the positive direction is shown. Within each source of heterogeneity, heterogeneous treatments followed by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) are significantly different from the homogeneous treatment and + are marginally significantly different ($p < 0.1$).

3.3.2 Nitrogen retention

Soil homogenization altered nitrogen retention but the effect varied based on the source of homogenization and between aboveground and belowground. Aboveground nitrogen retention significantly decreased by approximately 50 % with homogenization for the woodchip treatment ($p = 0.047$, $t_{22} = 2.1$) (Fig. 3.2a). As with productivity, topsoil and microedges in woodchip heterogeneous plots had significantly greater retention compared to woodchip patches (Table 3.2). Woodchip microedges had similar retention to topsoil patches alone, but greater retention than the average of woodchip and topsoil patches ($p = 0.072$, $t_{33} = -1.86$) (Table 3.2). Aboveground nitrogen retention increased by approximately 50 % with homogenization for the topography treatment (pit $p = 0.01$, $t_{50} = -2.5$ and mound $p = 0.07$, $t_{50} = -1.9$, respectively) (Fig. 3.2a). Belowground nitrogen retention was approximately double the aboveground retention overall but there were few significant treatment effects (Fig. 3.2b). Belowground nitrogen retention decreased in flat homogeneous plots by 40 % compared to the mound topography treatment ($p = 0.036$, $t_{30} = 2.2$) (Fig. 3.2b). Neither aboveground or belowground retention was significantly different among microsites present within pit and mound heterogeneous plots (Table 3.2).

Belowground total nitrogen decreased by approximately 20 % with homogenization for the sand treatment ($p = 0.01$, $t_{17} = 3$) (Table 3.3). Belowground total nitrogen was greatest in the topsoil patch in the sand heterogeneous plots, and the microedge was intermediate between the sand and topsoil patches (Table 3.2). Soil homogenization increased belowground total nitrogen by approximately 8 % for the topography treatment (pit compared to flat topsoil, $p = 0.002$, $t_{33} = -3.5$) (Table 3.3). Belowground total nitrogen was lowest in the pits within the heterogeneous plots, and the microedge was intermediate between the pit and topsoil patches (Table 3.2). Belowground total nitrogen was lowest along the mound microedges (Table 3.2).

Table 3.2 Mean N retention and total N (standard error) in microsites present in heterogeneous plots.

		N Retention (%)		Total N (%)	
		Aboveground	Belowground	Aboveground	Belowground
Woodchips	Topsoil	3.17 (0.98)a	18.50 (2.89)	0.73 (0.07)a	0.19 (0.01)
	Microedge	3.01 (1.22)a	11.30 (1.82)	0.77 (0.07)ab	0.18 (0.01)
	2:1 Woodchips topsoil patch	0.49 (0.20)b	12.49 (3.12)	1.04 (0.15)b	0.19 (0.01)
Sand	Topsoil	3.63 (0.82)	13.45 (3.33)	0.60 (0.05)a	0.19 (0.01)a
	Microedge	3.32 (0.66)	13.79 (2.19)	0.74 (0.07)ab	0.15 (0.01)b
	4:1 Sand topsoil patch	4.56 (0.82)	19.89 (3.65)	0.77 (0.07)b	0.08 (0.01)c
Topography					
Pit	Topsoil	4.02 (0.82)	19.52 (2.67)	0.75 (0.08)	0.20 (0.01)a
	Microedge	2.96 (1.19)	15.02 (2.26)	0.86 (0.11)	0.18 (0.01)b
	Pit	1.93 (0.50)	17.24 (2.05)	1.01 (0.20)	0.14 (0.01)c
Mound	Topsoil	3.59 (0.75)	20.88 (2.67)	0.86 (0.08)	0.20 (0.01)a
	Microedge	3.86 (1.20)	15.22 (3.28)	0.81 (0.09)	0.18 (0.01)b
	Mound	2.20 (0.59)	14.49 (2.30)	0.80 (0.11)	0.19 (0.01)a

Numbers were back-transformed from the log scale $\log_{10}(y+1)$, standard error in the positive direction is shown. Within each source of heterogeneity, microsites with different letters are significantly different ($p < 0.05$).

Table 3.3 Mean percent aboveground and belowground total nitrogen (standard error) (n = 18).

		Aboveground total N (%)	Belowground total N (%)
Woodchips	Heterogeneous	0.88 (0.07)	0.19 (0.01)
	Homogeneous	0.90 (0.08)	0.19 (0.01)
Sand	Heterogeneous	0.72 (0.04)	0.14 (0.01)*****
	Homogeneous	0.75 (0.05)	0.12 (0.01)
Topography	Pit	**0.89 (0.45)**	0.18 (0.01)*****
	Mound	**0.85 (0.14)**	0.19 (0.01)
	Flat topsoil (homogeneous)	**0.73 (0.15)**	0.20 (0.01)

Numbers in bold were back-transformed from the log scale $\log_{10}(y+1)$, standard error in the positive direction is shown.
Within each source of heterogeneity, the heterogeneous treatment followed by ***** is significantly different ($p < 0.05$) from the homogeneous treatment.

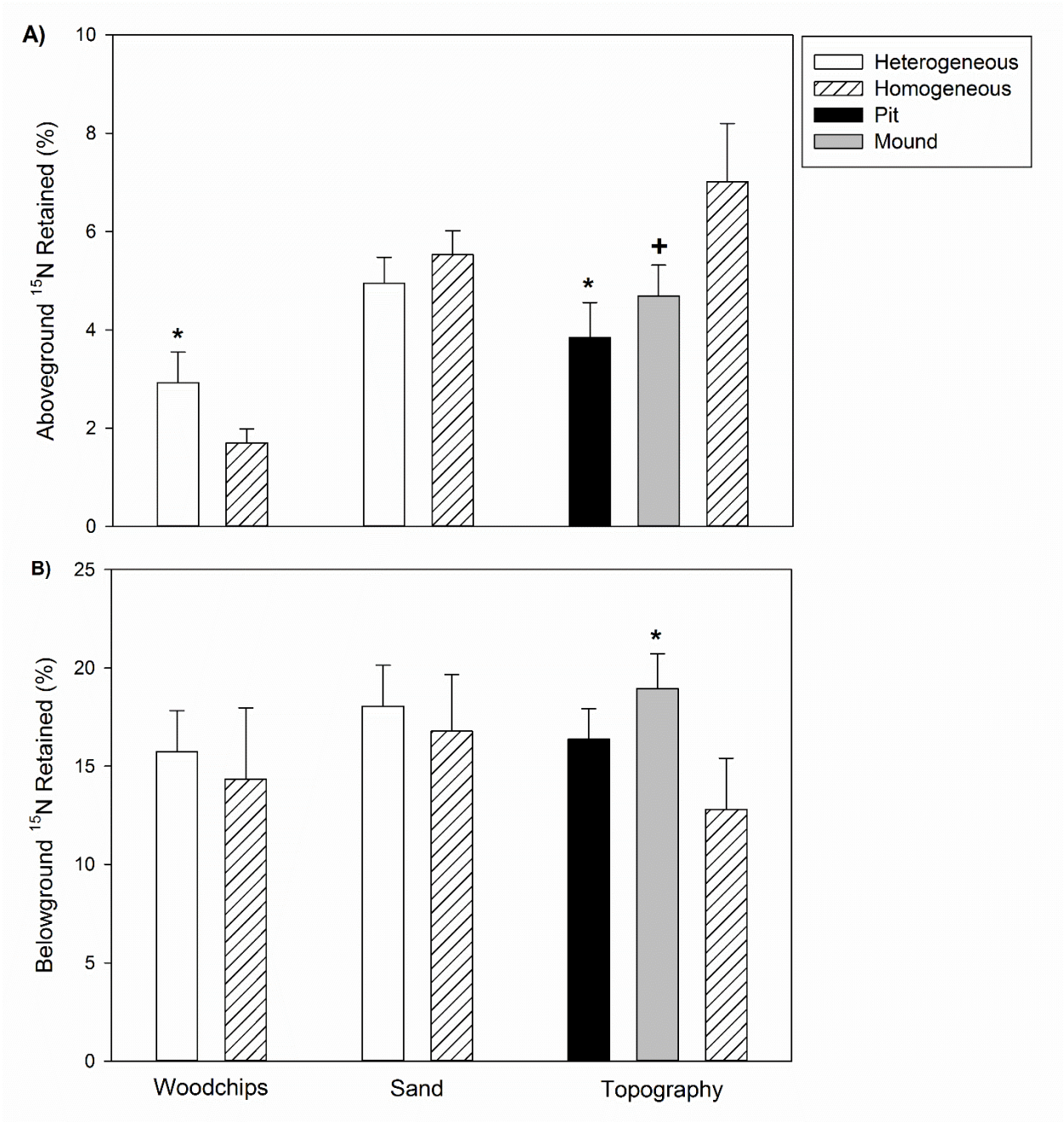

Figure 3.2 Percent ^{15}N retained (over summer / growing season nitrogen retention) in a) aboveground and b) belowground pools. Means were back-transformed from the log scale $\log_{10}(y+1)$ and standard error in the positive direction is shown. Within each source of heterogeneity, heterogeneous treatments followed by * ($p < 0.05$) are significantly different from the homogeneous treatment and + are marginally significantly different ($p < 0.1$).

3.3.3 Decomposition

Homogenization significantly increased mass loss by 8 % in the woodchip treatment ($p = 0.026$, $t_{24} = 2.37$) (Fig. 3.3). Mass loss was similar (averaging around 35 %) among topsoil, microedge and woodchip patches in woodchip heterogeneous plots (Table 3.4). No other significant treatment effects on mass loss were observed. However, within sand heterogeneous plots, mass loss was significantly lower in sand patches compared to topsoil patches (Table 3.4).

Table 3.4 Mean percent mass loss (standard error) in microsites present in heterogeneous plots.

		Mass Loss (%)
Woodchips	Topsoil	38.96 (3.13)
	Microedge	34.56 (3.08)
	2:1 Woodchips topsoil patch	33.44 (4.93)
Sand	Topsoil	40.15 (2.02) a
	Microedge	35.68 (3.31) ab
	4:1 Sand topsoil patch	29.68 (4.04) b
Topography		
Pit	Topsoil	**36.46 (6.21)**
	Microedge	**26.88 (9.92)**
	Pit	**27.26 (11.88)**
Mound	Topsoil	41.36 (3.22)
	Microedge	43.73 (4.54)
	Mound	45.31 (3.41)

Numbers in bold were back-transformed from the log scale $\log_{10}(y+1)$, standard error in the positive direction is shown.
Within each source of heterogeneity, microsites with different letters are significantly different ($p < 0.05$).

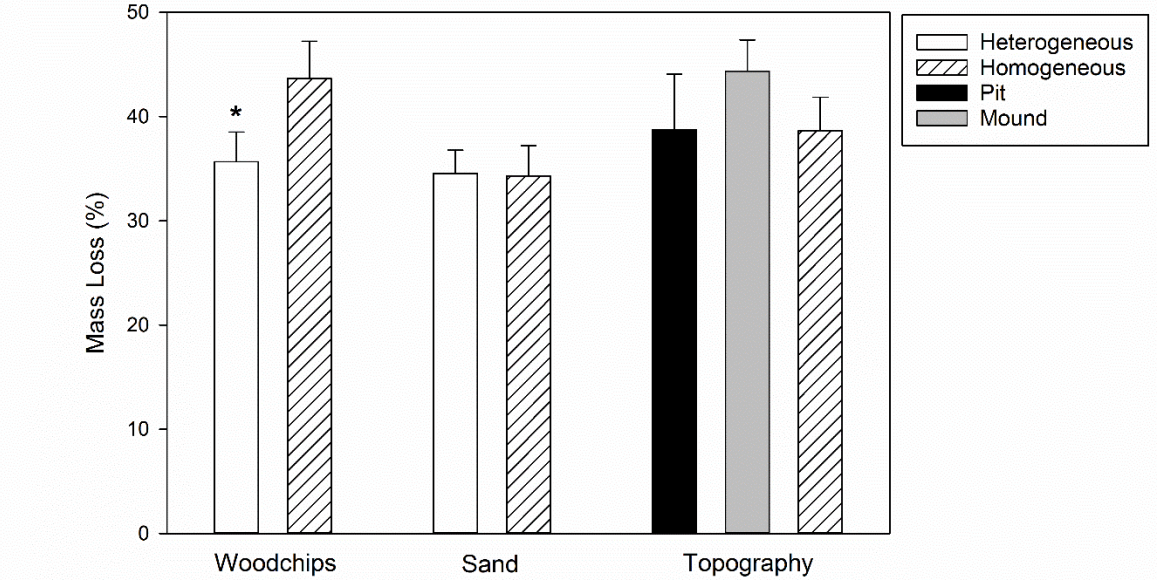

Figure 3.3 Mean percent mass loss in litter bags for decomposition experiment. Initial mass was 0.3 g and percentage mass loss after 12 months is presented. Higher mass loss infers greater decomposition rate. Error bars are standard error. Within each source of heterogeneity, heterogeneous treatments followed by * are significantly different from the homogeneous treatment ($p < 0.05$).

3.4 Discussion

I tested the hypothesis that soil homogenization decreases plant functional group diversity and substrate diversity, which decreases productivity, nitrogen retention and decomposition. I also hypothesized that microedges contribute to increased ecosystem function in heterogeneous soils due to complementarity between adjacent microsites. To my knowledge, these hypotheses have not been tested with multiple, diverse sources of heterogeneity and in a grassland restoration field experiment in the context of soil homogenization from agricultural disturbance. My results showed ecosystem responses varied based on the source of heterogeneity. Homogenization decreased productivity when woodchips were used to create heterogeneity, increased productivity for microtopographic heterogeneity and resulted in no overall change in productivity in the sand treatment. Productivity and other ecosystem responses were associated with differences among individual microsites and edges, confirming substrate heterogeneity contributes to changes in ecosystem function. Decreased productivity has important implications for ecological succession as it creates gaps in the canopy for future species to colonize, which could facilitate greater biodiversity over time. In other studies, productivity also has varied depending on the type of heterogeneity studied, and increased productivity with increasing soil nutrient heterogeneity generally leads to greater plant nitrogen uptake (Maestre et al. 2005, 2006, 2007, Maestre and Reynolds 2006b, 2007a).

Greater abundance of forbs with greater overall productivity was associated with increased aboveground N retention in this study. Homogenization, via reduction in productivity, decreased aboveground N retention in the woodchips treatment. Heterogeneous woodchip plots had a patch of topsoil that was associated with increased aboveground productivity, since homogeneous woodchip plots had woodchips spread throughout, suppressing plant growth. The exact opposite occurred in the topography treatment where flat topsoil plots (homogenization) led to increased aboveground biomass compared to the suppressed growth in pit and mounds. Pits were also associated with lower belowground nitrogen compared to flat topsoil, which may be due to suppressed root growth in the wetter conditions. Microedges in the woodchip and mound heterogeneous treatments exhibited greater forb productivity and aboveground N retention than the average of their neighboring patches, suggesting microedges may be an important mechanism whereby soil heterogeneity increases ecosystem function. There was also an inverse relationship between aboveground and

belowground nitrogen retention with double the belowground retention overall, which is logical given the greater capacity for soil storage of nitrogen. Decreased belowground retention may be related to increased plant uptake and allocation to aboveground tissues.

Changes in functional group diversity (the relative abundances of grasses, forbs and legumes) caused by soil homogenization was a stronger predictor of changes in aboveground productivity and nitrogen retention than changes in plant species diversity (Chapter 2). In 2016, the year productivity, nitrogen retention and decomposition were measured, plant species diversity was greater in pit and mound plots than flat topsoil, lower in woodchip heterogeneous than homogeneous and there was no difference in the sand treatment (Chapter 2). In no case was there a clear link between species diversity and function, agreeing with other studies that suggest functional group diversity is a greater predictor of function (Maestre et al. 2006, de Vries and Bardgett 2016). Under conditions of heterogeneity in the sand treatment, the abundance of grasses increased with increased belowground nitrogen. Grasses are recognized as having dense root systems and are resource conservative species in prairie with greater N retention in more mature ecosystems (Phoenix et al. 2008, Suding et al. 2008). The greater belowground nitrogen retention in sand heterogeneous supports the functional diversity hypothesis (Johnson et al. 1996) and suggests that heterogeneous conditions in the sand treatment promoted increased diversity of plant functional groups (a more balanced community of grasses and forbs compared to the forb dominated homogenized plots). Increased abundance of grasses increased complementarity in terms of belowground sequestration of N from dense grass roots and aboveground capture via “top-heavy” forbs. However, my findings for aboveground N retention suggest the dominant and most productive functional group (forbs) predicted the outcome, in line with the mass ratio hypothesis that dominant plant species control ecosystem processes (Grime 1998). The relative abundance of forbs, grasses and legumes in herbaceous grassland communities has variable but significant influences on N retention (de Vries and Bardgett 2016). While results vary widely among studies, a common theme, as we observed for aboveground N retention, may be that the functional group with greatest productivity is associated with increased plant N retention (Maestre and Reynolds 2006a).

In the mound heterogeneous plots, belowground nitrogen retention was significantly greater than in the flat topsoil plots and forbs were greatest along mound edges and grasses in topsoil patches.

Thus, heterogeneous conditions in the mound treatment increased functional group diversity, which could have increased complementarity in belowground N sequestration. Topographic heterogeneity can promote the development of temporal and spatial vegetation variability in grassland restoration (Biederman and Whisenant 2011). Mounds also can increase forb abundance during grassland restoration, and although the effect may be short lived, it may occur due to increased water infiltration rate (Grant et al. 1980, Naeth et al. 2018). Mounding also has been demonstrated to affect nutrient cycling (Hough-Snee et al. 2011), and the elevated temperatures on mounds can increase decomposition and nutrient availability (Walker and del Moral 2003, Bruland and Richardson 2005), which may explain the greater belowground N retention in my study.

Contrary to my hypothesis, Big Bluestem litter mass loss significantly increased with homogenization in the woodchip treatment, although in heterogeneous plots with mounds mass loss was greater compared to homogeneous flat topsoil. The contrasting outcomes from the different sources of heterogeneity highlight the importance of testing more than one type of heterogeneity. Overall, a lower rate of decomposition was observed in plots with greater aboveground productivity. This may be related to a sheltering effect where more vegetative cover sheltered litterbags from degradation from sunlight, weathering, exposure to herbivores, etc. Field observations also suggested macrofauna were more abundant in plots with topographic heterogeneity. For example, several ant colonies were observed on mounds which may explain a complementarity-driven mechanism for greater decomposition in topographically heterogeneous plots compared to flat topsoil. Heterogeneous habitat conditions were previously shown to facilitate increased invertebrate species diversity and increased ecosystem function (Griffin et al. 2009). Field studies have found greater decomposition often occurs in areas with greater soil moisture (Zirbel et al. 2017) but the relationship between vegetative cover and decomposition is interesting and worth further research.

3.4.1 Conclusions

Changes in nitrogen retention, aboveground productivity and decomposition associated with soil homogenization were evaluated in a grassland restoration field experiment on former cropland beginning from time zero in secondary succession. To our knowledge, this is the first field experiment on the relationship between soil heterogeneity and ecosystem functioning, presenting important evidence that builds upon previous work in more controlled greenhouse settings (Maestre

et al. 2006). To build upon this work, more field experiments are required which also manipulate levels of plant species diversity and investigate the role of plant functional traits using the response effect trait framework (García-Palacios et al. 2012, 2013). Belowground root systems should also be further assessed to provide more explanation in terms of the mechanisms involved in these responses (Liu et al. 2017). The ecosystem functional responses investigated were likely interrelated as treatments with higher aboveground productivity tended to have a lower decomposition rate, belowground nitrogen retention and greater aboveground nitrogen retention. Plant communities are clearly closely involved in these ecosystem processes. The productivity and composition of the plant communities were significantly altered by soil homogenization, which had important consequences at the ecosystem level. The results of this study suggest addition of contrasting soil patches to restoration sites will aid in restoring multiple ecosystem functions while establishing structural diversity and biodiversity. Clearly, if no action is taken, direct planting of former cropland to restoration will result in more uniform functioning and plant community composition across restoration sites. Therefore, homogenization as a potential outcome of disturbance should be addressed during ecological restoration.

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Chapter 4

4 Interactions between soil heterogeneity and soil freezing: Implications for the diversity and relative abundances of grassland species

4.1 Introduction

Environmental heterogeneity is a ubiquitous feature of ecosystems at almost all temporal and spatial scales (Stein et al. 2014). Soil spatial heterogeneity occurs in the form of microsites, which are patches of soil with distinct features compared to their surroundings (e.g. soil depth to bedrock, hummocks, hollows, litter, rock, etc.) (Killham 1994). Microedges, the edges between soil patches, also may function as distinct microsites, with unique hydrological and biogeochemical properties that are not simply an additive combination of their adjacent patches (Stover and Henry 2018).

Stress is defined as any external constraint that limits the rate of dry matter production in vegetation (Grime 1979). Microsites can provide refugia from stressful conditions within plant communities (e.g. for seedling establishment) and this has long been recognized as a mechanism whereby increased patchiness can increase species richness (Grubb 1977). Microsites are recognized as important plant refugia in high stress environments such as limestone pavements and green roofs (Richardson et al. 2012, Heim and Lundholm 2014, Walker and Lundholm 2018). In the context of climate warming, microrefugia can be provided by microsites differing in soil depth, with deep microsites gaining species lost from shallow microsites (Fridley et al. 2011). Similarly, dryland plant communities showed resistance to a nine-year drought due to spatial heterogeneity (Tielbörger et al. 2014), and microtopographic variation and variability in aspect along mountain sides can result in a range of temperatures, providing potential microrefugia for alpine species stressed by climate warming (Scherrer and Körner 2010).

Soil heterogeneity also promotes coexistence/diversity by providing distinct plant niches (Laliberté et al. 2013). Increased plant species diversity can in turn minimize reductions in plant productivity that result from stress (e.g. as demonstrated by Tilman and Downing (1994) for grassland responses to drought). Specifically, with more species present, there is an increased likelihood of having at least some species remaining productive to compensate for those lost or suppressed during periods

of stress (Tilman 1999). However, the ability for microsites to function as microrefugia, or, for increased plant diversity derived from heterogeneous soils to buffer the effects of stress, have rarely if at all been tested in a field experiment where heterogeneity and stress are both manipulated.

Interactions between soil heterogeneity and stress also have important implications for studies of the effects of soil heterogeneity on plant productivity and diversity (i.e. not only does soil heterogeneity affect stress responses, but stress affects plant responses to soil heterogeneity). For example, evidence from pot experiments suggests that variation in nutrient or CO_2 availability can augment or subdue the influence of soil heterogeneity on plant productivity (Maestre and Reynolds 2006a, 2006b, 2007a, Maestre et al. 2007). Furthermore, Maestre and Reynolds (2007a) observed that greenhouse plant assemblages had increased biomass under heterogeneous nutrient supply, and this effect was most pronounced under low moisture conditions. Theoretical modelling suggests that the relationship between soil heterogeneity and plant species diversity varies along environmental gradients (Yang et al. 2015), and variation in the effects of soil heterogeneity on plant productivity have been observed along moisture, CO_2 and nutrient gradients (García-Palacios et al. 2012). Nevertheless, for studies of the interactive effects of soil heterogeneity and environmental stress on plant productivity, there has been a bias towards the study of variation in soil nutrient availability, as opposed to variation in substrate, and with some exceptions (e.g. Arnone 1997), these interactions have not been examined experimentally in the field.

Soil freezing is a source of plant stress that is expected to increase in some temperate regions over the next century due to reductions in snow cover caused by climate change (Groffman et al. 2001, Hardy et al. 2001, Henry 2007, 2008, Kapnick and Delworth 2013). The snowpack has an insulating effect, and in its absence, increases in soil freezing and soil freeze-thaw cycles can occur (Henry 2008), which can damage overwintering plant tissues and reduce plant growth in subsequent growing seasons (Vankoughnett and Henry 2014). Snow removal thus is employed in field experiments to increase soil freezing. Variation in soil characteristics among microsites in heterogeneous soils could lead to variability in the severity of soil freezing; for example, freezing effects can be particularly severe in wet soils, and can vary depending on soil texture (Oztas and Fayetorbay 2003, Wu et al. 2017). Therefore, increased soil heterogeneity may increase the frequency of microsites that function as refugia from severe frost effects, and if increased soil

heterogeneity begets increased species diversity, this may increase the pool of frost tolerant species in the community.

I used a field experiment in a recently restored tallgrass prairie on former cropland to examine the effects of soil heterogeneity, stress (soil freezing implemented via snow removal) and their interactions on plant abundance (percent cover, density) and species diversity. Patches of topsoil supplemented with sand or woodchips were used to construct heterogeneous plots, and the same materials were added in equal quantities then mixed to construct homogenized plots. Based on the assumptions that soil heterogeneity would increase plant species diversity and availability of microrefugia, I hypothesized that soil heterogeneity would buffer the effects of soil freezing, such that freezing effects on overall plant abundance in heterogeneous substrates would be less severe than in homogeneous substrates.

4.2 Methods

4.2.1 Field site and experimental design

This study took place at Environmental Sciences Western Field Station (ESW) near Ilderton, Ontario, Canada. ESW is located in a rural region with London clay loam soils, a mean annual precipitation of 1012 mm and a mean annual air temperature of 7.9 °C (Hagerty and Kingston 1992, Environment and Climate Change Canada 2018). A 4 ha prairie research site was established in a former crop field in May 2015, and 10 experimental blocks were marked out in this area. Each block contained eight 50 × 100 cm plots spaced 2 m apart (Fig. 4.1). Four of these plots contained added sand and four contained added woodchips, and for each substrate type there were both homogeneous and heterogeneous plots, half of which experienced snow removal and half of which experienced ambient snow cover (Fig. 4.1).

The heterogeneous plots were divided into two halves consisting of a 50 × 50 cm and 15 cm deep patch of topsoil (mixed to 15 cm with a shovel) and a 50 × 50 cm patch enriched with sand (4:1 sand:topsoil) or woodchips (2:1 woodchips:topsoil). The edge in the center of each plot between the two patches, was defined as the microedge (Chapter 2). To create homogeneous plots, the same heterogeneous plots were installed and then mixed thoroughly across the entire 50 × 100 cm plot area (Fig. 4.1). In early June 2015, after plot installation, equal amounts of tallgrass prairie grass and

forb seeds were planted in each plot. Areas outside of the plots were seeded with a separate mixture of seed from the same species. See Chapter 2 for further details, including figures for the experimental design, soil characteristics of microsites (e.g. pH, nutrients) and plant species and quantities of seed planted.

The plots were prepared for snow removal in autumn by covering the ground with a white plastic mesh (1 × 1 cm mesh size; Winter Wrap, Quest Plastics Ltd., Mississauga, ON, Canada) to prevent soil, plant and litter damage during snow removal. During the winters of 2015-2016 and 2016-2017, snow removal was performed opportunistically during periods of snowfall when the air temperature dropped to a minimum of -10 ^{o}C. Snow was removed with a shovel, stopping at approximately 2 cm depth to avoid soil disturbance. Snow removal ceased several weeks before spring melt to minimize snow removal effects on soil moisture over summer. Soil moisture was measured in the spring in each plot using a Thetaprobe (Delta-T Devices, UK) and these measurements confirmed there was no significant difference in soil moisture between the snow removal and ambient snow plots in either the second (p = 0.4, t_{89} = -0.8) or third (p = 0.7, t_{89} = -0.4) growing season after winter snow removal.

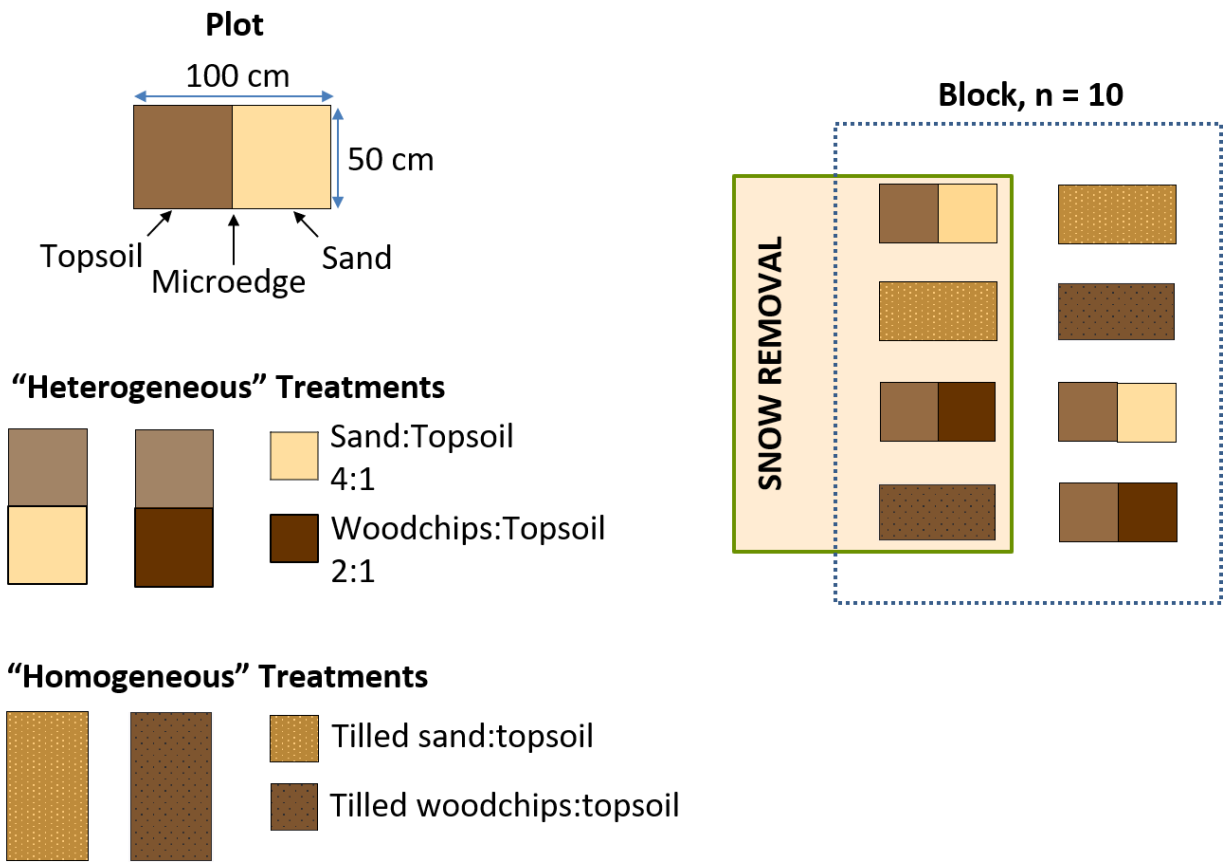

Figure 4.1 Experimental design. Plots were used as replicates of the eight different treatments. Plots were randomly assigned to 10 blocks.

4.2.2 Meteorological conditions

Soil temperature probes (LogTag TRIX-8, MicroDAQ, NH, USA) programmed for hourly sampling were buried at 5 cm depth at six of the snow removal plots and six ambient snow plots to determine snow removal effects on soil temperature (Fig. B.1). Meteorological data were obtained from the Environment and Climate Change Canada National Climate Data and Information Archive (Environment and Climate Change Canada 2018). Growing season mean daily temperature, total monthly precipitation and long-term climate normals for the time period of the study are provided in Table A.1. Mean monthly snowfall and temperature for the 2015-2016 and 2016-2017 winters, as well as long term averages, are provided in Table B.1. The two winters when snow removal was conducted (2015-2016 and 2016-2017) were warmer and experienced less snowfall than the climate normal (Table B.1). Despite these mild conditions, in both winters soil temperature probes indicated the snow removal plots experienced episodes of reduced minimum daily temperatures and increased freeze-thaw cycling relative to the ambient snow plots (Fig. B.1).

4.2.3 Vegetation sampling

Vegetation was monitored over three growing seasons (2015–2017) following plot establishment. The plots were surveyed in late spring and summer (June-July and September-October) each year to correspond with peak biomass for the different plant species. Three 25 × 25 cm (0.0625 m^2) sampling quadrats were overlaid on each plot in the center of each patch and along the microedge between the two patches (Fig. 4.1). The same number and position of quadrats was used in the homogeneous plots to provide a consistent and even sampling effort. Within each quadrat all plant species were identified and a visual estimate of individual species percent canopy cover, percent ground cover (bare ground, moss, vegetation, litter, rock) and density (number of individuals) was made. Nomenclature followed Voss and Reznicek (2012).

4.2.4 Statistical analyses

Treatment effects on total canopy cover, native (seeded) and adventive (non-seeded) graminoids, leguminous and non-leguminous forbs, total plant density (number of individuals), Shannon index and species richness were assessed. Total canopy cover was calculated by summing the individual species cover values for each quadrat. Shannon index of species diversity and species richness were

calculated in R using dplyr, vegan and rich (Rossi 2011, Oksanen et al. 2016, Wickham and Francois 2016). Responses were analysed at the spatial scale of the sampling quadrats: 0.0625 m^2. Data for each source of heterogeneity (sand and woodchips) were analyzed using a linear mixed model with block, and plot nested within block, as random effects, and soil freezing (no snow removal versus snow removal), soil treatment (heterogeneous versus homogeneous), year (2015, 2016, 2017) and season (early summer versus late summer) as fixed effects. An additional linear mixed model (the same as the model described above) was used for sand heterogeneous and woodchips heterogeneous treatments separately to assess differences among patches in the heterogeneous plots, except the 'soil treatment' was replaced with the fixed effect 'subplot,' which consisted of the levels topsoil patch, microedge, and sand or woodchips patch. To investigate interactions among soil treatment, soil freezing and year, data were divided into subsets based on one of the factors of the interaction and then analyzed with the linear mixed model above. An *a-priori* contrast (assessed with a t-test) was used to compare the heterogeneous plot mean to the homogeneous mean (or snow removal to no snow removal) within years; the same approach was also used to compare means among subplots. A $\log_{10}(y+1)$ transformation was used for residuals that did not meet assumptions of normality or homogeneity of variance. Friedman's tests and Wilcoxon rank sum tests were used for cases when transformation could not be used. All statistical analyses were conducted with R v. 3.3.3 (R Core Team 2017). For all statistical tests an alpha of 0.05 was used. However, marginally significant differences at $p < 0.1$ were also considered biologically important and reported.

4.3 Results

4.3.1 Total canopy cover and density

For several response variables, significant interactions among soil freezing, heterogeneity and year were detected (Table B.2). For total canopy cover, there were no significant effects of snow removal or soil heterogeneity for the woodchip treatment (Fig. 4.2a). However, for the sand treatment, snow removal decreased total cover in the homogeneous plots in the second ($p = 0.03$, $t_{58} = 2.2$) and third year ($p = 0.02$, $t_{58} = 2.5$) (Fig. 4.2b), and in the snow removal plots, soil heterogeneity increased total cover in the second ($p = 0.006$, $t_{69} = 2.8$) and third year ($p = 0.009$, $t_{69} = 2.7$) (Fig. 2b). Within

the sand heterogeneous plots, snow removal increased total cover in the topsoil patches in the second year (p = 0.008, t_{23} = -2.9) (Table 4.1).

For the woodchip treatment, soil heterogeneity decreased plant density in all three years (p = 0.0002, t_{38} = -4), but there was no snow removal effect or interaction between soil heterogeneity, soil freezing or year (Fig. 4.2c). For the sand treatment, snow removal increased plant density in the homogeneous plots in the second year (p = 0.007, t_{15} = -3.1) (Fig. 4.2d) and plant density was also greater than heterogeneous snow removal plots in the second year (p = 0.002, t_{15} = -3.7) (Fig. 4.2d).

Table 4.1 Mean total percent canopy cover (standard error) in sand heterogeneous microsites with and without snow removal.

	Topsoil patch	Microedge	Sand patch
2015			
No snow removal	15.0 (4.0)	28.2 (7.5)	31.1 (8.1)
Snow removal	27.4 (6.5)	27.3 (5.6)	27.6 (10.5)
2016			
No snow removal	66.6 (9.2)	85.1 (12.4)	100.0 (10.1)
Snow removal	109.5 (14.3)*	99.7 (12.1)	79.1 (9.5)
2017			
No snow removal	77.8 (10.1)	78.5 (10.0)	161.5 (7.5)
Snow removal	98.1 (11.8)	88.0 (7.8)	138.4 (14.6)

Within microsite type, means followed by * ($p < 0.05$) are significantly different from the control treatment (no snow removal).
2015 was the pre-snow removal year and 2016 and 2017 represent the growing seasons after one and two winters of snow removal, respectively.

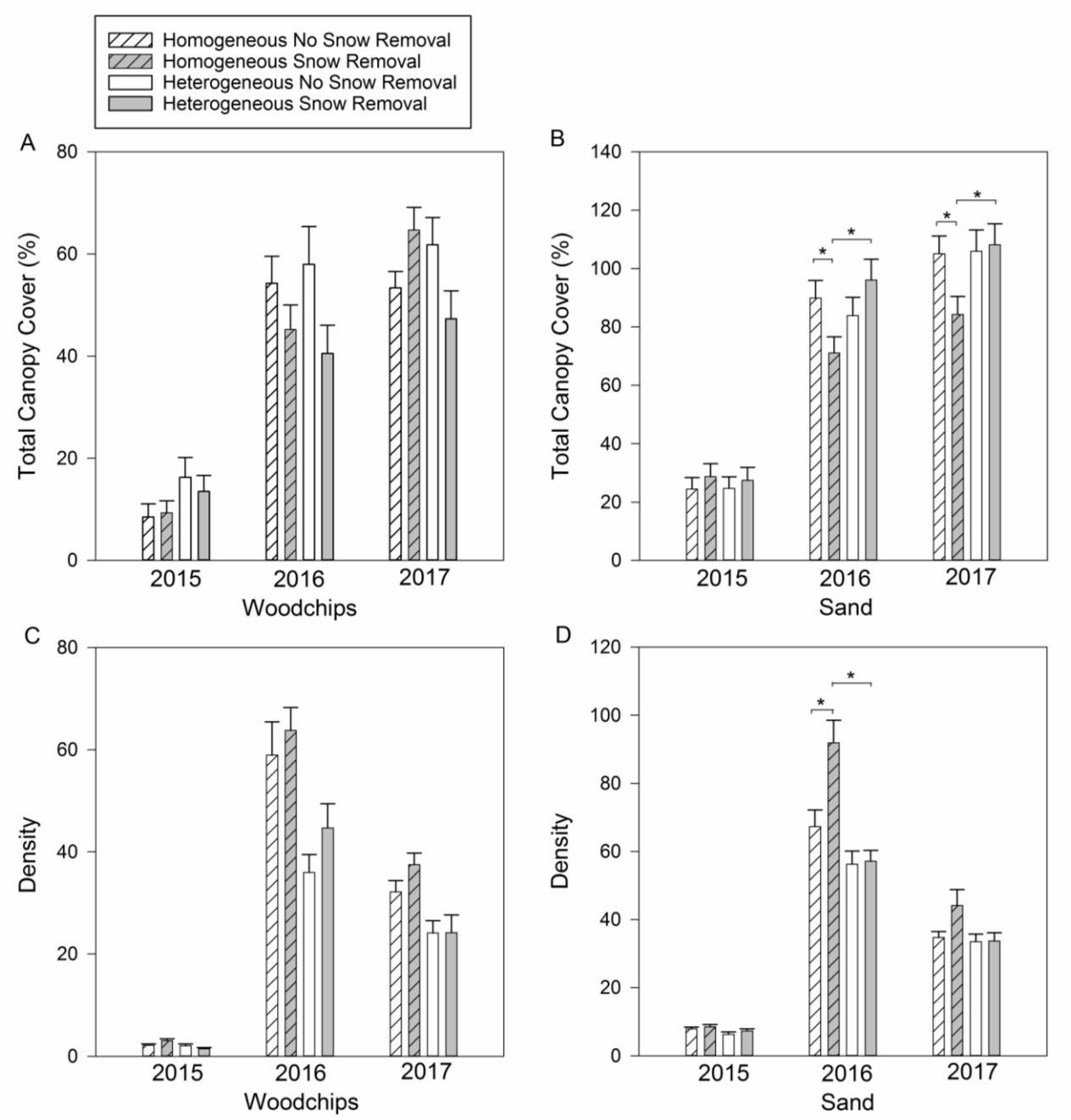

Figure 4.2 Total percent canopy cover (a) and (b), density (total number of individuals) (c) and (d) in woodchips (a) and (c) and sand (b) and (d) soil heterogeneity treatments. Means were back-transformed from the log scale $\log_{10}(y+1)$ and standard error in the positive direction is shown. Significant differences between means are indicated by * ($p < 0.05$) according to t-tests of *a-priori* contrasts. 2015 was the pre-snow removal year and 2016 and 2017 represent the growing seasons after one and two winters of snow removal, respectively.

4.3.2 Plant community composition

The cover of native seeded grasses and non-leguminous forbs followed the same trends and were therefore summed into a single category. Seeded native legume cover was extremely low throughout the study in both sand and woodchip plots (overall mean 0.2 %, std error 0.06 %), and therefore did not contribute meaningfully to the analysis. For the woodchip treatment, snow removal decreased cover of native grasses and forbs in the heterogeneous plots in the second year ($p = 0.0156$, $t_{50} = 2.5$) (Fig. 4.3a), and heterogeneous had lower cover than homogeneous snow removal plots in the second ($p = 0.0004$, $t_{45} = -3.85$) and third year ($p = 0.0057$, $t_{45} = -2.91$) (Fig. 4.3a). Within the woodchip heterogeneous plots, snow removal decreased native cover in the topsoil patches and microedges (Table B.3). For the sand treatment, snow removal decreased native grass and forb cover in the homogeneous plots in the second and third year ($p = 0.05$, $t_{20} = 2.1$) (Fig. 4.3b), and homogeneous had lower cover than heterogeneous snow removal plots in the second and third year ($p = 0.02$, $t_{18} = 2.6$) (Fig. 4.3b). Within the sand heterogeneous plots, snow removal increased native cover in the topsoil patches in the second year (Table B.3).

For adventive grasses, snow removal increased cover in the heterogeneous woodchip plots in the late growing season of the second year ($p = 0.02$, $F_R = 5.4$) (Fig. 4.4a) and heterogeneous had greater cover than homogeneous snow removal plots in the late growing season of the third year ($p = 0.01$, $F_R = 6.4$) (Fig. 4.4a). Within the woodchip heterogeneous plots, snow removal increased adventive grass cover in the topsoil microsites and microedges in the second and third year (Table B.4). Snow removal decreased adventive grass cover in sand heterogeneous plots in the late growing season of the second year ($p = 0.096$, $F_R = 2.8$) (Fig. 4.4b), which corresponded with a decrease in adventive grass cover with snow removal in microedges at the same time (Table B.4).

For adventive forbs, there was a marginally significant decrease in cover following snow removal in the homogeneous woodchip plots in the early growing seasons of the second and third year ($p = 0.058$, $F_R = 3.6$) (Fig. 4.4c). In the ambient snow woodchip plots, homogeneous had greater adventive forb cover than heterogeneous during the late growing season of the second year ($p = 0.058$, $F_R = 3.6$) (Fig. 4.4d). For the sand treatment, snow removal decreased adventive forb cover in the homogeneous plots late in the growing season of the third year ($p = 0.025$, $F_R = 5$) (Fig. 4.4e), and in the ambient snow plots, homogeneous plots had greater cover than heterogeneous plots at this

time (p = 0.03, F_R = 4.5) (Fig. 4.4e). Despite the overall differences observed at the plot level, no significant differences in adventive forb cover among microsites were found for either the woodchip or sand treatment (Table B.5). For adventive legumes, cover was very low overall (mean 1.2 %, std error 0.2 %) and no significant treatment effects were observed.

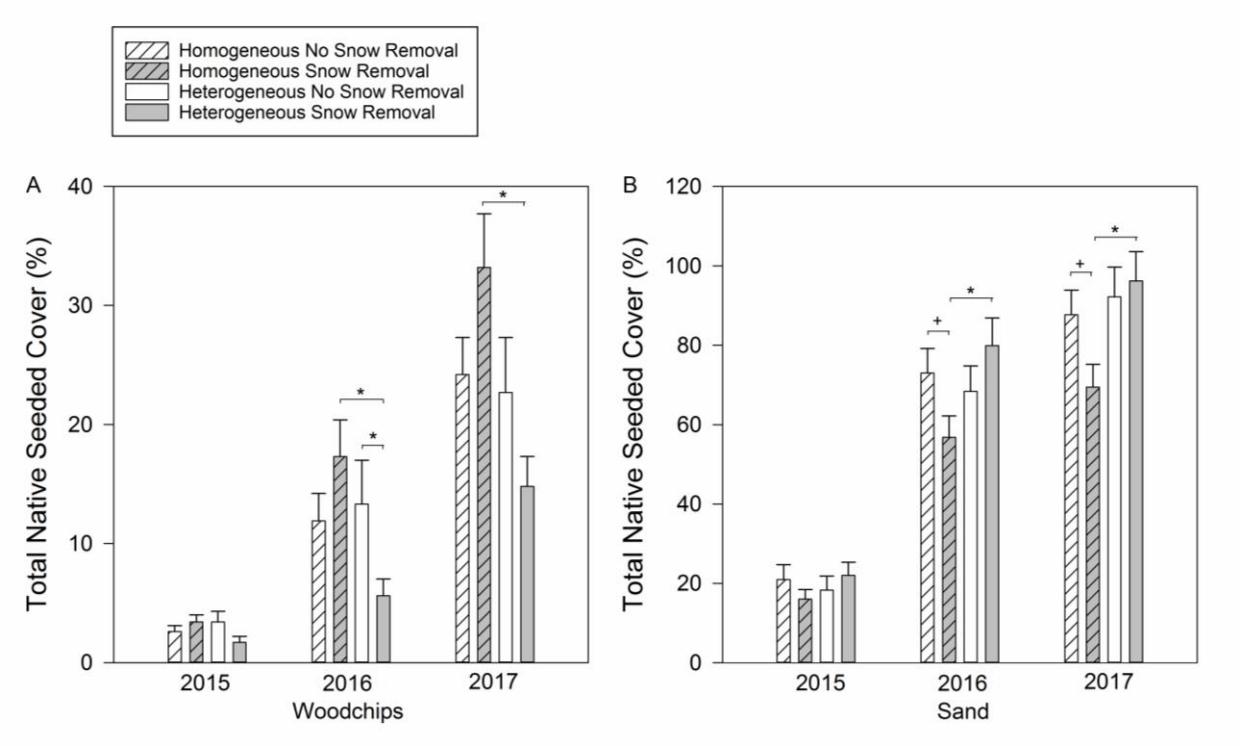

Figure 4.3 Mean total native seeded forb and grass cover (%) in woodchip (a) and sand (b) treatments. Within years, significant differences between means are indicated by * ($p < 0.05$) and + are marginally significantly different ($p < 0.1$) according to t-tests of *a-priori* contrasts. Means were back-transformed from the log scale $\log_{10}(y+1)$ and standard error in the positive direction is shown. 2015 was the pre-snow removal year and 2016 and 2017 represent the growing seasons after one and two winters of snow removal, respectively.

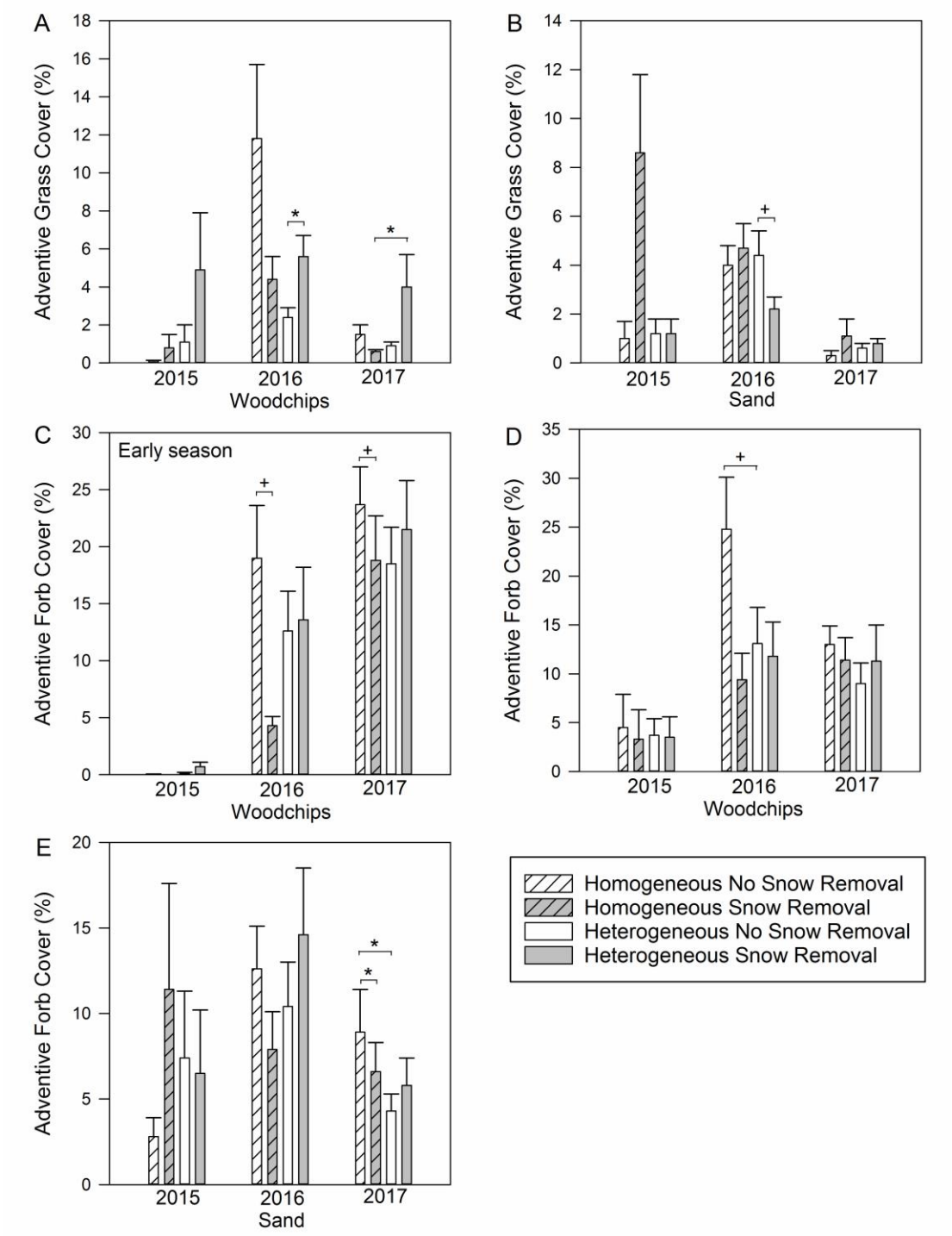

Figure 4.4 Mean adventive (non-seeded) grass (a-b) and forb cover (c-e) in woodchip (a, c-d) and sand (b, e) treatments. Other than panel C, all means are late growing season. Within years, significant differences between means are indicated by * ($p < 0.05$) and + are marginally significantly different ($p < 0.1$) according to t-tests of *a-priori* contrasts.

4.3.3 Species richness and Shannon index of species diversity

For the woodchip treatment, soil heterogeneity decreased the Shannon index in all three years (p = 0.0008, t_{30} = -3.7), but there was no significant snow removal effect or interaction between soil heterogeneity, soil freezing or year (Fig. 4.5a). However, soil heterogeneity significantly decreased species richness in the snow removal plots in the second (p = 0.0002, t_{24} = -4.4) and third year (p = 0.0002, t_{24} = -4.5) (Fig. 4.5b). Within the woodchip heterogeneous plots, in the second year, snow removal significantly decreased species richness in topsoil microsites (Table B.6). For the sand treatment, there were no significant effects of soil heterogeneity or snow removal on species richness or the Shannon index.

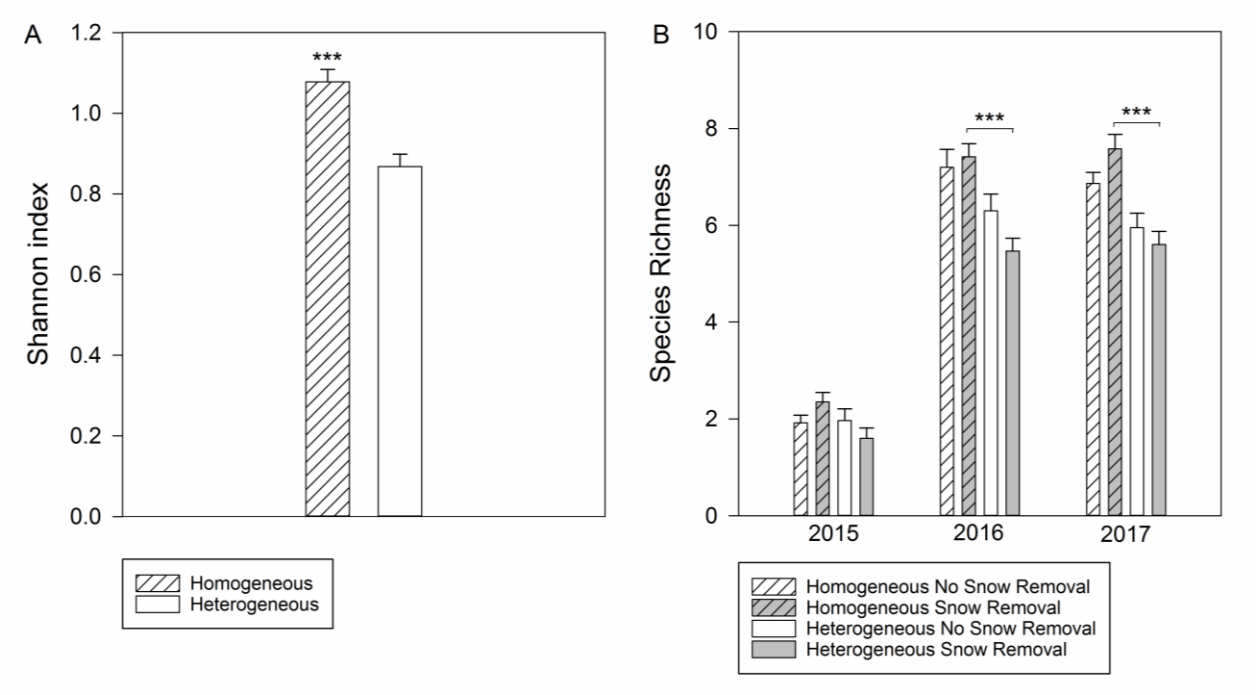

Figure 4.5 Shannon index (a) and species richness (b) in homogeneous and heterogeneous woodchip treatments. Significant differences between means are indicated by *** ($p < 0.001$) according to t-tests of *a-priori* contrasts. For Shannon index (a), there was no significant year × soil treatment × soil freezing interaction, only a significant main effect of soil treatment.

4.4 Discussion

Consistent with my hypothesis, soil heterogeneity buffered the effects of soil freezing, such that freezing effects on total cover, native forb and grass cover, and plant density were not evident at the plot scale in the heterogeneous sand treatment. Because heterogeneity did not increase diversity in the sand treatment, diversity was not likely involved in this buffering effect. Instead, cover increased in topsoil microsites with snow removal, suggesting that the topsoil microsites within the heterogeneous sand plots could have functioned as microrefugia to soil freezing. However, snow removal decreased native cover and species richness in the topsoil microsites and microedges within the heterogeneous woodchip plots, which suggests the ability for topsoil microsites to function as microrefugia is dependent upon neighboring conditions. The isolated topsoil patch in the heterogeneous woodchip plots neighbored a sparsely vegetated woodchip microsite, whereas there was continuous cover in the heterogeneous sand plots. Thus, the decreased cover in response to soil freezing in the topsoil and edge microsites within the heterogeneous woodchip plots may have been due to reduced vegetation cover in the woodchip patch, which would reduce snow accumulation and insulation in the plot area.

Conversely, soil heterogeneity increased the sensitivity of native cover to freezing in the woodchip plots. Thus, depending on the source of heterogeneity and plant response examined, soil heterogeneity either suppressed or exacerbated soil freezing effects. The increased species diversity in the homogeneous woodchip plots may have been associated with the maintenance of native species cover during soil freezing. In the heterogeneous woodchip plots, there was no net change in total cover, because adventive grasses compensated for the decrease in native cover. Therefore, both plant species diversity and functional group diversity resulting from varying levels of soil heterogeneity may buffer responses to stress.

Naturally occurring microrefugia can help maintain species diversity and composition in plant communities affected by stress (Scherrer and Körner 2010, Fridley et al. 2011, Tielbörger et al. 2014). In my study, the maintenance of cover in the homogeneous woodchip plots suggested woodchips may have ameliorated soil freezing effects, but decreases in cover observed in the homogeneous sand plots suggested that sand may have intensified the effects of soil freezing. Therefore, while some microsites indeed functioned as safe sites with respect to soil freezing

damage, others functioned as traps. In addition, edges between distinct soil zones can exhibit prolonged wet and dry periods due to the contrast in soil pore sizes (Stover and Henry 2018). The potential for relatively wet conditions along woodchip microedges may have contributed to more severe freezing effects, which could explain the observed decreases in cover. Coarse textured and wet soils are typically most susceptible to physical disruption caused by soil freezing (Oztas and Fayetorbay 2003), which could affect belowground plant tissues detrimentally. In contrast, the addition of woodchips to topsoil can have beneficial effects, such as moderating soil surface temperatures and reducing water loss and erosion (Naeth et al. 2018), which could have increased the buffering capacity to freezing damage.

Different plant functional groups (native seeded, and adventive forbs and grasses) also differed in their responses to freezing among microsites. For example, adventive forbs were most abundant under ambient snow cover, and most susceptible to soil freezing in the homogeneous substrates. In the woodchip plots, soil freezing decreased adventive forb cover and increased species richness in the homogeneity treatment compared to the heterogeneity treatment, and it is possible that this increase in species richness resulted from release from competition with adventive forbs (namely the invasive Canada thistle, *Cirsium arvense* L.). Moving forward, studies of interactions between heterogeneity and stress could benefit not only from more explicit testing of these species interactions, but from the inclusion of plant functional trait and functional diversity data to better understand the mechanisms involved (García-Palacios et al. 2013). Leguminous forbs may be particularly sensitive to soil freezing (Joseph and Henry 2008, Henry et al. 2018), but they established at extremely low abundance in my study, despite being introduced by broadcast seeding, so more deliberate efforts would be needed in future studies to examine the freezing responses of this functional group.

My results demonstrated that soil heterogeneity interacts with soil freezing in a substrate dependent manner to either suppress or exacerbate stress responses. However, this interaction also means that stress can influence plant responses to heterogeneity, which is an important caveat in the context of studies of soil heterogeneity effects on plant communities. For example, in both the woodchip and sand plots, adventive forb cover differed between the homogeneity and heterogeneity treatments under ambient snow cover, but this effect was not significant when soil freezing was increased via

snow removal. Likewise, the change in total cover, density and native cover in the homogeneous sand plots in response to soil freezing led to a soil heterogeneity effect that was otherwise absent in the ambient snow plots. Soil freezing also facilitated greater species richness in the homogeneous woodchip plots compared to heterogeneous woodchip plots. These results are consistent with greenhouse experiments, where stress has either augmented or subdued the influence of soil nutrient heterogeneity on plant productivity (Maestre and Reynolds 2006a, 2006b, 2007b, Maestre et al. 2007, Xi et al. 2015). Therefore, there is strong generality across diverse stresses (e.g. frost, drought, warming), microsites (e.g. soil nutrients, texture, depth) and systems (e.g. greenhouse, field) that stress can modulate the effects of heterogeneity.

Overall, my results demonstrated significant interactions between soil heterogeneity and soil freezing for plant community responses in a field experiment. The majority of research on stress in the context of topics such as global change has not taken environmental heterogeneity into account (García-Palacios et al. 2012). Likewise, studies of heterogeneity-plant relationships often do not consider the potentially confounding influence of stress or environmental extremes (Yang et al. 2015). Beyond providing a more thorough understanding of the ecological processes associated with interactions between heterogeneity and stress, my results have broader ramifications for plant communities establishing on homogenized soils. Approximately 40 % of Earth's land area is in agricultural use, and old fields (abandoned cropland) cover over 200 million hectares (Foley et al. 2005, Cramer et al. 2008). The legacy of soil mixing due to the tillage of crop fields can increase soil homogenization (Anderson and Coleman 1985, Elliott 1986) which could reduce microrefugia available to plants during stressful periods, resulting in detrimental effects on plant species diversity and community stability. Furthermore, global change is increasing the frequency and intensity of stressful events in ecological communities (Barnosky et al. 2011, IPCC 2014). The deliberate addition of microsites could provide microrefugia to plant communities succeeding into former agricultural land and thus could be a useful management tool in ecological restoration. However, design of microsites for a particular system may need to be considered carefully based on the species present and potential stresses, given that the interactions demonstrated in the current study were substrate dependent. In addition, the effects observed occurred in the context of early community assembly and secondary succession, and it remains to be demonstrated how these effects may persist over the longer term.

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Chapter 5

5 General discussion

5.1 Summary: Interconnections among soil homogenization, plant species diversity, ecosystem function and stress

The overall goal of my thesis was to examine relationships among soil homogenization, plant diversity, ecosystem properties and stress during ecological succession. I used a newly restored tallgrass prairie on former cropland as a study system and conducted field experiments during the first three growing seasons after establishment of the restoration. I investigated soil homogenization by comparing plant cover, plant species diversity, aboveground productivity, plant litter decomposition and nitrogen retention (^{15}N tracer) between homogeneous and heterogeneous treatment plots. I also compared treatment plots exposed to soil freezing, implemented via snow removal in winter, to plots which received ambient snow cover. Findings presented in Chapter 2 suggest heterogeneity-diversity relationships are dependent upon plant productivity, spatial scale and environmental conditions, such as drought. Chapters 2 and 3 showed elevated and intermediate levels of plant cover and ^{15}N retention along microedges, indicating microedges may act as unique microsites and small scale ecological transition zones. Chapter 3 further suggested the importance of productivity responses to soil homogenization in influencing ecosystem level responses of ^{15}N retention and plant litter decomposition to homogenization. Chapter 4 showed loss of microrefugia via soil homogenization could have detrimental effects on plant production during periods of stress and that multiple interactions can occur between stress and soil heterogeneity. All responses to soil homogenization examined were substrate dependent (i.e. sand versus woodchips versus microtopography). Overall, these experiments provided insight into interactions among soil heterogeneity (or homogenization), plant species diversity, ecosystem function and environmental stresses.

The microsites facilitated the establishment of different plant functional groups and varying levels of severity of soil freezing, which influenced species diversity, ecosystem function and community-level freezing effects. My findings demonstrated, in the restored tallgrass prairie ecosystem at large, a strong interconnectedness of soil heterogeneity (or homogenization), plant community diversity,

ecosystem function and stress. The relationship between soil heterogeneity, ecosystem function and stress has been recognized theoretically and examined observationally and in pot experiments (Maestre et al. 2006, García-Palacios et al. 2011), but my results represent one of the first field experiments of this nature.

A final conceptual figure is presented in Fig. 5.1 which builds upon the preliminary figure in the introduction (Fig. 1.1). Fig. 1.1 includes the preliminary hypotheses at the outset of the thesis and Fig. 5.1 includes a summary of the key thesis results.

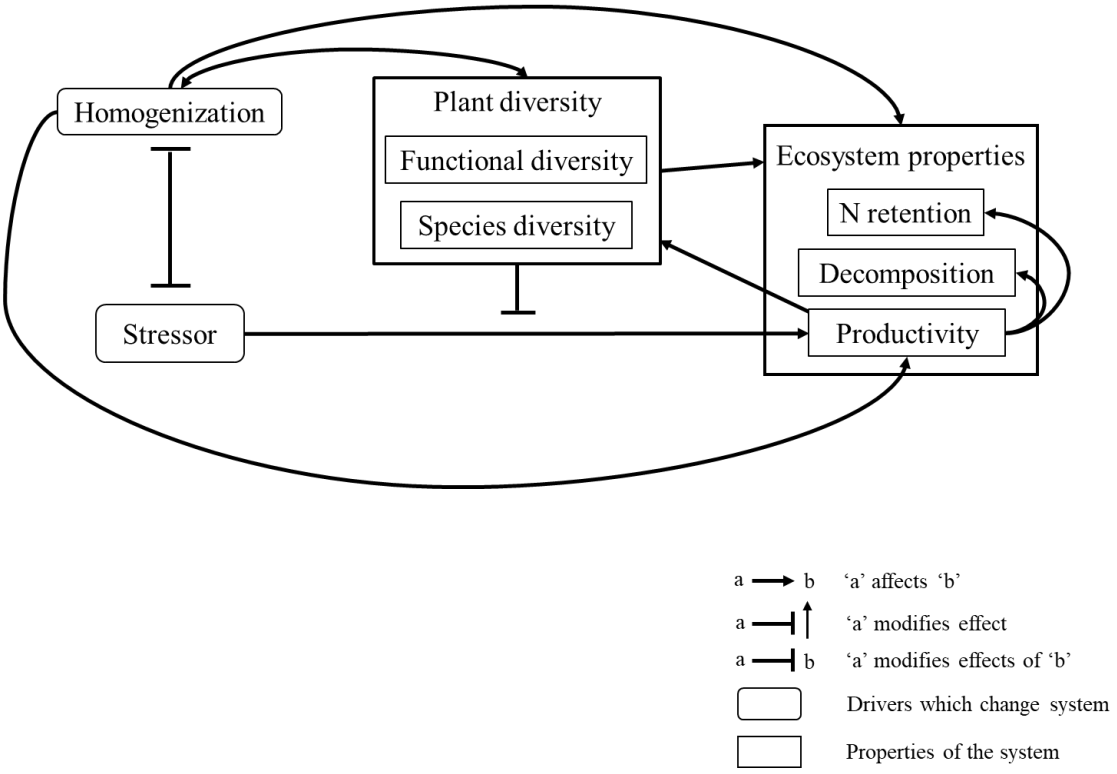

Figure 5.1 Final conceptual model linking the associations among soil homogenization, stress, plant diversity and ecosystem properties (e.g. nitrogen retention, decomposition, productivity). Homogenization alters diversity and ecosystem properties compared to heterogeneous substrates with distinct microsites and microedges. Homogenization effects are substrate dependent and may be positive, negative or neutral. Homogenization effects on productivity influence other ecosystem properties and species diversity with an inverse relationship between productivity and species diversity. Homogenization can negatively affect plant functional diversity, which can decrease ecosystem properties like nitrogen retention. Stress negatively influences productivity but increased diversity resulting from homogenization (or heterogeneity) may lessen this effect and should be further researched. Stress and homogenization interact and can modify the effects of one another on plant community responses like productivity. Plant diversity decreases homogeneity by increasing 'plant induced' heterogeneity. Although substrate, stress, productivity and spatial scale (patch size) were identified as important predictors of variability in homogenization relationships, the overall knowledge gap to be addressed in the system is refining prediction of the direction of effects.

5.2 A central role for productivity in heterogeneity relationships?

Beyond niche effects, an important finding of my thesis was a potentially central role of productivity responses to soil homogenization in indirectly influencing the responses of plant species diversity, nitrogen retention and plant litter decomposition to soil homogenization. Productivity could be a central factor linking diversity and ecosystem functional responses to soil homogenization in ecosystems (Fig. 5.1). The plant species diversity differences between the homogeneous and heterogeneous treatments were related to total plant cover and density, suggesting the effects of heterogeneity on plant size influenced the resulting diversity patterns. Soil nutrient heterogeneity is believed to have a negative influence on diversity at fine spatial scales, because it favors the growth of more productive, competitive species (Gazol et al. 2013, Price et al. 2014, Baer et al. 2016, Tamme et al. 2016). Whichever treatment, homogeneous or heterogeneous, that results in increased plant density, could have greater diversity because of sampling effects and stochastic/neutral processes (i.e. with more individuals comes a greater probability of more species) (Williams and Houseman 2014, Walker and Lundholm 2018).

Underlying productivity differences between homogeneous and heterogeneous treatments extended to ecosystem level effects; as with increased aboveground productivity, increased aboveground ^{15}N retention and decreased mass loss occurred. It has long been recognized that soil heterogeneity can modify net primary productivity (Maestre et al. 2006), but my results suggest potential for this relationship to play an indirect role in the subsequent development of diversity and other ecosystem responses (Fig. 5.1). It was previously recognized that plant compositional diversity, rather than species diversity, may benefit ecosystem function in the context of soil heterogeneity (Maestre et al. 2006). Carbon, phosphorus and nitrogen cycling (measured via beta-glucosidase and acid phosphatase enzyme activity, and *in situ* N availability) were not influenced directly by soil nutrient heterogeneity (García-Palacios et al. 2011), but specific plant functional groups and traits had large effects on the cycling of these nutrients in response to nutrient heterogeneity (García-Palacios et al. 2013).

5.3 The importance of environmental stresses in heterogeneity relationships

My thesis is one of the first long term field investigations of the potential interactive effects of soil homogenization and environmental stress and probably the first to directly manipulate a source of heterogeneity (but see Arnone 1997). Some of the results suggest heterogeneous microsites offer microrefugia to plant communities during periods of stress (Chapter 4). Soil homogenization could result in the loss of microrefugia and increased sensitivity of plant communities during stressful periods. Soil homogenization effects on plant species diversity also could indirectly influence sensitivity of plant communities to stress, with an inverse relationship between sensitivity and diversity. Although there was no significant effect of soil homogenization or soil freezing on diversity in the sand treatment, soil homogenization significantly increased plant species diversity in the woodchip treatment. The increased species diversity in woodchip homogeneous plots may have been associated with the maintenance of native forb and grass cover in homogeneous plots during soil freezing. In Chapter 2, at the same site and with the same heterogeneity treatments, species diversity was greater in sand heterogeneous than homogeneous. This was possibly because the effect was more detectable due to a larger sample size (n=28) and because there was a greater pool of species (in Chapter 4, only one seeding took place in 2015). Chapter 2 also showed plant species diversity was greater in topographically heterogeneous plots with pits compared to flat soils during drought. My results demonstrate that the creation of soil microsites which function to increase species diversity and provide refuge from stressful conditions could be used as a management tool. I also demonstrated that stress can affect plant responses to soil heterogeneity, such that environmental extremes lead to augmented or subdued differences between plant communities in homogeneous versus heterogeneous environments. The importance of interactions between stress and environmental heterogeneity cannot be ignored because of the ubiquitous nature of the two.

5.4 The significance of microedges in heterogeneous soils for plant species diversity and ecosystem function

Just as heterogeneity-diversity relationships occur at both small and landscape-level spatial scales, several ecological processes also may have spatial analogues. At the spatial scale of within plant communities, research on soil heterogeneity has traditionally considered its impact as a summative

effect of microsites, without considering the edges between them (Stover and Henry 2018). Prior to my PhD research, few (if any) studies had examined the influence of microedges on plant communities or ecosystem processes. However, microedges may be analogous to the zones of overlap between vertical strata found within soil profiles, recognized as distinct sublayers in the soil taxonomy hierarchy (Weil and Brady 2016). Diversity, plant cover, soil properties and ecosystem responses varied along microedges over time and among heterogeneity sources but in several cases exhibited levels of responses that were intermediate, lower or elevated compared to their adjacent patches. Therefore, my results suggest microedges may function as 'microecotones' and provide unique ecological niches that could contribute to increased plant diversity. My results also suggest microedges exhibit ecosystem function that is not a simple additive effect of the adjacent microsites, which could occur due to complementarity between neighboring microsites. Experimental research on soil heterogeneity that takes microedges into account could identify more specific mechanisms involved in soil heterogeneity-plant diversity and ecosystem function relationships.

5.5 Methodological considerations and future research

In terms of community composition, in Chapter 2, the abundances of specific species among treatments are provided in the Appendix (Tables A.6-A.11). Preliminary analyses showed species abundances were redundant at the level of plant functional groups (native seeded and adventive forbs and grasses). In other words, relative abundances of species within functional groups did not vary greatly among treatments. Differences in plant functional group abundances among microsites and treatments were explored in detail in Chapter 2 using a linear mixed model, in which changes in community composition and functional group affinities for microsites and homogeneous and heterogeneous treatments at the level of plant functional groups is described.

The experimental treatments resulted in uniform replication of microsites that were discrete patches. This was necessary in order to statistically replicate the treatments and draw conclusions about the effects of homogenization. In reality, microsites are much more variable in size, shape and composition across space in ecosystems and may be continuous gradients rather than the discrete patches employed in the experimental design.

Although plant communities were monitored for three growing seasons in my thesis, my research took place during early succession, so the permanence and significance of the trends observed in the long term (> 5 years) needs to be investigated. Further research on microedges is required to determine their role in both promoting increased plant diversity and in creating unique interfaces for driving ecosystem processes. To build upon this work, more field experiments are required that also manipulate levels of plant species diversity and investigate the role of plant functional traits using the response effect trait framework (García-Palacios et al. 2012, 2013). Belowground root systems and three-dimensional heterogeneity should be further assessed to provide more explanation in terms of the mechanisms involved in the observed responses (Liu et al. 2017a, Liu et al. 2017b). Future research should consider experiments that more directly control productivity levels to better understand the role of productivity in influencing diversity and ecosystem responses to heterogeneity. In addition to field study and experimentation, modelling and theoretical development is needed in parallel to provide a clearer framework for interpreting the complexity of trends observed. It is still unclear how species should be selected for heterogeneity experiments, and how the number and type of species influences resulting investigations (but see Conradi et al. 2016, Liu et al. 2017b). In addition to including multiple diverse sources of heterogeneity within a single investigation, future researchers also may want to consider integrating the study of 'plant-induced' and 'environmentally-induced' heterogeneity. Overall, future research should continue to build upon the current framework of understanding variability in heterogeneity-plant relationships.

5.6 Implications for ecological restoration

My results clearly illustrate that habitat heterogeneity, created by the addition of diverse soil microsites, has the potential to aid efforts to increase biodiversity during ecological restoration. Microsites which promote the growth of specific plant species also can be used to facilitate the establishment of target species. Furthermore, microsites were not only beneficial for diversity, but also for ecosystem function, and buffered plant communities from drought and soil freezing. The pits, mounds and woodchip patches had sparse growth initially, and this 'delayed succession' may lead to alternate successional trajectories and facilitate establishment of later successional species. The 4:1 sand-topsoil microsites within the sand heterogeneity plots supported consistently high plant diversity throughout the study, and they were dominated by seeded native tallgrass prairie

forbs, unlike the topsoil patches, which supported a higher proportion of seeded prairie grasses. Seed source/ecotypes specific to soil types (i.e. clay topsoil and sandy soils) were sensitive to the microsites used, indicating that soil microsites should be designed in restoration that are compatible with the ecotypes of planted species. Many of the sown species important to conservation, such as native legumes, did not establish or were at extremely low abundance. Many vulnerable native flora have been restricted to marginal habitat on sandy soils, limiting their ability to establish on richer soils. Therefore, organizations which secure natural lands for conservation must target a greater diversity of soil habitat types so the ranges of these species can be further extended when restoration land becomes available.

5.7 Concluding remarks

Soil homogenization has not been explicitly considered in experimental investigations of heterogeneity-plant relationships to date, but my PhD research demonstrated that it may represent an important outcome of disturbance. My research is one of the first initiatives to test if soil homogenization influences productivity, nitrogen retention, decomposition and response to soil freezing, thereby addressing the greater implications beyond plant community and diversity effects. Overall, my results indicate that there is a significant relationship between soil homogenization, plant diversity, ecosystem responses and stress during ecological succession. Human addition of microsites and microedges could be used to benefit plant community stability and diversity in the context of ecological restoration. Finally, my thesis is important in terms of understanding the lack of clarity surrounding variability in heterogeneity-plant relationships, and it contributes to a promising framework that has been developing across studies, which will ultimately lead to better implementation of microsites as a valuable management tool.

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Appendices

Appendix A: Chapter 2 Supplementary data

Table A.1 Plant species seeded in research plots.

Year planted: 2015						Year planted: 2016				
Species Name	**Vernacular Name (Source*)**	**Life Form***	**Seed (No.) per plot**	**Seed (g) per outer block area**	**Viable seed (%)**	**Species Name**	**Vernacular Name (Source)**	**Life Form**	**Seed (No.) per plot**	**Viable seed (%)**
Ceanothus americanus	New Jersey Tea (W)	s	10		8	*Ceanothus americanus*	New Jersey Tea (W)	s	60	8
Andropogon gerardii	Big Bluestem (C)	g	60	11	95	*Bouteloua curtipendula*	Side-oats Gramma (W)	g	60	18
Bouteloua curtipendula	Side-oats Gramma (W)	g	28		18	*Carex vulpinoidea*	Fox sedge (C)	g	30	87
Elymus canadensis	Canada Wild Rye (W)	g	28		0	*Elymus hystrix*	Bottlebrush grass (C)	g	60	97
Elymus trachycaulus	Slender Wheatgrass (W)	g	28		26	*Elymus virginicus*	Virginia Wild Rye (W)	g	60	17
Schizachyrium scoparium	Little Bluestem (C)	g	67	10	62	*Juncus tenuis*	Path rush (C)	g	60	96
Sorghastrum nutans	Indian Grass (C)	g	67	10	6	*Leersia oryzoides*	Rice-cut grass (C)	g	60	53
Asclepias tuberosa	Butterflyweed (W)	f	19		47	*Scirpus atrovirens*	Black-fruited bulrush (C)	g	60	93
Desmodium canadense	Showy Ticktrefoil (W)	f	10		30	*Scirpus pendulus*	Lined Rush (W)	g	60	1
Heliopsis helianthoides	Sweet Oxeye (W)	f	6	2.5	13	*Spartina pectinata*	Prairie Chordgrass (C)	g	60	95
Lespedeza capitata	Round-headed Bushclover (C)	f	5		91	*Achillea millefolium*	Common Yarrow (C)	f	20	85
Liatris cylandracea	Dwarf Blazing-star (W)	f	10		22	*Agalinus tenuifolia*	Slender-leaved Agalinus (W)	f	60	1
Lupinus perennis	Wild Lupine (W)	f	10		25	*Asclepias incarnata*	Swamp milkweed (C)	f	60	91
Monarda fistulosa	Wild Bergamot (C)	f	11		95	*Asclepias syriaca*	Common milkweed (C)	f	60	98
Oenothera biennis	Evening Primrose (W)	f	6	1.5	NA	*Asclepias tuberosa*	Butterflyweed (C)	f	60	95
Penstemon digitalis	Foxglove Beardtongue (W)	f	12		5	*Desmodium canadense*	Showy Ticktrefoil (W)	f	60	30
Pycnanthemum virginianum	Virginia Mountain-mint (W)	f	19		28	*Doellingeria umbellata*	Flat topped Aster (C)	f	60	90
Ratibida pinnata	Grey-headed Coneflower (W)	f	10		9	*Euthamia graminifolia*	Grass-leaved goldenrod (C)	f	30	90
Silphium laciniatum	Compass-plant (W)	f	19		70	*Eutrochium maculatum*	Joe-Pye Weed (W)	f	60	13
Solidago juncea	Early Goldenrod (W)	f	12	2	2	*Gentiana andrewsii*	Bottle Gentian (W)	f	60	1
Solidago nemoralis	Grey Goldenrod (W)	f	12	2	2	*Gnaphalium obtusifolium*	Sweet Everlasting (W)	f	60	18
Solidago rigida	Stiff Goldenrod (W)	f	10		0.5	*Helianthus giganteus*	Tall Sunflower (C)	f	60	96
Symphyotrichum laeve	Smooth Blue Aster (C)	f	40		42	*Heliopsis helianthoides*	Sweet Oxeye (C)	f	30	91
Symphyotrichum novae-angliae	New England Aster (W)	f	10	2	1	*Lespedeza capitata*	Round-headed Bushclover (C)	f	60	96
Verbena hastata	Blue Vervain (W)	f	10		6	*Liatris spicata*	Dense Blazing Star (W)	f	60	68
Verbena stricta	Hoary Vervain (W)	f	10		18	*Lobelia siphilitica*	Great Lobelia (W)	f	60	25
Verbesina alternifolia	Wingstem (W)	f	10		11	*Lupinus perennis*	Wild Lupine (W)	f	30	25
Vernonia missurica	Prairie Ironweed (W)	f	10		3	*Ratibida pinnata*	Grey-headed Coneflower (C)	f	60	97
						Rudbeckia hirta	Black-eyed Susan (C)	f	60	97
						Sisyrinchium montanum	Blue-eyed grass (W)	f	60	0
						Verbesina alternifolia	Wingstem (C)	f	60	81

Source: W = wild collected, C = commercial; Life form: f = forb, g = graminoid, s = shrub
NA: seed for this species could not be tested

Table A.2 Mean soil properties (standard error) of substrates used to create homogeneous and heterogeneous treatments.

	Woodchips (pure substrate)	Sand (pure substrate)	Topsoil (pure substrate)	Woodchip-topsoil 2:1 (heterogeneous microsite)	Woodchip-topsoil 1:2 (homogeneous)	Sand:topsoil 4:1 (heterogeneous microsite)	Sand:topsoil 2:3 (homogeneous)
Soil organic matter (%)	-	2.2 (0.1)	7.3 (0.2)	-	-	-	-
Sand (%)	-	90 (1)	34 (1)	-	-	-	-
Silt (%)	-	7 (2)	51 (1)	-	-	-	-
Clay (%)	-	3 (1)	12 (1)	-	-	-	-
$N\text{-}NO_3$ µg G dry $soil^{-1}$	0.43 (0.06)	0.64 (0.09)	7.04 (0.11)	-	-	-	-
$N\text{-}NH_3$ µg G dry $soil^{-1}$	0.75 (0.26)	0.25 (0.15)	0.07 (0.15)	-	-	-	-
pH	6.60 (0.20)	8.50 (0.02)	7.60 (0.10)	6.90 (0.10)	7.10 (0.04)	8.00 (0.06)	7.80 (0.03)

Table A.3 Mean soil total carbon and nitrogen (standard error) in homogeneous and heterogeneous treatments and microsites present in heterogeneous plots.

		Total Carbon (%)	Total Nitrogen (%)
Woodchips	Heterogeneous	2.97 (0.23)	0.19 (0.01)
	Homogeneous	3.44 (0.29)	0.20 (0.01)
Heterogeneous microsites	Topsoil	2.68 (0.21)a	0.19 (0.01)
	Microedge	2.9 (0.24)ab	0.18 (0.01)
	2:1 Woodchips topsoil patch	3.33 (0.33)b	0.19 (0.01)
Sand	Heterogeneous	3.48 (0.14)	0.15 (0.01)*****
	Homogeneous	3.81 (0.26)	0.12 (0.01)
Heterogeneous microsites	Topsoil	2.73 (0.14)a	0.20 (0.01)a
	Microedge	3.27 (0.22)a	0.17 (0.02)b
	4:1 Sand topsoil patch	4.63 (0.23)b	0.09 (0.01)c
Topography	Pit	2.59 (0.21)	0.18 (0.01)*****
	Mound	2.81 (0.13)	0.20 (0.01)
	Homogeneous flat topsoil	2.91 (0.25)	0.19 (0.01)
Heterogeneous microsites			
Pit	Topsoil	2.69 (0.14)	0.20 (0.01)a
	Microedge	2.65 (0.20)	0.19 (0.02)a
	Pit	2.44 (0.31)	0.15 (0.01)b
Mound	Topsoil	2.84 (0.12)	0.21 (0.01)a
	Microedge	2.90 (0.18)	0.19 (0.01)b
	Mound	2.68 (0.13)	0.20 (0.01)ab

Numbers were back-transformed from the log scale $\log_{10}(y+1)$, standard error in the positive direction is shown.

Within heterogeneity source, heterogeneous treatments followed by ***** are significantly different from the homogeneous treatment ($p < 0.05$).

For heterogeneous microsites, within heterogeneity source, microsites with different letters are significantly different ($p < 0.05$).

Table A.4 Growing season (April-October) precipitation, temperature and long-term climate normals in the study area during the period research was conducted.

		Environment Canada Weather Station[1]		Environmental Sciences Western Field Station site records
Year	Month	Mean Temperature (°C)	Total Monthly Precipitation (mm)	Total Monthly Precipitation (mm)
2015	April	6.7	64.9	100.6
	May	15.7	60.2	70.5
	June	17.7	173.1	182.5
	July	19.9	61.8	64.5
	August	19.2	34.6	80.5
	September	18.5	63.0	56.0
	October	9.6	77.4	141.5
			2015 Total: 535.0	2015 Total: 696.1
2016	April	5.0	48.6	65.3
	May	14.1	31.1	50.0
	June	18.0	63.1	30.3
	July	21.4	91.1	98.5
	August	22.0	170.4	153.5
	September	18.0	70.3	44.5
	October	11.0	50.1	54.5
			2016 Total: 524.7	2016 Total: 496.6
2017	April	9.5	113.0	119.5
	May	12.0	133.4	84.0
	June	18.7	67.6	98.5
	July	20.5	49.6	30.0
	August	18.6	42.4	44.0
	September	17.4	32.0	41.5
	October	12.2	84.9	57.0
			2017 Total: 522.9	2017 Total: 474.5
Long Term Normals[2]	April	7.3	81.2	1988–2017
	May	13.9	92.0	Mean: 658.1
	June	19.0	85.1	Standard deviation: 206.2
	July	21.2	89.8	Minimum: 383.5
	August	20.1	97.6	Maximum: 1179.5
	September	16.1	104.4	
	October	9.9	77.6	
			Total: 627.7	

[1]2015–2017 London, Ontario 43°02'00.000" N, 81°09'00.000" W, elevation 278 m.
[2]1981– 2010 Ilderton Bear Creek, Ontario, 43°03' N, 81°26' W, elevation 267 m.

Table A.5 Mean functional group percent cover (standard error) for microsites present in heterogeneous plots.

	Woodchips			Topography (Pit)			Topography (Mound)		
	Topsoil	Microedge	2:1 Woodchips Topsoil	Topsoil	Microedge	Pit	Topsoil	Microedge	Mound
Seeded Grasses									
2016	5.4 (1.1) a	2.4 (0.6) b	0.2 (0.1) c	5.1 (1.4) a	3.8 (0.9) a	1.3 (0.3) b	7.7 (1.6) a	2.5 (0.6) b	3.7 (1.0) b
2017	11.2 (2.2) a	6.8 (1.5) b	1.7 (0.3) c	14.9 (3.9) a	7.8 (1.9) b	8.2 (1.9) b	15.9 (3.0) a	6.9 (1.7) b	10.6 (2.6) c
Seeded Forbs									
Early Season									
2017	24.3 (7.1) a	12.0 (3.7) b	3.4 (1.1) c	39.5 (10.8) a	15.2 (6.3) b	8.2 (3.3) b	43.0 (8.7) a	31.1 (10.2) ab	17.7 (6.7) b
Late Season									
2015	12.2 (4.1) a	3.4 (1.4) b	0.5 (0.2) c	9.7 (4.1) a	4.6 (2.9) b	2.2 (1.1) b	18.4 (6.9) a	15.7 (6.9) a	4.0 (2.3) b
2016	30.5 (10.3) a	7.7 (3.2) b	2.4 (0.8) c	39.2 (14.8) a	11.7 (5.9) b	8.0 (2.9) b	48.1 (13.8) a	34.6 (13.7) a	11.4 (6.1) b
2017	23.0 (7.2) a	25.0 (6.7) a	8.5 (2.4) b	63.2 (10.2) a	38.3 (7.9) a	13.9 (4.4) b	50.8 (16.2)	41.8 (14.6)	31.5 (8.4)
Adventive Grasses									
Early Season									
2015	1.2 (1.1)	0.3 (0.2)	0 (0)	0.1 (0.1)	0 (0)	0.6 (0.5)	1.8 (0.8) a	1.2 (0.6) ab	0.1 (0.1) b
2016	2.0 (0.4)	1.6 (0.3)	1.6 (0.4)	3.1 (0.8) ab	2.4 (0.7) a	5.0 (1.1) b	1.7 (0.6)	1.6 (0.3)	2.5 (0.7)
2017	0.6 (0.1) a	1.1 (0.2) b	0.9 (0.2) ab	0.9 (0.2) a	1.0 (0.3) a	1.9 (0.3) b	0.8 (0.2) ab	0.7 (0.8) b	1.2 (0.2) a
Late Season									
2015	4.0 (2.4)	1.0 (0.7)	0.1 (0.1)	0.8 (0.6)	0 (0)	2.2 (1.5)	4.5 (2.1)	4.2 (2.6)	1.2 (0.7)
2016	5.2 (1.1)	3.8 (1.0)	3.3 (0.7)	10.8 (3.8) ab	5.8 (1.6) a	14.6 (3.3) b	4.4 (1.2)	4.7 (1.2)	8.1 (2.4)
2017	1.1 (0.3)	1.2 (0.3)	0.7 (0.2)	0.8 (0.3)	0.5 (0.8)	1.6 (0.6)	0.5 (0.2)	0.4 (0.2)	1.3 (0.3)
Adventive Forbs									
2015	0.7 (0.2)	0.4 (0.2)	0.4 (0.1)	2.0 (0.8)	1.1 (0.5)	1.7 (0.7)	1.7 (0.6) a	0.4 (0.2) b	1.1 (0.4) a
2016	4.8 (0.8) a	4.9 (0.8) a	2.6 (0.6) b	3.8 (1.0) a	6.5 (1.3) a	11.4 (2.0) b	4.9 (1.2)	3.7 (0.7)	5.3 (1.3)
2017	7.0 (1.4)	6.0 (0.9)	7.1 (1.2)	4.8 (1.3) a	7.1 (1.4) a	16.6 (3.3) b	4.6 (1.3) a	3.6 (0.9) a	9.1 (2.1) b

Numbers were back-transformed from the log scale $\log_{10}(y+1)$, standard error in the positive direction is shown. Within year, season and heterogeneity source, means followed by different letters are significantly different ($p < 0.05$). Only significant treatment years and seasons are presented.

Table A.6 Mean percent cover (2015–2017) of native seeded grasses found in research plots. Species with 'x' have mean percent cover of less than 0.1 % and greater than 0 %.

Botanical name	Vernacular name	WC het	WC homog	Sand het	Sand homog	Pit	Mound	Flat soil
Andropogon gerardii	Big bluestem	0.9	0.9	3.2	2.8	2.3	2.0	1.7
Bouteloua curtipendula	Side-oats gramma	0	0	x	0	0	x	0
Elymus canadensis	Canada wild rye	0	0	0	x	0	0.2	0
Elymus hystrix	Bottlebrush grass	0	x	0	0	x	x	x
Elymus trachycaulis	Slender wheatgrass	2.3	1.5	3.7	2.9	2.1	3.1	2.6
Elymus virginicus	Virginia wild rye	0.2	0.3	0.2	0.2	0.5	0.4	0.3
Schizachyrium scoparium	Little bluestem	0.2	0.5	1.3	1.3	0.6	0.7	1.1
Sorghastrum nutans	Indian grass	3.8	2.3	5.8	4.8	3.4	3.6	6.3

WC=woodchips, het=heterogeneous, homog=homogeneous

Table A.7 Mean percent cover (2015–2017) of native seeded leguminous forbs found in research plots. Species with 'x' have mean percent cover of less than 0.1 % and greater than 0 %.

Botanical name	Vernacular name	WC het	WC homog	Sand het	Sand homog	Pit	Mound	Flat soil
Desmodium canadense	Showy ticktrefoil	0.4	0.6	0.6	0.6	0.5	1.2	1.1
Lespedeza capitata	Round-headed bushclover	0	0	0	0	x	0	0
Lupinus perennis	Wild lupine	x	x	x	x	x	x	x

WC=woodchips, het=heterogeneous, homog=homogeneous

Table A.8 Mean percent cover (2015–2017) of native seeded non-leguminous forbs found in research plots. Species with 'x' have mean percent cover of less than 0.1 % and greater than 0 %.

Botanical name	Vernacular name	WC het	WC homog	Sand het	Sand homog	Pit	Mound	Flat soil
Achillea millefolium	Common yarrow	0	x	x	x	x	x	x
Agalinus tenuifolia	Slender false foxglove	x	0.3	0.2	0.2	0.2	0.3	0.4
Asclepias incarnata	Swamp milkweed	x	0	x	x	x	0	0
Asclepias syriaca	Common milkweed	0	0	x	x	0	0	0
Asclepias tuberosa	Butterflyweed	x	x	x	x	x	x	x
Ceanothus americanus	New Jersey tea	x	x	x	x	0	x	x
Euthamia graminifolia	Grass-leaved goldenrod	0	x	0	0	0	0	0.2
Gentiana andrewsii	Bottle gentian	0	x	0	0	0	0	0
Heliathus giganteus	Tall sunflower	0	x	x	x	x	x	0
Heliopsis helianthoides	Sweet oxeye	x	x	x	0.1	x	x	x
Liatris cylandracea	Dwarf blazing-star	0	0	x	x	0	0	0
Liatris spicata	Dense blazing-star	x	x	x	x	x	0	0
Lobelia siphilitica	Great blue lobelia	0	0	0	0	x	0	0
Monarda fistulosa	Wild bergamot	0.4	0.2	0.9	0.7	1.2	1.9	1.5
Oenothera biennis	Evening primrose	1.3	1.5	2.1	4.0	1.2	2.6	4.5
Penstemon digitalis	Foxglove beardtongue	0.2	0.3	0.1	0.2	0.2	0.3	0.1
Pycnanthemum virginianum	Virginia mountain-mint	0.5	1.1	2.6	2.2	1.5	1.1	2.6
Ratibida pinnata	Grey-headed Coneflower	0.3	0.3	0.8	0.5	0.2	0.2	0.2
Rudbeckia hirta	Brown eyed Susan	0.4	0.3	0.1	0.2	0.2	0.5	0.3
Rudbeckia laciniata	Cutleaf coneflower	0	0	0	x	0	0	0
Silphium laciniatum	Compass-plant	7.6	9.7	13.4	12.0	10.5	9.7	11.5
Sisyrinchium montanum	Blue-eyed grass	0	x	0	x	0	x	0

WC=woodchips, het=heterogeneous, homog=homogeneous

Table A.8, con'd Mean percent cover (2015–2017) of native seeded non-leguminous forbs found in research plots. Species with 'x' have mean percent cover of less than 0.1 % and greater than 0 %.

Botanical name	Vernacular name	WC het	WC homog	Sand het	Sand homog	Pit	Mound	Flat soil
Solidago juncea	Early goldenrod	4.8	0.4	10.4	5.8	3.5	5.0	6.8
Solidago nemoralis	Grey goldenrod	0.3	0.8	2.6	4.5	2.3	3.2	1.7
Solidago rigida	Stiff goldenrod	0	0	0.3	0.2	0	0.3	x
Symphyotrichum laeve	Smooth blue aster	x	x	1.6	2.2	0.4	0.9	0.2
Symphyotrichum novae-angliae	New England aster	1.5	1.8	3.2	4.9	1.5	3.2	3.5
Verbena hastata	Blue vervain	5.1	3.1	5.4	5.2	3.7	7.3	9.9
Verbena stricta	Hoary vervain	1.0	0.1	0.6	0.5	1.1	0.6	1.4
Verbesina alternifolia	Wingstem	0.1	x	0.4	0.6	0.3	1.8	0.3
Vernonia missurica	Prairie ironweed	x	x	0.4	0.3	x	0.1	1.0

WC=woodchips, het=heterogeneous, homog=homogeneous

Table A.9 Mean percent cover (2015–2017) of adventive non-seeded graminoids found in research plots. Species with 'x' have mean percent cover of less than 0.1 % and greater than 0 %.

Botanical name	Vernacular name	WC het	WC homog	Sand het	Sand homog	Pit	Mound	Flat soil
Cyperus esculentus	Yellow nutsedge	x	0	0	x	x	x	0
Digitaria sanguinalis	Hairy crabgrass	x	x	0	0	0	0	0
Panicum capillare	Witch grass	x	x	0.2	x	x	x	x
Poa compressa	Canada bluejoint	0	0	0	0	0	0	x
Setaria viridis	Green foxtail	1.6	3.1	2.0	2.3	2.8	2.2	1.1

WC=woodchips, het=heterogeneous, homog=homogeneous

Table A.10 Mean percent cover (2015–2017) of adventive non-seeded leguminous forbs found in research plots. Species with 'x' have mean percent cover of less than 0.1 % and greater than 0 %.

Botanical name	Vernacular name	WC het	WC homog	Sand het	Sand homog	Pit	Mound	Flat soil
Medicago lupulina	Black medic	1.0	2.5	0.8	1.2	1.5	1.1	1.7
Melilotus alba	White sweet clover	0	0	0	0	0	0	0
Melilotus sp.	Sweet clover	0	0	0	0	0	0	0
Trifolium pratense	Red clover	x	x	x	0.3	0.3	0.2	0.2
Trifolium repens	White clover	0	x	0	0	x	0	0

WC=woodchips, het=heterogeneous, homog=homogeneous

Table A.11 Mean percent cover (2015–2017) of adventive non-seeded forbs found in research plots. Species with 'x' have mean percent cover of less than 0.1 % and greater than 0 %.

Botanical name	Vernacular name	WC het	WC homog	Sand het	Sand homog	Pit	Mound	Flat soil
Amaranthus retroflexus	Redroot pigweed	x	x	0.2	0.2	0	0.4	0.3
Ambrosia artemisiifolia	Common ragweed	x	0.1	x	0.3	x	0	0
Anagallis arvensis	Scarlet pimpernel	0.3	0.1	0.2	0.6	1.3	x	0.4
Cerastium arvense	Field chickweed	x	x	x	x	0	0	x
Chenopodium album	Lamb's quarters	0.2	0.2	x	0.3	0.3	0.1	0.2
Cirsium arvense	Canada thistle	2.4	3.2	2.3	2.2	2.6	2.4	3.3
Cirsium vulgare	Bull thistle	0	0	x	0	0	0	x
Convolvulus arvensis	Field bindweed	0	0	0	0	x	0	0
Conyza canadensis	Horseweed	0.1	x	0.1	x	0.2	0.2	x
Cornus stolonifera	Red osier dogwood	0	x	0	0	0	0	0
Daucus carota	Wild carrot	0.2	0.4	x	0.4	0.3	x	0.4
Epilobium ciliatum	Fringed willowherb	x	x	x	0.1	x	x	x
Epilobium sp.	Willowherb	0	x	x	x	x	x	0
Erigeron annuus	Annual fleabane	0.5	0.6	0.4	0.1	0.5	0.6	0.4
Erigeron pulchellus	Robin's Plantain	0	0	0	0	x	0.1	0
Fragaria virginiana	Meadow strawberry	0	x	x	0	x	x	x
Holosteum umbellatum	Jagged chickweed	0	0	0	0	0	0	x
Hypericum perforatum	Common St. Johnswort	0	0	0	x	0	0	0
Oxalis dillenii	Slender yellow woodsorrel	x	x	x	x	x	x	x
Plantago lanceolata	Narrow-leaved plantain	0	0	0	x	0	0	0
Plantago major	Common plantain	0.4	0.5	0.6	0.7	1.5	0.6	0.5
Polygonum sp.	Knotweed	0	0	x	x	0	0	0
Senecio vulgaris	Common groundsel	0.1	x	x	x	0.2	0.1	x
Solidago altissima	Late goldenrod	0	0	x	0	0	0	0

WC=woodchips, het=heterogeneous, homog=homogeneous

Table A.11, con'd Mean percent cover (2015–2017) of adventive non-seeded forbs found in research plots. Species with 'x' have mean percent cover of less than 0.1 % and greater than 0 %.

Botanical name	Vernacular name	WC het	WC homog	Sand het	Sand homog	Pit	Mound	Flat soil
Solidago canadensis	Canada goldenrod	0.7	0.6	0.8	0.5	0.5	0.2	2.0
Solanum carolinense	Horse nettle	0.5	0	x	x	x	0.9	0
Solanum nigrum	Black nightshade	0	0	x	0	0	0	0
Sonchus arvensis	Perennial sow thistle	x	x	0.3	0.1	0.1	x	x
Sonchus asper	Prickly sow thistle	0.2	x	0.2	0.3	0.5	0.3	x
Sonchus oleraceus	Common sow thistle	1.4	1.3	1.2	0.7	1.0	1.1	0.5
Symphyotrichum lanceolatum	Tall panicled aster	0.3	0.6	0.2	0.2	1.0	0.1	0.6
Taraxacum officinale	Danelion	0.6	1.0	0.4	0.5	1.2	0.7	0.3
Vitis riparia	Riverbank Grape	0	0	x	0	0	0	0
Verbascum thapsus	Common Mullein	0	0	0	x	0	0	0
Veronica arvensis	Corn speedwell	0	0	0	0	0	x	0

WC=woodchips, het=heterogeneous, homog=homogeneous

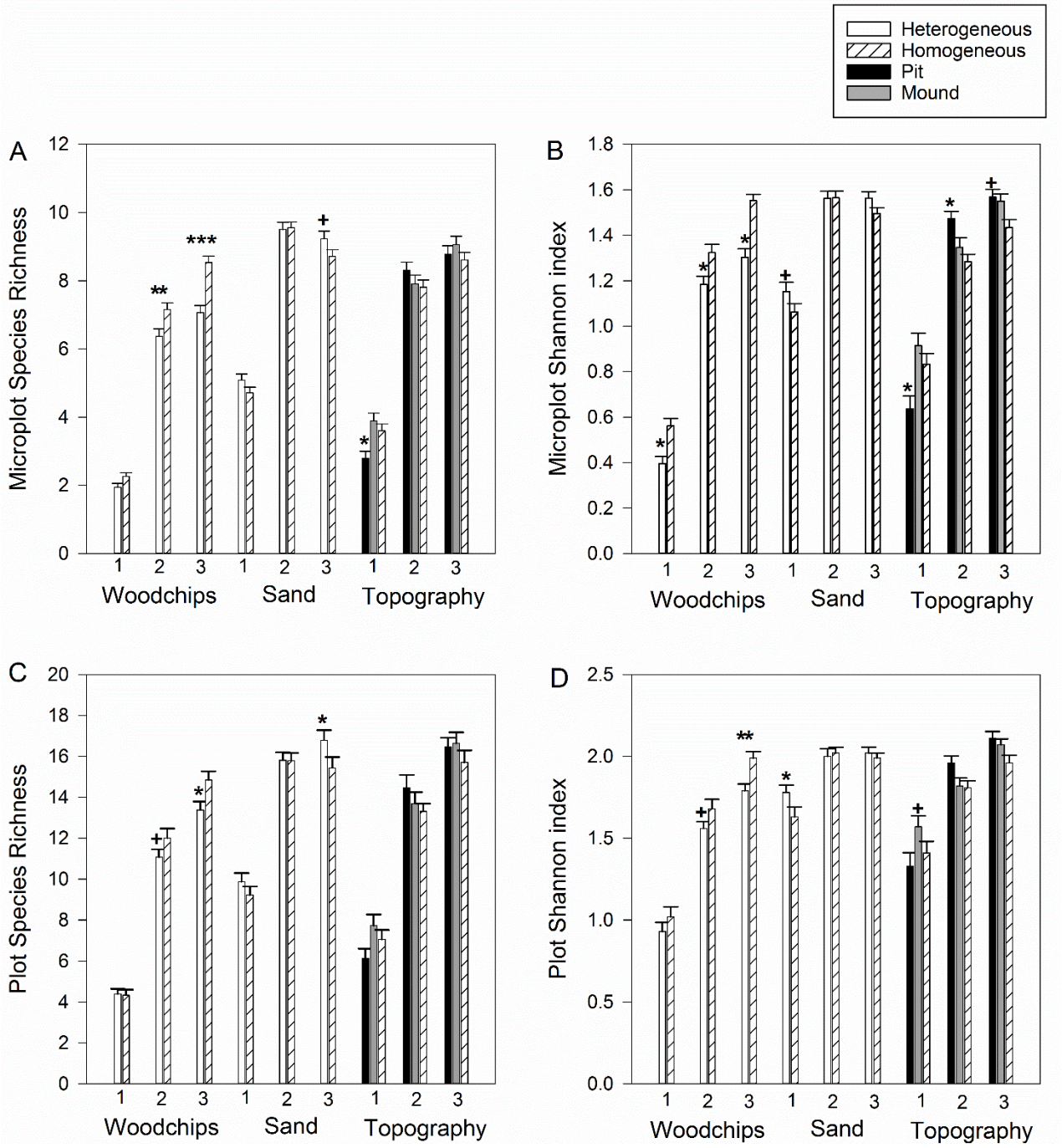

Figure A.1 Mean species diversity over three years (1 = year 1, 2015; 2 = year 2, 2016; 3 = year 3, 2017) in the three different sources of heterogeneity studied. Parts A and C are species richness, B and D are Shannon index, A and B are mean microplot diversity (spatial scale 0.0625 m^2) and C and D are plot diversity (0.1875 m^2). Error bars are standard error. Within year and heterogeneity source, heterogeneous treatments followed by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) are significantly different from the homogeneous treatment and + are marginally significantly different ($p < 0.1$).

Appendix B: Chapter 4 Supplementary data

Table B.1 Winter (December-March) precipitation, temperature and long-term climate normals in the study area during the period research was conducted.

Winter	Month	Mean Temperature[1] (°C)	Total Rainfall (mm)	Total Snow (mm)
2015-2016	December	3.3	60.9	9.0
	January	-4.4	32.3	42.5
	February	-2.7	41.4	58.5
	March	3.5	138.0	18.5
2016-2017	December	-2.6	36.4	64.0
	January	-2.0	62.4	41.6
	February	0.1	84.0	22.0
	March	0.2	58.5	26.0
	December	-2.6	44.5	45.4
Long Term	January	-5.6	28.7	45.6
Normals[2]	February	-4.4	30.6	32.0
	March	0.4	41.8	22.7

[1]2015-2017 London International Airport, London, Ontario, 43°01'59.000" N, 81°09'04.000" W, 278 m. [2]1981– 2010 Ilderton Bear Creek, Ontario, 43°03' N, 81°26' W, 267 m.

Table B.2 Interactions detected among soil freezing, heterogeneity and year for the plant responses examined[1].

Treatment	Response	Interaction	*p*	*F*	*d.f.*
Sand	Total cover	heterogeneity × freezing × year	0.094	1.8	671
		heterogeneity × freezing	0.045	4.3	36
Sand	Density	heterogeneity × freezing × year	0.0063	5.1	680
Sand	Native cover	heterogeneity × freezing	0.049	4.1	40
Woodchips	Native cover	heterogeneity × freezing	0.009	7.5	36
Woodchips	Species richness	heterogeneity × freezing	0.047	4.3	30
		heterogeneity × year	0.001	6.96	680

[1]Adventive forb and grass cover were analyzed non-parametrically and a heterogeneity × freezing × year interaction was confirmed for both responses in both sand and woodchip treatments visually with graphical plots.

Table B.3 Mean percent cover (standard error) of native seeded grasses and forbs in sand and woodchip heterogeneous microsites with and without snow removal.

	Topsoil patch	Microedge	Woodchips patch	Topsoil patch	Microedge	Sand patch
2015						
No snow removal	12.8 (5.3)	2.9 (1.3)	0.6 (0.2)	11.2 (3.7)	20.0 (6.4)	23.8 (7.6)
Snow removal	4.1 (1.9)*	1.8 (1.0)	0.4 (0.2)	24.7 (6.4)	21.1 (5.1)	20.3 (6.0)
2016						
No snow removal	68.0 (13.8)	12.0 (5.9)	2.3 (0.9)	51.0 (10.0)	65.2 (11.7)	89.0 (10.5)
Snow removal	17.5 (7.5)*	4.8 (2.2)	1.7 (0.6)	92.2 (14.4)*	82.0 (12.6)	65.6 (8.5)
2017						
No snow removal	39.6 (11.8)	38.4 (10.1)	7.3 (2.8)	65.8 (10.3)	61.4 (9.5)	149.5 (8.4)
Snow removal	42.3 (7.4)	10.0 (2.9)*	7.3 (1.9)	79.4 (11.3)	80.8 (8.0)	128.5 (15.3)

Within microsite type, means followed by * ($p < 0.05$) are significantly different from the control treatment (no snow removal).
2015 was the pre-snow removal year and 2016 and 2017 represent the growing seasons after one and two winters of snow removal, respectively.
Means were back-transformed from the log scale $\log_{10}(y+1)$ and standard error in the positive direction is shown.

Table B.4 Mean percent cover (standard error) of adventive grasses in sand and woodchip heterogeneous microsites with and without snow removal during the late growing season.

	Topsoil patch	Microedge	Woodchips patch	Topsoil patch	Microedge	Sand patch
2015						
No snow removal	3.3 (2.5)	0 (0)	0 (0)	0.6 (0.3)	3.0 (1.6)	0.1 (0.1)
Snow removal	12.0 (8.3)	2.5 (2.5)	0.3 (0.3)	1.9 (1.3)	1.6 (1.5)	0 (0)
2016						
No snow removal	3.6 (1.1)	1.6 (0.5)	1.9 (0.5)	6.6 (2.4)	4.1 (1.2)	2.6 (1.0)
Snow removal	8.1 (1.6)*	5.2 (1.9)*	3.4 (1.9)	3.4 (1.2)	1.6 (0.5)+	1.5 (0.5)
2017						
No snow removal	1.0 (0.5)	0.9 (0.3)	0.8 (0.4)	0.5 (0.2)	0.8 (0.5)	0.4 (0.2)
Snow removal	6.6 (4.8)+	3.8 (1.9)+	1.5 (1.0)	0.9 (0.5)	0.7 (0.3)	0.9 (0.3)

Within microsite type, means followed by * ($p < 0.05$) or + ($p < 0.1$) are significantly different or marginally significantly different, respectively, from the control treatment (no snow removal).
2015 was the pre-snow removal year and 2016 and 2017 represent the growing seasons after one and two winters of snow removal, respectively.

Table B.5 Mean percent cover (standard error) of adventive forbs in sand and woodchip heterogeneous microsites with and without snow removal.

	Topsoil patch	Microedge	Woodchips patch	Topsoil patch	Microedge	Sand patch
Late growing season						
2015						
No snow removal	10.1 (4.7)	1.0 (0.7)	0.1 (0.1)	4.6 (4.5)	9.0 (7.9)	8.6 (7.9)
Snow removal	1.4 (1.2)	7.2 (6.0)	2.0 (1.1)	1.5 (1.5)	7.9 (5.1)	10.2 (10.0)
2016						
No snow removal	15.4 (8.3)	15.7 (6.2)	8.2 (5.0)	12.1 (3.1)	15.2 (7.0)	4.0 (1.0)
Snow removal	14.6 (5.7)	5.9 (2.1)	14.8 (8.8)	15.6 (7.3)	16.9 (8.5)	11.2 (4.2)
2017						
No snow removal	10.7 (4.9)	8.3 (3.2)	7.9 (2.4)	3.2 (1.0)	5.0 (2.1)	4.8 (2.1)
Snow removal	15.7 (10.0)	6.6 (1.4)	11.6 (5.2)	9.8 (4.1)	2.7 (0.7)	4.9 (2.0)
Early growing season[1]						
2015						
No snow removal	0.4 (0.4)	0 (0)	0 (0)			
Snow removal	0.6 (0.6)	1.2 (0.8)	0.4 (0.4)			
2016						
No snow removal	10.2 (3.4)	13.1 (4.9)	14.6 (9.0)			
Snow removal	16.2 (7.9)	8.1 (4.9)	16.5 (10.6)			

[1]Early growing season data is shown for the woodchip treatment only since significant homogeneous and heterogeneous treatment differences were not detected in the sand treatment.

2015 was the pre-snow removal year and 2016 and 2017 represent the growing seasons after one and two winters of snow removal, respectively.

Table B.6 Mean species richness (standard error) in woodchip heterogeneous microsites with and without snow removal.

	Topsoil patch	Microedge	Woodchips patch
2015			
No snow removal	3.5 (0.4)	1.6 (0.3)	0.8 (0.2)
Snow removal	2.9 (0.4)	1.3 (0.2)	0.7 (0.2)
2016			
No snow removal	8.5 (0.6)	6.2 (0.5)	4.3 (0.3)
Snow removal	6.8 (0.4)*	5.5 (0.5)	4.2 (0.3)
2017			
No snow removal	7.2 (0.5)	6.2 (0.4)	4.6 (0.5)
Snow removal	6.9 (0.5)	5.5 (0.4)	4.5 (0.4)

Within microsite type, means followed by * ($p < 0.05$) are significantly different from the control treatment (no snow removal).

2015 was the pre-snow removal year and 2016 and 2017 represent the growing seasons after one and two winters of snow removal, respectively.

Means were back-transformed from the log scale $\log_{10}(y+1)$ and standard error in the positive direction is shown.

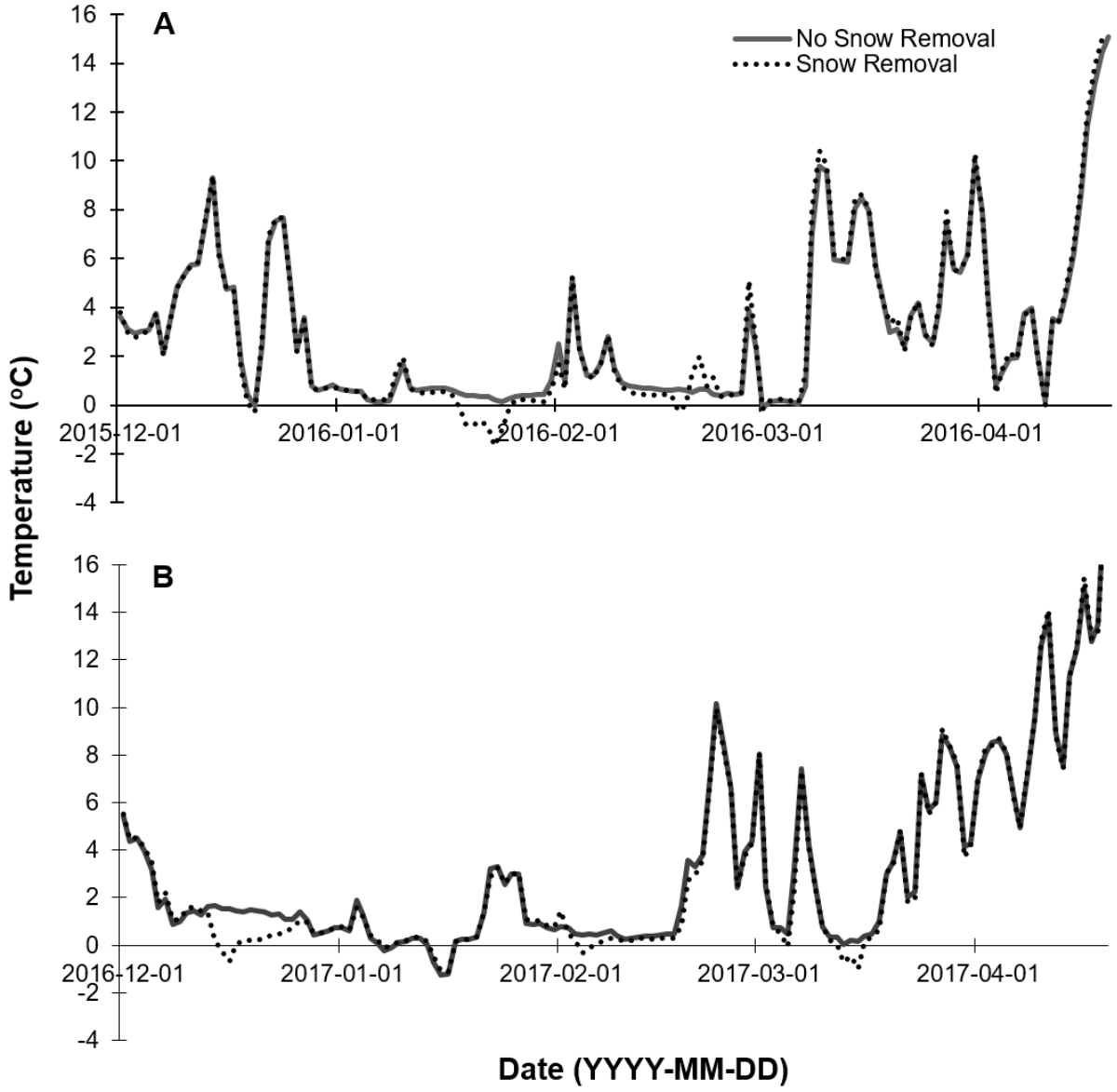

Figure B.1 Mean soil temperature in no snow removal (solid line) and snow removal (dotted line) plots during the two winters of snow removal, A) 2015-2016 and B) 2016-2017. Soil temperature sensors were buried at 5 cm soil depth (n = 6).

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Some of the Chapter 1 content was published in the journal *Ecosphere*.

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Post-secondary Education and Degrees:	The University of Western Ontario London, Ontario, Canada 2005-2010 Honors B.Sc. (Science Internship)
	The University of Alberta Edmonton, Alberta, Canada 2010-2013 M.Sc. Land Reclamation
	The University of Western Ontario London, Ontario, Canada 2014-current Ph.D. Biology
Honours and Awards:	Collaborative Program in Environment & Sustainability Graduate Student Travel Award (2018)
	Collaborative Program in Environment & Sustainability Graduate Student Award (2017)
	Collaborative Program in Environment & Sustainability Graduate Travel Award (2017)
	Department of Biology Travel Award (2017)
	Field Botanists of Ontario Student Award (2016)
	Doctoral Excellence Research Award, Western Graduate & Postdoctoral Studies (2016-2017)
	Collaborative Program in Environment & Sustainability A.E.R. Scholarship (2016)
	Vice President's Research Award, Research Western (2015-2018)
	David E. Laudenbach Scholarship (2015-2016)
	Future Environmental Professional Award, Air & Waste Management Association Ontario Section (2015)
	Vanier Canada Graduate Scholarship, Natural Sciences and Engineering Research Council of Canada (NSERC) (2015-2018)
	Postgraduate Scholarship-Doctoral level, NSERC (declined 2015)
	Ontario Graduate Scholarship-Doctoral level (declined 2015)
	Collaborative Program in Environment & Sustainability Graduate Student Award (2015)

Ruth Horner Arnold Fellowship (2014)

Graduate Student Award of Merit, Senior Women Academic Administrators of Canada (nominated 2014)

Mary Louise Imrie Graduate Student Travel Award (2012)

Alberta Government Advanced Education and Technology Graduate Scholarship (2012)

Shell Self-Enhanced Learning Fund (2012)

Graduate Students Professional Development Grant (2012)

Queen Elizabeth II Graduate Scholarship (2011)

Soil Ecology Society Parkinson Travel Grant (2011)

Starfish Canada Top 25 Environmentalists Under 25 (2011)

Western Scholars Degree Designation (2010)

NSERC Undergraduate Student Research Award (2009)

Millennium Excellence Award (2007)

Dean's Honor List (2005-2010)

Award of Distinction Western University Entry Scholarship (2005)

Zonta Young Women in Public Affairs Award (2005)

Related Work Experience

Research Assistant
The University of Western Ontario
Assisted in projects in laboratory of Supervisor Dr. Hugh Henry (International Drought Experiment, Nutrient Network Experiment, nitrogen deposition in tallgrass prairie restoration, Environmental Sciences Western Field Station tallgrass prairie restoration)
2014-present

Organizing Committee Member (Volunteer)
Sustainability Panel Discussion Series & EnviroCon (graduate student-run conference)
The University of Western Ontario
2014-2018

Guest Lecturer
Restoration Ecology
The University of Western Ontario
2014-2017

Co-leader, Great Canadian Shoreline Clean Up (Volunteer)
2016-2017

Teaching Assistant
Courses: Research Hypothesis Testing, Environmental Biology, Restoration Ecology
The University of Western Ontario
2014-2017

Volunteer, Thames Talbot Land Trust
2014-2017

Advisory Committee Member
Biology 4999E Honors Research Thesis
2015-2016

Biologist, Carolinian Canada Coalition
Assisted with field survey for Species At Risk
2014

Botany Intern, Royal Botanical Gardens
2013-2014

Teaching Assistant
Courses: Land Reclamation & Revegetation, Special Topics in Land Reclamation, Wildlife Ecology & Conservation
The University of Alberta
2011-2013

Research Assistant
The University of Alberta
Assisted in land reclamation research projects in laboratory of Dr. M. Anne Naeth
2011-2012

Research Assistant & NSERC USRA student
The University of Western Ontario
Assisted in research projects in laboratory of Dr. R. G. Thorn
2009-2010

EnviroWestern Coordinator, University Students' Council
The University of Western Ontario
2009-2010

Remediation Associate (Science Internship)
Imperial Oil Limited
2008-2009

Publications:

Stover, H.J., M.A. Naeth and K. Boldt-Burisch. In Press. Soil disturbance changes arbuscular mycorrhizal fungi richness and composition in a Fescue grassland in Alberta Canada. Applied Soil Ecology.

Stover, H.J. and H.A.L. Henry. 2018. Soil homogenization and microedges: perspectives on soil-based drivers of plant diversity and ecosystem processes. Ecosphere. doi: 10.1002/ecs2.2289.

Stover, H.J., M.A. Naeth and S.R. Wilkinson. 2018. Transplanting following non-native plant control in foothills fescue grassland restoration. Ecological Restoration. 36(1): 19–27.

Henry, H.A.L. M. Abedi, C.L. Alados, K.H. Beard, L.H. Fraser, A. Jentsch, J. Kreyling, A. Kulmatiski, E.G. Lamb, W. Sun, M.R. Vankoughnett, S. Venn, C. Werner, I. Beil, I. Blindow, S. Dahlke, M. Dubbert, A. Effinger, H.W. Garris, M. Gartzia, T. Gebauer, M. Holdrege, M.A.S.A. Khan, A.V. Malyshev, J.P. Paulson, Y. Pueyo, **H.J. Stover** and X. Yang. 2018. Snow removal versus rain-out shelters as components of reduced precipitation effects on plants: results from a globally-coordinated distributed field experiment. Ecosystems. doi: https://doi.org/10.1007/s10021-018-0231-7.

Stover, H.J., M.A. Naeth and S.R. Wilkinson. 2017. Glyphosate, steam and cutting for non-native plant control in Alberta fescue grassland restoration. Applied Vegetation Science. 20: 608–619. doi: 10.1111/avsc.12316.

Stover, H.J., M.A. Naeth and S.R. Wilkinson. 2017. Relative performance of native cultivar and wild collected seed for native grassland restoration. Ecological Engineering. 103: 141–145. doi: 10.1016/j.ecoleng.2017.03.012.

Stover, H.J., R.G. Thorn, J.M. Bowles, M.A. Bernards and C.R. Jacobs. 2012. Arbuscular mycorrhizal fungi and vascular plant species abundance and community structure in tallgrass prairies with varying agricultural disturbance histories. Applied Soil Ecology. 60: 61–70.