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The effects of global climate change on Canadian boreal forest Collembola
communities

(Thesis format: Integrated Article)

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
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Abstract

Soil fauna are an integral component of terrestrial ecosystem function. The effects of global environmental change on soil biodiversity are poorly studied, particularly interactions among temperature, atmospheric CO_2, precipitation intensity, and nutrient loading. Body size distributions can be used to quantify soil community responses to perturbation and consequences for ecosystem function. I quantified top-down and bottom-up effects of environmental change on the abundance, richness, and size distribution of the soil microarthropod group Collembola. I demonstrated negative effects in a lab experiment of increased precipitation on collembolan density and richness across all size groups. I demonstrated positive effects in a field experiment of N addition on collembolan richness, and a positive effect of C addition on evenness. These findings demonstrate that precipitation can act as a disturbance to soil communities, as well as the importance of bottom-up control in soils, and the responsiveness of body size distributions to environmental change.

Keywords

Co-Authorship Statement

This thesis contains discussion of a soil community assessment approach known as the body size spectrum (BSS), the methodology and application of which was previously described in a review paper co-authored with Paul George and Dr. Zoë Lindo (Turnbull et al. 2014). All co-authors contributed substantially to literature collection and synthesis, writing, and editing. Portions of the research presented in chapters 2 and 3 of this thesis represent a use of the techniques and ideas described in the Turnbull et al. (2014) review. I plan to adapt the data presented in chapters 2 and 3 of this thesis for publication in scientific journals in cooperation with Dr. Lindo.

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A portion of my research involved a field experiment at a beautiful black spruce forest experimental site, the Forêt d'Enseignement et de Recherche Simoncouche, run by the University of Quebec at Chicoutimi. My work at this site was arranged and facilitated by Dr. J.P. Bellenger at the University of Sherbrooke, to whom I am grateful. I would also like to thank the members of his lab, Mathieu Dufresne and Romain Darnajoux, for their assistance and friendship in my field experiences. My field research would have been far less efficient without the help of our summer assistant Caterina Buratta.

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Chapter 1

1 Soils and global environmental change

1.1 Soil systems and soil biodiversity

Soils are an integral part of all terrestrial ecosystems. Soil formation is one of the first stages of primary succession, where weathering of rock forms substrate for biological colonization and subsequent organic matter decomposition; soils continue to develop for thousands of years as generations of organisms contribute to their formation. In addition to acting as the substrate for almost all terrestrial primary productivity, soils are also the primary site of terrestrial decomposition, and it is estimated that 10 times as much CO_2 is released from soils as from anthropogenic processes (Nielsen *et al*. 2011). At the same time, soils are the world's largest biologically interactive terrestrial carbon sink, storing over 80% of accessible carbon (IPCC 2007). These functions in soil systems are driven by soil organisms.

Soils are thought to be the most species-rich component of terrestrial ecosystems (Wall *et al*. 2005). For instance, in one gram of forest soil there can be up to 10^4 species of bacteria (Torsvik *et al*. 1990), in a handful there can be hundreds of species of animals (Whalen & Sampedro 2011), and there are an estimated 1.5×10^6 fungal species worldwide (Hawksworth & Rossman 1997). It is estimated that 40-80% of terrestrial animal biomass is found in soil (Fierer *et al*. 2009). Yet an estimated 95% of soil diversity is still undescribed (Wall *et al*. 2005). This wealth of biodiversity has led to soils being referred to as "the poor man's tropical rainforest" (Giller 1996) and is largely driven by high habitat heterogeneity at small spatial scales (Berg 2012). Soil biodiversity is determined by a range of biotic and abiotic factors (e.g., soil pH, moisture, parent material), and is typically greatest in well-developed soils with high organic matter such as coniferous forests or fertile prairies (Petersen & Luxton 1982).

Soil fauna are generally classified into different size groups ranging 10 orders of magnitude (Table 1.1). Microbial soil fauna (e.g., rotifers, other protists) and microfauna (e.g., nematodes, tardigrades) inhabit water films around or inside soil aggregates,

Table 1.1. Size classes, size ranges, and notable groups of soil fauna. Note size ranges represent largest and smallest fauna discovered from groups typically categorized in that size class. Numbers in brackets after group name indicate typical sizes. Adapted from Whalen and Sampedro (2011).

Size Class	Size range	Key taxa
Macrofauna	>2 mm	Centipedes (1.5 - 50 mm) Isopods (2 - 20 mm) Spiders (0.7 - 20 mm) Earthworms (0.7 - 10 mm)
Mesofauna	150 µm - 10 mm	Enchytraeids (0.2 - 5.0 mm) Acari (0.1 - 2 mm) Collembola (0.1 - 2 mm)
Microfauna	2 µm - 5.5 mm	Nematodes (5 - 100 µm) Protists (5 - 50 µm)
Microorganisms	>2 µm	Algae (5 - 50 µm) Fungi (1 - 50µm) (hyphal width) Bacteria (0.5 - 2.0 µm)

whereas mesofauna (e.g., Acari, Collembola) inhabit air-filled pore spaces around these aggregates. Many macrofauna (e.g., earthworms, insect larvae) engineer their own habitable space among soil particles. There are an estimated 80,000 species of nematodes described, over 45,000 described species of mites, and in a relatively well defined group, approximately 8,000 species of the primitive arthropods in the subclass Collembola (Whalen & Sampedro 2011). However, for each of these groups, described species are likely to be only a small proportion of the true number of extant species (Behan-Pelletier & Bisset 1992).

Soil fauna are involved in a vast array of ecosystem functions including releasing nutrients held within organic matter, increasing litter surface area through communition

of primary detritus, altering litter nutrient ratios to more digestible forms, distributing litter through the soil profile, and contributing to soil structure through fecal pellet deposition (Sylvain & Wall 2011). Interactions of soil mesofauna with the microbial community are also an important indirect factor in decomposition and nutrient cycling processes (Seastedt 1984). Soil fauna grazing on bacteria and fungi leads to compensatory growth, and therefore, higher microbial activity (Hedlund & Öhrn 2000). Collembola also disperse microbial propagules to new substrates (Klironomos *et al.* 1992). These activities make soil fauna a major component of decomposition rates, and therefore, terrestrial nutrient cycling.

1.2   Global environmental change affects biodiversity

While the importance of soil fauna in soil processes has been demonstrated, there is relatively little known about how soil faunal biodiversity will change under global environmental change, and what the consequences of these changes will be for soil structure and function. I refer to global environmental change (GEC) as the suite of changes in climate, nutrient deposition, and land use currently occurring at global spatial scales due to anthropogenic activities. The primary drivers of GEC are radiative forcing caused by anthropogenic emissions of greenhouse gases and the increasing area of land used for human activities (IPCC 2013). It is predicted that global mean surface temperature will increase by 2-6 °C by the year 2100, compared to 1900. One of the primary causes for this increase in temperature is increased atmospheric concentration of carbon dioxide (CO_2) due to anthropogenic emissions predicted to rise between 510 and 7005 Gt per year by 2100 depending on scenarios used (IPCC 2013). There are also changes expected for the hydrological cycle, with precipitation in middle latitudes becoming more sporadic and more intense when it does occur. In boreal forest regions of North America, precipitation has already increased between 1.4 and 3.8 mm yr^{-1} per decade between 1901 and 2008 (IPCC 2013). It is also expected that there will be an increased likelihood of extreme weather events in the next century, meaning a higher probability of drought and flooding, and periods of exceptionally high and low temperatures (IPCC 2007, 2013).

In addition to these changes in climate, human outputs and biotic interactions are expected to cause changes in nutrient availability. Humans are mobilizing large amounts of nitrogen as a consequence of industrial processes, energy generation, and agricultural practices, and it is estimated that anthropogenic reactive nitrogen (N) outputs will increase to 270 Tg yr^{-1} by 2050 (up from 156 Tg yr^{-1} in 1993), which would have enormous impacts on global N cycles (Galloway *et al*. 2004). Increased temperatures and atmospheric [CO_2] may also increase rates of plant growth, which will cause plants to absorb more nutrients and increase the amount of productivity available for other organisms (Hansen *et al*. 2001). These changes in temperature, CO_2, precipitation, and nutrient availability are all expected to detrimentally affect biodiversity, even in natural communities not directly subject to habitat destruction (Garcia *et al*. 2014).

Biodiversity, in general, may be directly affected by environmental factors in two ways. Changes to environmental conditions may create "filters" that limit or reduce biodiversity because only certain organisms are able to tolerate the new conditions (Freedman *et al*. 2014). For instance, rapid changes in climate may create new conditions that organisms are poorly adapted to, which reduces their ability to compete effectively with native or invasive species leading to competitive exclusion or extinction (Garcia *et al*. 2014). Environmental changes may also directly affect biodiversity through disturbance. For the purposes of my research I define disturbance as a discrete biogenic or environmental event that causes loss (e.g., of individuals or species) in a biotic community (Freedman *et al*. 2014). Individual disturbance events are often sudden and their effects are not immediately reversible. Examples under GEC may include extreme weather events such as major storms, flooding, and droughts, all of which are expected to become more common in the future (IPCC 2013).

The two components that determine the effect of a disturbance are its *intensity*, which is the degree of the disturbance relative to prevailing conditions, and its *frequency*, which is how often the disturbance occurs (Freedman *et al*. 2014). Disturbance regimes in which disturbances are more intense and frequent are said to be more *severe*. In classic ecological theory, the Intermediate Disturbance Hypothesis (Connell 1978) predicts that maximum biodiversity should exist at an intermediate severity of disturbances: systems

with high severity of disturbance are expected to contain less diversity as few species can tolerate the environmental conditions, while systems with low severity of disturbance are expected to have less diversity as superior competitors exclude tolerant but less competitive species (Grime 1973).

To further describe effects of environmental change on biological communities, it is important to identify which organisms are affected. Effects may be described as being top-down or bottom-up (*sensu* Hairston *et al*. 1960). Top-down effects alter the abundance, diversity, or distribution of organisms in higher trophic positions, i.e., predators and dominant omnivores with relatively fewer trophic positions above them (Freedman *et al*. 2014). This can lead to trophic cascades, whereby decreases in the abundance and diversity of predatory species allows an increase in the abundance the species they prey on, which may in turn decrease the abundance of the next lowest trophic level, and so on (Figure 1.1). The strength of a trophic cascade depends on the strength of trophic connections between predators and prey, i.e., the extent to which the population growth of a prey species is limited by its predators, and the extent to which a predator is reliant on a given prey species (Moore & Hunt 1988; Scheu 2002). Top-down effects on ecosystems are common under GEC because larger bodied, higher trophic level organisms are generally less abundant than their prey and less tolerant of disturbance, leading to a higher extinction proneness following perturbation (Lindo *et al*. 2012). Predators are also expected to be more vulnerable to environmental change than grazers.

Bottom-up effects are changes to biotic communities caused by changes in resource availability (Freedman *et al*. 2014), which is often considered to be net primary productivity but at a more basic level comprises the nutrients, light, and temperature conditions required to drive productivity. Bottom-up effects may cause a general increase or decrease in the abundance of all fauna as resources become more or less available. However, bottom-up effects can also cause cascades at higher trophic levels particularly through competition, as producers better adapted to more fertile conditions (faster growers) or less fertile conditions (superior extractors) gain an advantage over their competitors (Figure 1.1). Humans are causing bottom-up effects through fertilization of

commercial crops, pollution through resource extraction, and emissions from industrial processes. In addition, all other things being equal, increased global temperatures are likely to lead to increased decomposition rates, and therefore, higher nutrient availability as more nutrients from recalcitrant litter are mobilized (IPCC 2013).

Despite the immense importance of soils to global productivity and nutrient cycling, and the key role of soil fauna in soil process rates, soil fauna are not currently included in GEC models (Nielsen *et al*. 2011). Therefore, it is important to improve our ability to predict changes in soil faunal biodiversity and how this biodiversity feeds back into terrestrial ecosystem function to better predict future conditions under GEC. Although changes in prevailing environmental temperature, CO_2, precipitation, and nutrient conditions may result in loss of biodiversity and changes in community composition, their consequences for soils are poorly understood (Wall *et al*. 2008; Blankinship *et al*. 2011; Kardol *et al*. 2011). The inclusion of soil fauna in GEC predictions is problematic because the relationship between biodiversity and ecosystem function is poorly defined in soil systems (Hunt & Wall 2002; Wardle *et al*. 2004; Hooper *et al*. 2005). However, it is known that soil fauna respond to both top-down (Schneider & Maraun 2009) and bottom-up control (Scheu 2002). The effects of disturbance have also been demonstrated on soil communities, with agricultural practices such as tillage and soil compaction by livestock decreasing the diversity and density of soil mesofauna (Dittmer & Schrader 2000; Cole *et al*. 2008), but the effects of disturbance on non-agricultural soil communities have been poorly studied.
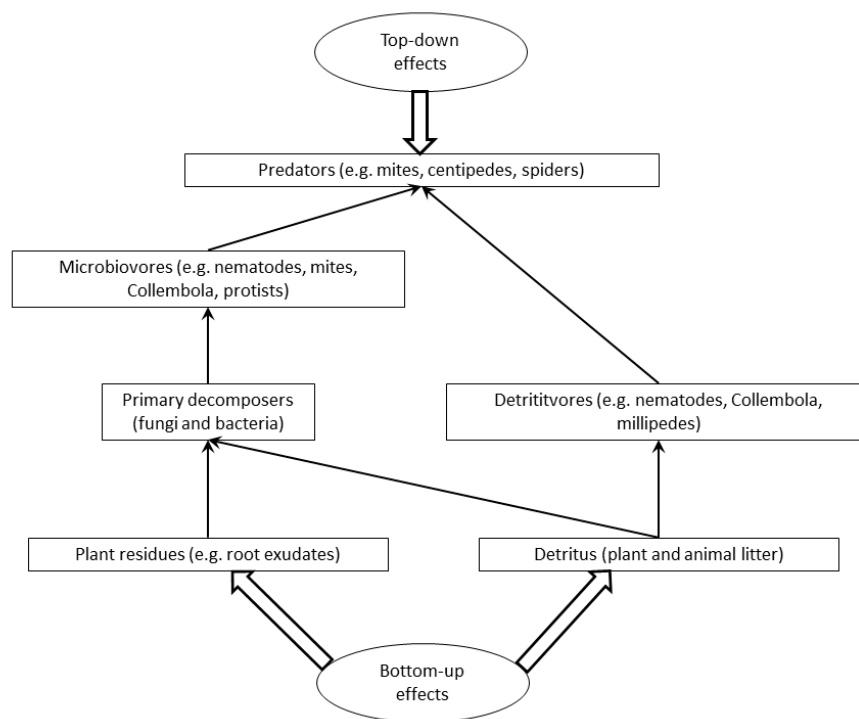
Fig 1.1. A simplified conceptual model of top-down and bottom-up effects in soil. Solid arrows represent direction of energy flow and empty arrows represent the direction of effects. Both bottom-up and top-down effects can cascade to different trophic levels by altering resource availability or predation pressure, respectively. For example, a loss of predators following disturbance may increase the abundances of microbiovores, which would then decrease microbial biomass. In a bottom-up cascade, increased plant growth may increase the quality and amount of detritus and root exudates, which would increase microbial biomass. These effects may be modified by competition at each trophic level. Adapted from Scheu (2002).

1.3   Collembola as an ecological model

Collembola (Hexapoda: Entognatha) are soil mesofauna ranging from 0.12 – 17 mm in length, with most species in the 1-5 mm body length range (Bellinger *et al*. 2014). Collembola are commonly called springtails because of a forked abdominal appendage (furcula) used for springing away from predators. There are approximately 8,000 described species worldwide (Bellinger *et al*. 2014) and they are found in almost every soil with densities up to 10^5 individuals per m^2 (Whalen & Sampedro 2011), making them one of the most common animals on the planet alongside two other common soil taxa: Nematoda (nematodes) and Acari (mites). Collembolan taxonomy in Canada is

relatively manageable, with the most recent checklist naming 412 species nationwide (Skidmore 1995).

Collembola are generally considered mid-trophic level generalist feeders in the soil foodweb. Although they are often described as fungivores, Collembola exhibit a wide variety of feeding strategies and may consume plant litter, plant roots, bacteria, fungi, animal waste, and even nematodes (Whalen & Sampedro 2011). Collembola partly determine fungal succession (Klironomos *et al*. 1992), stimulate fungal respiration (Bengtsson & Rundgren 1983), and their abundances are positively correlated with microbial biomass (Addison *et al* 2003) and plant diversity (Sabais *et al.* 2011). The presence of mesofauna including Collembola also decreases the retention of photosynthetically fixed C in soils, due to their grazing on microbes (Bradford *et al*. 2007). Thus, Collembola play an important, albeit mostly indirect role, in decomposition and nutrient cycling of soils (Seastedt 1984), as well as serving as prey for other mesofauna (mites) and larger soil fauna. Collembola also respond to changes in GEC factors including increases in temperature (Briones *et al*. 2009), precipitation (Tsiafouli *et al*. 2005), and nutrient availability (Cole *et al*. 2005).

How collembolan communities respond to GEC factors depends on interspecific differences and variation in collembolan traits. For the purposes of my research I define a trait as any morphological, physiological, or phenological characteristic of an organism that determines that organism's response to environmental conditions and the effect of that organism on the rate of ecosystem processes (*sensu* Violle *et al*. 2007; Lavorel & Grigulis 2012). Previously studied collembolan traits include mainly morphological characteristics that denote the preferred vertical distribution of collembolan species in the soil profile. This includes body size (as measured by length or body mass), number of eyes, ratio of antenna length to body length, development of the furcula, level of pigmentation, reproductive strategy (sexual or parthenogenic), and preferred food source (Whalen & Sampedro 2011). Surface–dwelling (epigeic) species are typically larger and more pigmented, have many eyes, often reproduce sexually, and feed directly on plant-litter. Species dwelling deeper in the soil (euedaphic) are often smaller, eyeless, parthenogenic, and fungivorous. Epigeic and euedaphic Collembola differ both in their

response to environmental conditions (Krab *et al*. 2010; Makkonen *et al*. 2011), and recovery from disturbance (Huebner *et al*. 2012; Malmström 2012). This combination of diversity, ubiquity, trophic importance, and trait variety make Collembola a superlative study group.

1.4   Thesis rationale and overview

To enhance our understanding of how GEC will influence soil biodiversity on a local scale, I studied the interactive effects of increased temperature, increased atmospheric CO_2, more sporadic and intense precipitation, and increased nutrient loading on collembolan communities in the Canadian boreal forest. I chose to focus on and develop my taxonomic expertise on the Collembola because they play an important role in soil functioning and there is a dearth of national and global taxonomic expertise for this group. I conducted two separate experiments which are thematically linked by their simulation of GEC conditions.

In the first experiment (Chapter 2) I focused on the direct effects of GEC factors, namely increased temperature, elevated atmospheric CO_2, and altered precipitation regimes. I studied these factors in a full-factorial design to determine the interactive effects on collembolan communities. I varied precipitation treatments in both frequency and intensity in order to simulate the effects of drought or saturation as a disturbance on soil communities. To quantify the effects of these factors I measured changes in collembolan abundance, species richness, species diversity, community composition, and body size distributions.

In the second experiment (Chapter 3) I focused on the bottom-up effects of nitrogen and carbon addition, once again in a full-factorial design. I used both short- and long-term nutrient addition to assess how collembolan communities will respond to nutrient inputs as both a perturbation and as a chronic change in conditions. As in the first experiment, I quantified changes in collembolan communities in terms of abundance, species richness, community composition, and body size distributions.

In Chapter 4, I discuss how collembolan communities may change in natural scenarios when temperature, CO_2, precipitation, and nutrient loads are simultaneously increasing, incorporating both previous literature and key findings from Chapters 2 and 3. I discuss ways in which these GEC factors are likely to interact in the future to affect soil communities in novel ways. I extend the findings from Chapter 2 and 3 to predict how the observed changes in collembolan community composition may alter soil function under future GEC scenarios. To conclude, I suggest further avenues of research that may help to reconcile my novel findings and incorporate them into our understanding of the effects of GEC on collembolan communities in particular, and soil communities in general.

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Chapter 2

2 Effects of temperature, CO$_2$, and precipitation on collembolan community structure and body size distribution

2.1 Introduction

2.1.1 Global environmental change and the boreal forest

The Canadian boreal forest is predicted to be strongly affected by global environmental change (GEC) factors such as increases in temperature, atmospheric CO$_2$ levels, and precipitation (IPCC 2013). For instance, the IPCC (2013) predicts a gradual but substantial increase in temperature between 2-8 °C by 2100 for the Quebec boreal forest, and global increases in atmospheric CO$_2$ concentrations, rising to 540-970 ppm by the end of the century. Concurrent with these changes, there is also a high likelihood in the Quebec boreal forest of a 0.1-0.4 mm increase in annual mean daily precipitation rate (normalized for surface temperature) by 2100, delivered by more sporadic and intense precipitation events compared to the historic norms (IPCC 2013). Previous studies have identified changes in severity and frequency of precipitation events as a disturbance that can impact forest biodiversity by means of both drought (Archaux & Wolters 2006) and flooding (Chaneton & Facelli 1991; Bornette & Amoros 1996) events.

The cumulative effects of GEC factors on the boreal forest are especially important because boreal systems represent a major global carbon sink (Nielsen *et al*. 2011) and contain substantial biodiversity, particularly in soils (Chagnon *et al*. 2000). It is suggested that soil fauna in these systems, and soil mesofauna (Acari and Collembola) in particular, are especially important drivers of microbial community structure and ecosystem function because of the relatively low diversity and abundance of macrofauna which would normally stimulate microbial communities and prey on mesofauna (Swift *et al*. 1979). Interactions between these mesofauna and microbes are known to be a determinant of microbial diversity and activity, and therefore, decomposition rates (Seastedt 1984; Klirosomos *et al*. 1992; Hedlund & Öhrn 2000). Changes in soil

biodiversity and community structure as a result of GEC could be expected to change soil function, with ramifications for global C cycles. It is important to understand how long-term changes in prevailing conditions, such as increased temperature and atmospheric CO_2 concentrations, will affect the biodiversity of soil fauna. Shorter-term events which act as disturbances, such as drought or soil saturation caused by sporadic and intense precipitation, could have different effects on soil biodiversity and also require study. There is also strong potential for interactions between GEC factors that are currently unquantified. For example, increased temperatures are expected to decrease soil moisture content (IPCC 2013), but increased atmospheric CO_2 levels may decrease evapotranspiration by plants, and therefore, increase soil moisture content (Dermody *et al*. 2007). These interactions make it difficult to predict what the net effect of GEC factors will be have soil biodiversity and functioning.

2.1.2   Current knowledge of GEC effects on soil fauna

Previous studies on the effects of GEC factors on soils, and on mesofauna in particular, have generally examined the effects of temperature, CO_2, and precipitation regimes separately, despite the fact that they will each be changing simultaneously (e.g., Hansen *et al*. 2001, Tsiafouli *et al*. 2005; Briones *et al*. 2009). Previous studies have highlighted the sensitivity of Collembola (Hexapoda: Entognatha) to desiccation, reporting decreased abundance and species richness under drought conditions (Pflug & Wolters 2001; Tsiafouli *et al*. 2005; Kardol *et al*. 2011; Makkonen *et al*. 2011). Collembola are thought to be especially vulnerable to desiccation due to their small size (0.1 – 2 mm), cutaneous respiration method, and relatively thin exoskeleton (Tsiafouli *et al*. 2005; Bellinger *et al*. 2014). In particular, smaller species with lower surface area to volume ratios are generally euedaphic (soil-dwelling) and especially susceptible to desiccation, should it occur (Krab *et al*. 2010). Makkonen and colleagues (2011) found a proportionally greater loss in abundance of smaller euedaphic Collembola under drought conditions.

The effects of changes in temperature and atmospheric CO_2 conditions on Collembola are not as well studied as soil moisture effects. A previous study on the interactive effects of increased temperature (+3 °C), increased CO_2 (+300 ppm), and drought conditions (-3.9% moisture) on the abundance and richness of soil microarthropods found a positive

correlation between moisture content and collembolan richness; however, there were no effects of increased temperature or CO_2 found when the effects on soil moisture were controlled for (Kardol *et al*. 2011). This finding was echoed by a review of the effects of GEC factors on soil fauna community structure, which concluded that detritivore abundances in cold and dry forest ecosystems, such as the Canadian boreal, can be affected by increased CO_2 concentration, increased temperatures, and decreased precipitation, but that decreased precipitation in particular substantially decreases the abundance of soil fauna in forest systems (Blankinship *et al*. 2011). Blankinship and colleagues (2011) also note the lack of studies on the interactive effects of GEC factors on soil communities and the need for higher taxonomic resolution when describing responses in community composition.

2.1.3 Trait-based approaches in soils

Studies that consider the effects of GEC on soil microarthropod communities typically quantify responses in abundance, species richness, and diversity. Studies with high (species-level) taxonomic resolution (e.g., Pflug & Wolters 2001; Krab *et al*. 2014; Salmon *et al*. 2014) are better equipped to assess changes in community structure than studies which consider soil fauna at coarser levels (i.e., class or order) of taxonomic resolution (e.g., Niklaus *et al*. 2003; Briones *et al*. 2009). The latter may underestimate community response to GEC factors (Krab *et al*. 2010; Makkonen *et al*. 2011) and consequences for soil function (Addison *et al*. 2003). Although taxonomic approaches are the most common, shifts in the richness and abundance of species does not necessarily equate to shifts in ecosystem function due to the uncertain (and hotly debated) relationship between soil species diversity and ecosystem function (Lavelle *et al*. 2006; Coleman 2008; Lavelle 2009). In order to quantify these shifts, we require some proxy of an organism's ecological role. To more concretely quantify the relationship between biodiversity and ecosystem function, several ecologists have begun to quantify the diversity of traits in biological communities (see McGill *et al*. 2006). It has been shown that traits are better able to predict responses to disturbance than species identities (Mouillot *et al*. 2013), and recent research also indicates that trait diversity is a better

predictor of ecosystem function than species diversity (Petchey *et al*. 2004; Cadotte *et al*. 2011; Lavorel & Grigulis 2012).

Trait-based approaches have only very recently been applied to collembolan communities, but these studies have demonstrated a strong relationship between traits and response to climate change (Makkonen *et al*. 2011), and the community-level recovery from disturbance by fire (Huebner *et al*. 2012). In particular, recent studies highlight how large-bodied, epigeic (surface-dwelling) species are drought tolerant (Makkonen *et al*. 2011) but more sensitive to fire disturbance (Huebner *et al*. 2012). However, one problem with trait-based analyses is that not all traits are equally descriptive of an organism's response to environmental conditions or their effect on ecosystem function; different traits may be more suitable proxies depending on the environmental stressor or ecosystem function of interest (Lavorel & Grigulis 2012). Therefore, traits must be selected that are universal, quantifiable, responsive to multiple disturbances, and determine the organism's influence on the rate of ecosystem functions.

One morphological trait that has been proposed for this purpose is body size, which may act as a "universal indicator" of an organism's ecological role and susceptibility to perturbation (Petchey & Belgrano 2010). Body size is an easily measurable characteristic of most organisms, and typically relates to many aspects of physiology and life history through allometry: larger organisms generally have lower mass-specific metabolic rates, smaller population sizes, higher extinction proneness, and slower population recovery from perturbation than smaller organisms (Brown *et al*. 2004). Quantifying organism body size requires less expertise than taxonomic-intensive approaches and may allow approximation of trophic complexity and stability. At the community level, the distribution of organismal body sizes ($\log_{10}$ transformed) may be plotted against the abundance of each body size ($\log_{10}$ transformed) to generate a body size spectrum (BSS). A regression line may be drawn for these data, and this line can provide quantitative information of changes in community structure and function. For instance, a more even distribution of body sizes in a biological community (i.e., a shallow BSS slope) is thought to be positively correlated with the efficiency with which nutrients and energy from resource pools are transferred to higher trophic positions (trophic transfer efficiency)

(Jennings *et al*. 2002; Jennings & Mackinson 2003; Barnes *et al*. 2010). The intercept of the BSS is also valuable for visualizing the abundance of different organism sizes. It has been demonstrated that BSS slopes respond to environmental perturbation (Brose *et al*. 2012), and this allows visualization of differential loss of organism sizes under perturbation. For example, a steeper slope following environmental change may indicate increased abundances of small-bodied species and/or decreased abundances of larger-bodied species.

The ease of adding body size analyses to classic taxonomic approaches, the responsiveness of BSS to environmental change, and the relationship between body size distributions and ecosystem function has led to a proposal by Turnbull and colleagues (2014) to use the slope and intercept of BSS as a standard descriptor of soil communities. Currently, few soil studies have applied this framework, but those that have demonstrate the loss of larger bodied, higher trophic position in response to experimental warming (Brose *et al*. 2012) and drought (Lindo *et al.* 2012). Studies on soil fauna body size distributions have also demonstrated the principle of energy equivalence in soil communities, i.e., that energy usage is consistent between different size classes of organisms (*sensu* Damuth 1981). Although larger species contain more biomass per individual, smaller species are correspondingly more abundant, and increases in the abundance of these small species following the loss of larger bodied species due to perturbation keeps the energy used by soil fauna consistent in an energy equivalence scenario (Kampichler 1995; Meehan *et al*. 2006). This is an important finding because it indicates the potential for functional redundancy among soil fauna, thus, keeping the total resources used by taxa consistent. This would manifest on a BSS as an increased slope, indicating an increase in smaller-bodied organisms and corresponding decrease in larger-bodied organisms.

2.1.4   Objectives and predictions

The objective of this research was to evaluate the individual and interactive effects of the anthropogenic climate change factors of temperature, CO_2, and precipitation frequency and intensity on soil collembolan communities. Specifically, I evaluated the effects of these GEC factors on the abundance, richness, species diversity, species composition, and

body size distribution of Collembola in moss mesocosms using a full-factorial experimental design. First, I hypothesized that the most important influence on the soil community would be through changes in precipitation events and predicted drought conditions would result in a general loss in abundance and species richness (Pflug & Wolters 2001; Tsiafouli *et al*. 2005; Kardol *et al*. 2011). I predicted that this loss of abundance would be most pronounced in epigeic, and therefore, large Collembola species, resulting in a steeper BSS slope. Second, I predicted that mesocosms under increased temperature would have higher collembolan abundances due to greater metabolic activity and higher resource availability. Third, I hypothesized weak, indirect effects of atmospheric [CO_2] on Collembola communities (Niklaus *et al*. 2003; Blankinship *et al*. 2011) through changes in moss growth and resource availability, but no direct effect of elevated atmospheric CO_2 conditions, because collembolan communities tolerate CO_2 levels up to ten times atmospheric concentrations when underground (Wall *et al*. 2005).

The interactive effects of the GEC variables are harder to predict, but as increased temperature reduces soil moisture through increase evaporation between precipitation events (Harte *et al*. 1996), I predicted that the negative effects of drought treatments on collembolan abundances would be more pronounced at higher temperatures. Increased atmospheric CO_2 has been shown to decrease plant evapotranspiration, and therefore, increase soil moisture (Dermody *et al*. 2007). It is also possible for increased plant growth caused by higher CO_2 to increase exudation of labile carbon from plant roots, which has been shown to increase C availability for microbes; this C can be transferred all the way up to tertiary consumers in soil food webs (Ruf *et al*. 2006). Because of the potential for these indirect effects, I predicted collembolan abundances would be higher at elevated [CO_2] in drought and intermediate moisture conditions compared to ambient [CO_2].

2.2   Methods

2.2.1   Sample collection

Forest floor soil and moss samples were collected from a black spruce (*Picea mariana*
(Mill.) Britton, Sterns & Poggenb.) forest east of Lac St. Jean, Quebec (48° 23'N, 71°
25'W). The average temperature in this region is 2.6 °C with average total precipitation
of 864.9 mm; in October, when sampling was performed, it is generally warmer with an
average temperature of 5.4 °C with a monthly average of 63.5 mm precipitation
(Roberval A station, data 1981-2010, Environment Canada 2013). On October 2nd, 2012,
four forest floor patches measuring 30 cm × 50 cm × 15 cm deep were removed and
placed in individual Rubbermaid® bins. These were promptly transported to the
University of Western Ontario in London, ON, and stored in a cold room at 4 °C. Each
forest floor sample was covered by a 2-3 cm deep moss carpet dominated by the
feathermosses *Hylocomium splendens* (Hedw.) Schimp. and *Pleurozium schreberi* (Brid.)
Mitt.

2.2.2   Mesocosm set-up and experimental design

On November 23rd, 2012, the forest floor samples were removed from the cold room and
cut into 7 cm × 7 cm subsamples. Bin origin was recorded to check for between-bin
effects. Five subsamples from each bin ("Initial", 20 subsamples total) were weighed to
determine their fresh weight, and then placed in Tullgren funnels (Burkard Scientific) to
extract soil fauna and assess initial community composition. Tullgren funnels operate by
shining a 25w light bulb over a soil sample to create a 14 °C temperature gradient,
causing soil fauna to move deeper into the soil and fall through a mesh into a preservative
solution. Tullgren funnel extraction is one of the most common soil fauna extraction
techniques and is more efficient at capturing fauna than manual sifting (van Straalen &
Rijninks 1982). Fauna were extracted over 48 h into 75% ethanol preservative. After
extraction, the soil/moss (hereafter "soil") subsamples were dried at 60 °C for 48 h in an
oven (ThermoScientific Heratherm OGS180), and weighed to obtain the dry weight of
each sample. Moisture content of these initial samples was calculated using the equation:

(1) $$\text{Moisture content} = \left(\frac{FW-DW}{FW}\right) \times 100\%$$

where FW is fresh weight of the soil before drying, and DW is the dry weight of the soil and moss after it has been dried to a constant weight (i.e., all moisture has evaporated). Moisture content then represents the percentage of the weight of the fresh sample that consists of water.

Ninety 500 ml wide-mouth mason jars were prepared to each contain a single 7 cm x 7 cm subsample and the fauna community residing within for the duration of the experiment (mesocosms). These mesocosms were wrapped in black construction paper to prevent any effects of light and then weighed. Once filled with soil, each mesocosm was weighed and jar weight was subtracted to give initial soil weight. Mesocosms were randomly assigned to treatment groups.

 The controlled environment facilities (biomes) in the University of Western Ontario's Biotron Institute for Experimental Climate Change were used to create a full-factorial design of temperature, precipitation, and CO_2 conditions. Of the six biomes used, two biomes were maintained at each of the temperatures 11.5 °C, 15.5 °C, or 19.5 °C for the duration of the experiment. Of the two biomes at each temperature, one chamber had atmospheric CO_2 conditions maintained at ambient levels (430 ppm) and the other chamber had elevated CO_2 levels (750 ppm). Within each biome, five replicate mesocosms were exposed to one of three precipitation treatments: they were watered to 50% of their initial weight every 3 weeks (Drought), 100% of their initial weight every other week (Control), or 125% of their initial weight every week (Saturated) (5 replicates × 3 precipitation levels × 2 CO_2 levels × 3 temperatures = 90 total). Humidity was kept constant at 60% within each biome.

Mesocosms were installed on Nov. 26-30, 2012 and destructively sampled 18 weeks later on a rolling schedule to account for fauna extraction times at the end of the experiment (March 25-29, 2012). Moisture regimes were maintained by the treatment conditions described above, and all mesocosms were weighed every week to measure fluctuations in

soil moisture. In order to reduce any within-chamber effects due to position, jars were moved within the biomes each week.

2.2.1   Destructive sampling

Starting on March 25th, 2013, after 18 weeks in the biomes, mesocosms were removed, weighed, and invertebrates were extracted from the soil over 48 h by Tullgren funnel into 75% ethanol for preservation. Soils were then dried at 60 °C for 48 h to determine dry weight. This was used to calculate moisture content of the sample at each measurement point, using Equation (1). The amount of water added at each watering point was averaged for each mesocosm to determine average weekly water added. Weekly mesocosm weights were also used to calculate variance in moisture content for each mesocosm over the duration of the experiment using the equation:

$$(2) \qquad\qquad \sigma^2 = \frac{\sum(x-\bar{x})^2}{n}$$

where σ^2 is the variance, $\bar{x}$ is the sample mean, and n is the number of watering events experienced over the course of the experiment by the mesocosm. This equation was used to quantify the variation in soil moisture during the experiment for each mesocosm.

A 10-50 × magnification dissecting scope (Nikon SMZ745T) was used to sort and enumerate Collembola and a compound light microscope (Eclipse Ni-U) was used at 400 × magnification to identify Collembola to species level using the taxonomic key provided in Christiansen & Bellinger (1998). The total abundance and species richness of Collembola in each mesocosm were divided by the dry weight of the moss/soil in that mesocosm to standardize among samples and derive collembolan individual and species density. These standardized values are used in all subsequent calculations and analyses.

Species abundance and total richness were used to calculate Shannon-Weiner diversity index (H') values for each mesocosm using the equation:

$$(3) \qquad\qquad H' = -\sum(pi)(\ln(pi))$$

where p_i is the relative proportion of each species' abundance in terms of the total collembolan abundance (Shannon 1948). Pielou's evenness (J') was calculated for each mesocosm using the equation:

$$(4) \qquad\qquad J' = \frac{H'}{\ln(S)}$$

where S is the number of collembolan taxa in the community (Pielou 1975).

Average body size for each species was calculated by measuring the length and width of five-ten representative specimens from each species. Average length and width for each species was used to estimate body mass using the equation:

$$(5) \qquad\qquad W = (bL)^3$$

where W is average species mass in µg, L is length in µm, and b is an empirically derived coefficient determined for collembolan biomass estimates at the family level (Edwards 1967). Although other biomass estimates exist (Caballero *et al*. 2004), these calculations did not produce realistic average species masses and were incompatible with known values by several orders of magnitude.

2.2.2   Data analysis

Seven mesocosms were removed from analyses as outliers due to inconsistencies in substrate (e.g., large woody debris) noted during destructive sampling; these mesocosms also had drastically different community compositions compared to other mesocosms. An alpha level of 0.05 was used throughout analyses. Statistical tests were not statistically significant unless reported.

Three-way analyses of variance (ANOVA) were performed on collembolan density, standardized richness, H', and J' values, and moisture content with temperature, precipitation treatment, and CO_2 as fixed factors. For these analyses, collembolan density was log-transformed and standardized richness was square root-transformed to meet the assumptions of ANOVA. Percentage moisture content was transformed by taking the reciprocal to correct for skew. Significant differences between treatment groups were

identified using Tukey's HSD post-hoc test. Multiple regression using stepwise backwards selection (F value to remove <10.00) was used to examine the relationship between mesocosm moisture content, average water added, and moisture variance with collembolan density, standardized richness, H', and J' sequentially as dependent variables. These analyses were conducted in Statistica 7 (StatSoft Inc., 2004).

Community composition of each mesocosm was assessed and compared among treatments by generating similarity matrices using the standardized species abundance data and Bray-Curtis percent similarity values. Non-metric multidimensional scaling (NMDS) with 100 permutations was used to show community composition for temperature, CO_2, and watering level treatment groups. Significant differences between clusters of treatments were tested using an Analysis of Similarity (ANOSIM) with 10,000 randomized permutations. Simper analyses were used to quantify percent similarity between samples within groups. These analyses were performed using Primer 5 (PRIMER-E Ltd., 2001).

Body size spectra were created for each main factor treatment. Body size estimates for each species were log transformed and plotted against log transformed abundance of each species. A least-squares regression at the 75th quartile of the most common species was then plotted to estimate community body size distribution evenness (slope) and allow comparison between communities. Using the 75th quartile is a recommended practice because rare species can significantly affect the slope of the BSS regression line, even if only one or two individuals are present, because of the large range in sizes among collembolan species and the effect of log transformation on low abundances. These body size spectra were graphed in the context of temperature, CO_2, and watering levels to elucidate patterns for each treatment. A homoscedasticity of slopes test was used to check for significant differences between slopes. These analyses were completed in R using the 'quantreg' package (R Foundation for Statistical Computing, 2013).

2.3 Results

A total of 33,328 Collembola individuals in 23 species were extracted, identified, and analysed. Initial samples, assessed for any random pre-treatment effects, did not display
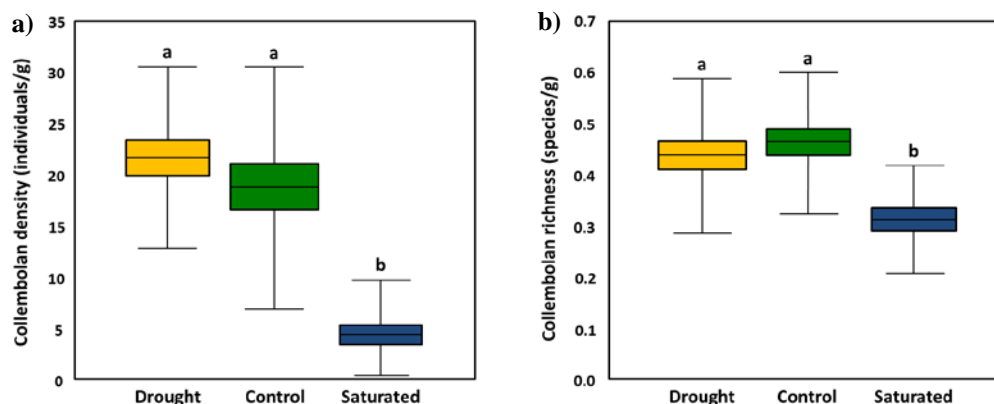
Fig 2.1. The effects of precipitation treatment on collembolan abundance (a) and species richness (b) per gram dry weight of soil. Mesocosms in Saturated precipitation treatments had significantly lower collembolan density (ANOVA, $F_{2,65}$ = 46.930, p < 0.001) and richness (ANOVA, $F_{2,65}$ = 12.596, p < 0.001) than Drought and Control treatments. Different letters denote significant differences between treatments. Box plot mid lines represent the mean, box limits are $\pm$SE, whiskers are $\pm$SD.

any significant treatment effects with respect to abundance, richness, evenness, or diversity. Control moisture conditions as a treatment level were not significantly different from the Initial samples in moisture content, abundance, richness, evenness, or diversity, which indicated that Control conditions were a good fit for Initial conditions. As such, the remainder of the results discussed are for differences between treatment groups only.

At the end of the experiment, overall collembolan densities were significantly affected by precipitation treatment whereby Saturated mesocosms had significantly lower density than the other precipitation treatments ($F_{2,65}$ = 46.930, p < 0.001; Figure 2.1a). Species richness was significantly lower in Saturated mesocosms compared to Control or Drought treated mesocosms ($F_{2,65}$ = 12.596, p < 0.001; Figure 2.1b). In terms of species diversity (H') , Drought treated mesocosms had significantly lower diversity than Control and Saturated mesocosms ($F_{2,65}$ = 18.677, p > 0.001; Figure 2.2a); there was also a statistically significant difference in H' between CO_2 treatments, with 430 ppm treated mesocosms containing significantly higher species diversity ($F_{1,65}$ = 4.767, p = 0.033) than elevated mesocosms. These differences in diversity were partly driven by differences in evenness. Drought treated mesocosms had significantly lower evenness
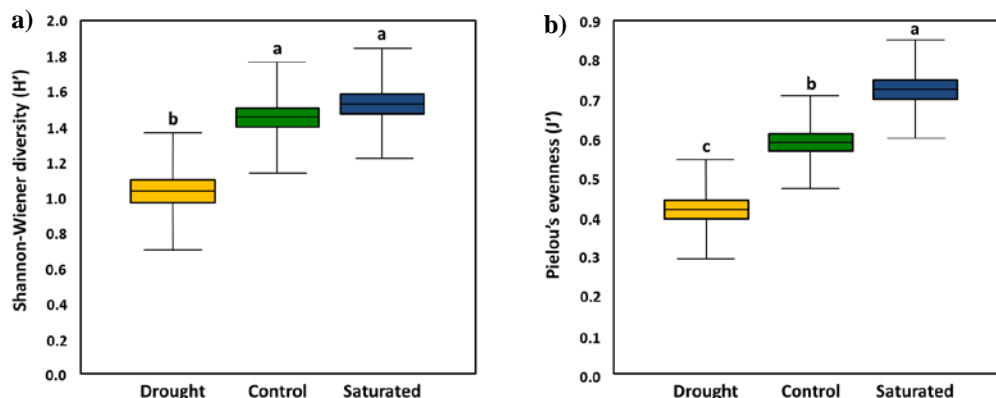
Fig. 2.2. The effects of precipitation treatment on collembolan community Shannon-Wiener diversity (H')
(a) and Pielou's evenness (J') (b). Mesocosms in Drought precipitation treatments had significantly lower
diversity (ANOVA, $F_{2,65} = 18.677$, p > 0.001). In graph (b), treatments different letters are significantly
different from one another (ANOVA, $F_{2,65} = 42.628$, p > 0.001). Drought treated mesocosms were less even
than Control mesocosms, which were in turn less even than Saturated mesocosms. Different letters indicate
significant differences between treatments. Box plot mid lines represent the mean, box limits are ±SE,
whiskers are ±SD.

than Control and Saturated mesocosms, and Control mesocosms had significantly lower
evenness than Saturated mesocosms ($F_{2,65} = 42.628$, p > 0.001; Figure 2.2b).

Collembolan density was significantly explained by moisture content and moisture
variance (multiple regression, $R^2 = 0.304$, $F_{2,80} = 17.442$, p < 0.001; Figure 2.3).
Collembolan species diversity was significantly explained by moisture content alone
(multiple regression, $R^2 = 0.166$, $F_{1,81} = 16.172$, p < 0.001). Evenness was also
significantly explained by moisture content alone (multiple regression, $R^2 = 0.250$, $F_{1,81} =$
26.941, p < 0.001). Richness was not significantly explained by these moisture variables.

Temperature within the biomes interacted with moisture conditions during the experiment
such that mesocosms at 11.5 °C had significantly higher average moisture content than
15.5 °C and 19.5 °C ($F_{2,82} = 67.755$, p < 0.001). This effect was consistent at each
watering level ($F_{4, 82} = 10.652$, p < 0.001). Mesocosms at 19.5 °C required significantly
more water per watering event on average to reach their target weights than 15.5 °C or
11.5 °C mesocosms ($F_{2,82} = 15.013$, p < 0.001). Variance in moisture content was also
significantly affected by watering level, with Saturated treatments experiencing
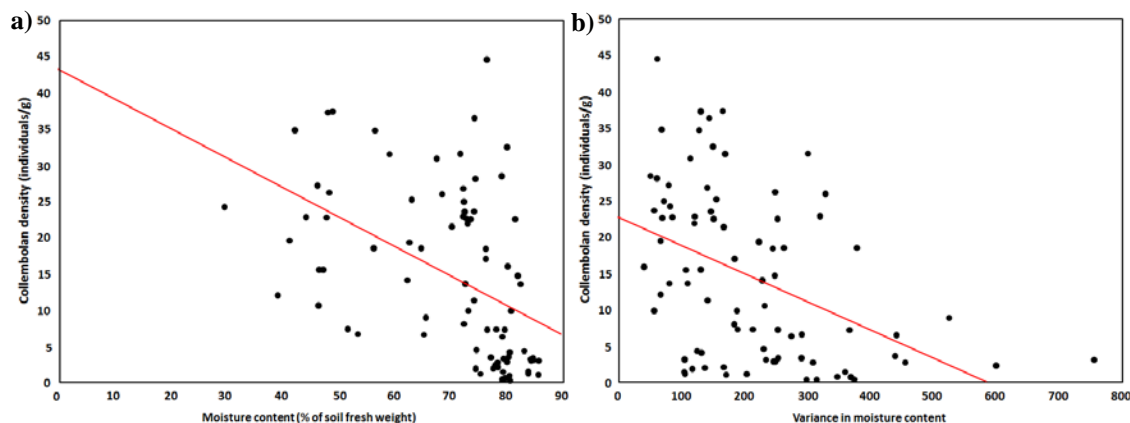
Fig. 2.3. Regressions between collembolan density and moisture content (a) and variance in moisture content (b). Moisture content and variance in moisture content significantly explained collembolan density (multiple regression, $R^2 = 0.304$, $F_{2,80} = 17.442$, $p < 0.001$). There was a negative relationship between density and moisture content ($R^2 = 0.223$, $y = 43.4253 – 0.4083x$) and variance in moisture content ($R^2 = 0.193$, $y = 22.8433 – 0.0386x$).

significantly higher moisture variance than Control or Drought treatments ($F_{2,82} = 15.160$, $p < 0.001$). Variance in moisture was also affected by the interaction between watering treatment and temperature, with warmer treatments causing less moisture variance in Drought treatments and more variance in Saturated treatments ($F_{4,65} = 3.840$, $p = 0.007$). There were no significant effects of CO_2 treatment on moisture content, moisture variance, or average water added.

In terms of community composition, all watering treatments were significantly different from one another (ANOSIM, global $R = 0.334$, $p < 0.001$), with each watering treatment becoming less similar between sample replicates (Figure 2.4). Saturated mesocosms were the least internally similar (average similarity 34.33%) followed by Control treated mesocosms (37.54%) and then Drought mesocosms (59.42%). When analysed by temperature, 11.5 °C treated mesocosms were significantly different in community composition compared to 19.5 °C mesocosms (ANOSIM, $R = 0.078$, $p = 0.012$). There was also a significant difference in community composition between ambient and elevated CO_2 treated mesocosms (ANOSIM, $R = 0.111$, $p < 0.001$).
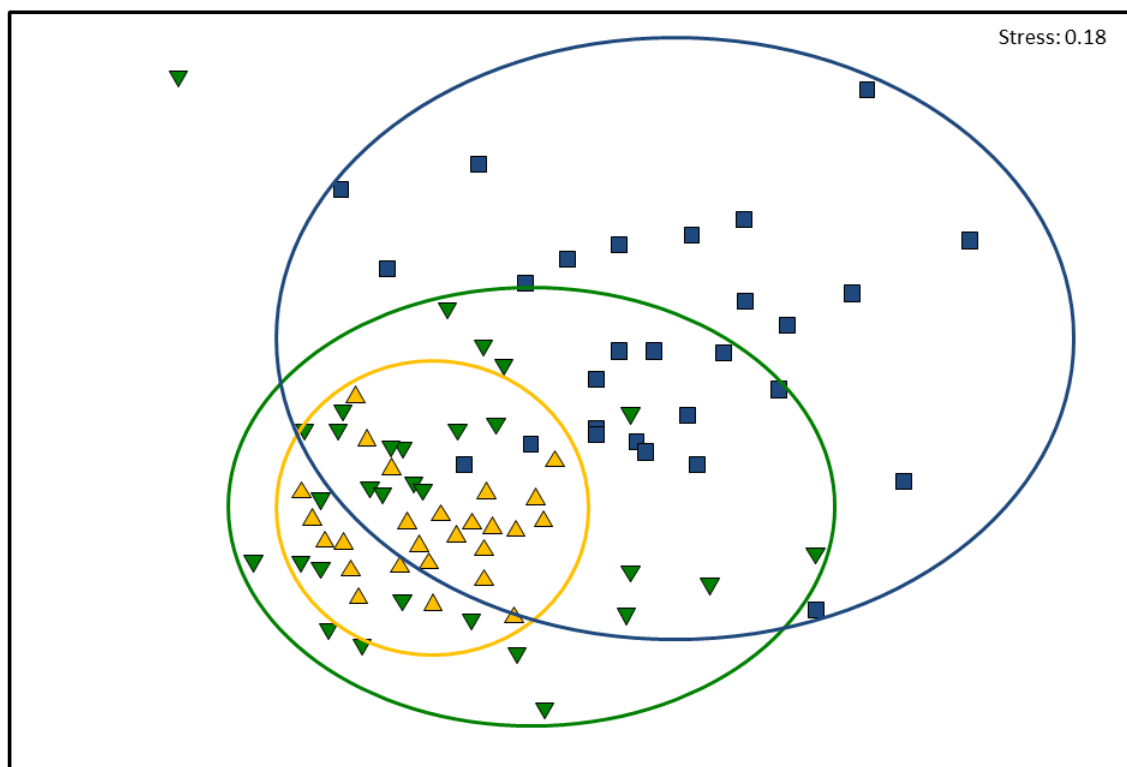
Fig. 2.4. Non-metric dimensional scaling (NMDS) of collembolan community composition by Bray-Curtis % similarity of species abundances by precipitation treatment (▲ = Drought treated mesocosms, ▼ = Control, ■ = Saturated). Each point represents one collembolan community and the distance between points represents the degree of similarity between communities in terms of relative proportion of species present. Drought communities are significantly different from Control communities (ANOSIM, R = 0.085, p = 0.006) which are in turn significantly different from Saturated communities (ANOSIM, R = 0.405, p < 0.001). Lines are for visualization purposes only.

Because watering treatments most consistently affected community composition characteristics, the BSS is demonstrated here with samples grouped for precipitation treatments. While the slopes of these lines were not significantly different, the intercept of the BSS for the Saturated watering treatment is visibly lower than that of the Control and Drought watering treatments, indicating a general loss of abundance with a similar community size evenness (Figure 2.5).
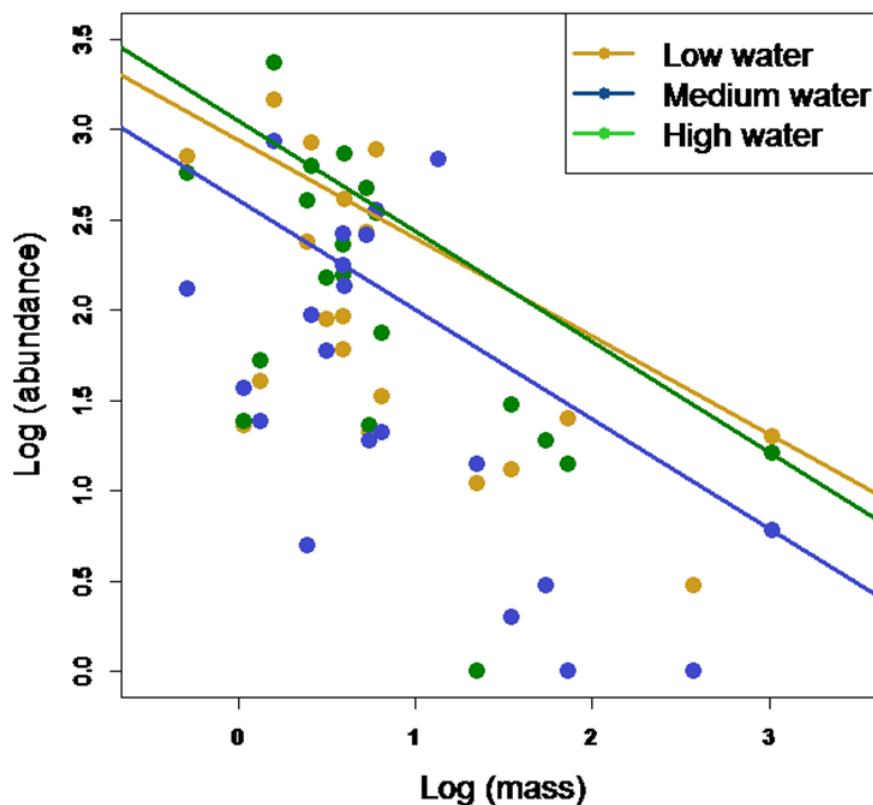
Fig. 2.5. Body size spectrum (BSS) for Collembola in response to precipitation manipulations. Collembolan lengths were derived from the average body length of 10 representative individuals from each species and biomass estimations were made using the equations of Edwards (1967). There was a general decrease in abundance in Saturated conditions for species of all body sizes.

2.4   Discussion

2.4.1   Frequency and intensity of precipitation events as disturbance

The increasing likelihood of extreme climate events predicted for the next century is expected to have major impacts on biodiversity at local scales (Garcia *et al*. 2014). These extremes will primarily consist of periods of extreme heat, extreme cold, drought, and flooding with greater severity and less predictability than historical norms (IPCC 2013). It is these extreme events that may act as a source of disturbance to decrease biodiversity at local spatial scales. In this study, increased frequency and intensity of precipitation events was a major driver of lost collembolan abundance and richness. This suggests that precipitation events may act as a disturbance for soil invertebrate communities, and is

contrary to the often stated drought sensitivity of Collembola (Tsiafouli *et al.* 2005; Kardol *et al.* 2011; Makkonen *et al.* 2011). Although this decrease in collembolan richness and density with increased severity of precipitation events was unexpected, it is not unprecedented. For example, in a study of variable moisture frequency on Mediterranean montane pine forest soil populations, frequent watering (every 3 days) significantly decreased collembolan abundance (by 14%) compared to control plots (Tsiafouli *et al.* 2005). However, many counter-examples exist: Starzomski and Srivastava (2007) used repeated soil fauna removal through soil drying as a disturbance event in moss mesocosms to demonstrate increased severity of drought events reduces the abundance and richness of soil microarthropods. In a study by Makkonen and colleagues (2011), a 33% decrease in soil moisture content in plots covered by open top chambers resulted in a 51% decrease in collembolan density, while Tsiafouli and colleagues (2005) found reduced species richness under infrequent precipitation regimes. Unfortunately, due to the experimental setup of my study it was not possible to directly differentiate between frequency and intensity effects of watering; future studies could cross these factors to better quantify whether variation or amount of precipitation is a more important determinant of collembolan community structure.

Moisture was not limiting in this experiment, even in low-precipitation mesocosms, and so precipitation events may have acted as a disturbance through several possible mechanisms. One explanation is that precipitation events may have disrupted feeding by changing fungal:bacterial ratios, as has been noted in previous studies (Filser 2002; Bardgett 2005). It is expected that under wetter conditions there will be a shift towards bacterial predominance and a change in collembolan diets as they exhibit switching, or a change in community composition as superior bacterial grazers out-compete other species (Filser 2002). It is also likely that increased precipitation decreased the amount of habitable air-filled pore space in the soil. This decrease in air-filled pore space, which mesofauna such as Collembola rely on, would primarily affect euedaphic species and have variable effects depending on soil structure (Bardgett 2005). This hypothesis is supported by observed changes in the community composition whereby abundances of dominant euedaphic species (primarily *Folsomia penicula*) were reduced and increased abundances of epigeic species (primarily in the family Sminthuridae, but also the
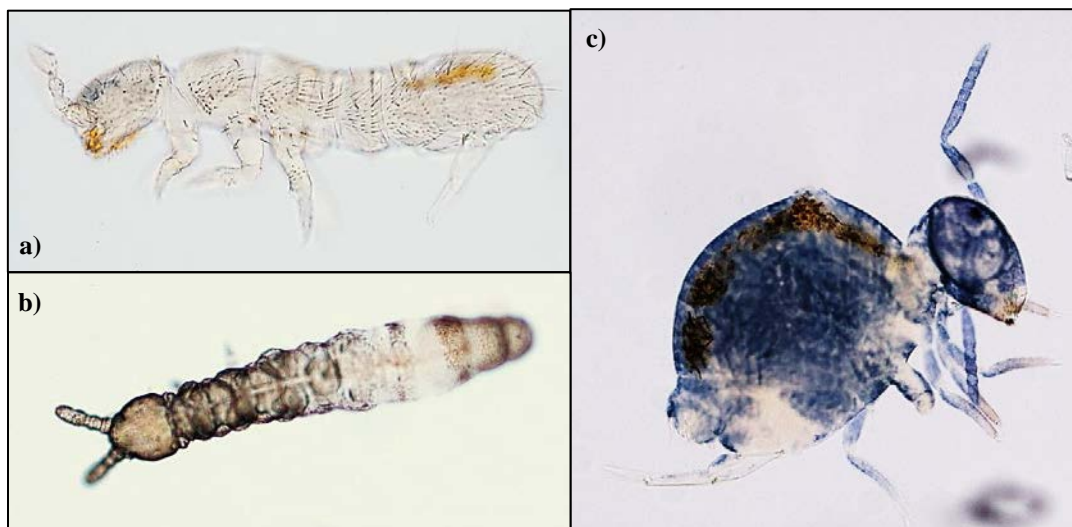
Plate 1. Collembolan species from experimental mesocosms, slide mounted in Hoyer's medium. A shift was observed from the highly abundant *Folsomia penicula* (a) and euedaphic species including *Tullbergia* (*M.*) *iowensis* (b) to sminthurid species including *Sminthurides* (*S.*) *violaceus* (c).

Entomyobryid *Argenia cyanura* and the Isotomid *Isotomiella minor*) were observed (Plate 1). Previous studies have also shown significant decreases in the abundance of euedaphic collembolan species in denser soil with less habitable pore space (Dittmer & Schrader 2000; Larsen *et al*. 2004). Although an increase in soil moisture and water-filled pore space may be expected to increase habitable area for microbial growth, it seems that this effect was not sufficient to overcome the lack of habitable space for Collembola. This finding also mirrors previous research indicating collembolan species are likely to be differentially affected by changes in environmental conditions such as increased precipitation events depending on morphology and life history, as has been reported previously on studies of collembolan trait distributions in response to warming and drying (Makkonen *et al*. 2011).

Trait-based approaches to understanding how ecological communities will change under global environmental change scenarios is an area of active research (Lavorel & Grigulis 2012; Lindo *et al*. 2012; Mouillot *et al*. 2013). Yet, identifying and defining appropriate traits can be problematic, especially traits for soil fauna that are reliable between groups and ecosystems (Pey *et al*. 2014). Body size has recently been proposed as a comprehensive trait metric for soil communities (Turnbull *et al*. 2014) because body size

correlates with many life history characteristics, and here we assessed its utility as an indicator of response to GEC in Collembola. While the BSS did not demonstrate a change in evenness in response to precipitation, it did demonstrate the general decrease in collembolan abundance. A previous study by Lindo and colleagues (2012) found that decreased moisture content was the most important explanatory variable in determining microarthropod abundance in a field climate change experiment, and that dry habitats had a lower intercept and steeper BSS slope, indicating a reduction in all species with large-bodied microarthropod species especially affected. Although trait-based approaches on soil fauna are relatively uncommon, the recent examples that do exist have either found significant relationships with body size in response to disturbance (Makkonen *et al*. 2011; Huebner *et al*. 2012; Malmström 2012) or as a predictor of distribution along latitudinal (Pflug & Wolters 2002) and a large range of environmental and soil property (Salmon & Ponge 2012; Salmon *et al*. 2014) gradients. These studies have repeatedly demonstrated association between large-bodied organisms and vertical distribution, drought tolerance, and susceptibility of disturbance from local to continental scales. While this experiment was unable to differentiate extinction proneness based on body size for extreme precipitation events, body size, as measured by mass, is still an excellent example of a continuous, ubiquitous, ecologically significant trait to allow comparison between ecosystems as requested by Pey and colleagues (2014).

2.4.2 Future temperature and CO_2 conditions on Collembola communities

While precipitation regimes were the overriding factor in patterns of collembolan diversity and community structure, elevated temperature and CO_2 levels in the experimental biomes had interactive effects with precipitation. Previous studies have shown that temperature and CO_2 do not strongly affect mesofaunal communities independently of their effects from soil moisture (Hodkinson *et al*. 1998; Blankinship *et al*. 2011; Kardol *et al*. 2011). This research highlights a similar interactive effect of precipitation with temperature, whereby warmer temperatures resulted in lowering the average moisture content, thereby increasing the amount of water added during precipitation events. Under future climate conditions, it is unclear to what extent

increased drying caused by warming will be counteracted by increased precipitation predicted for the boreal forest. However, this research highlights that increased variation in moisture content (more frequent and wider ranging wetting/drying cycles) is negatively correlated with collembolan abundance, indicating that the effect is likely to be negative.

The lack of temperature effect on richness or collembolan density is contrary to predictions that increased temperature would increase the metabolic activity, increasing population abundances and maintaining species richness. Briones and colleagues (2009) found that +3.5 °C of experimental warming resulted in an increase in the relative proportion of fungivorous microarthropods and an increase in epigeic microarthropod diversity, while Lindo and colleagues (2012) found increased temperatures of 2 °C increased abundance and richness of soil microarthropods. This contrasts with the results of this study where soil moisture appeared to be the overriding factor in determining collembolan biodiversity, as any positive effects of increased temperature on decomposition (and therefore, resource availability) or collembolan activity were counteracted by the increased watering intensity and decreased moisture content in these mesocosms.

Under elevated temperatures, there is the potential for biotic acclimation. It has been shown that Collembola reared under higher temperatures (15 °C) lose significantly less water under drought conditions than those reared under cooler temperatures (5 °C) (Leinaas *et al*. 2009). However, this acclimation also had an interaction with body size: smaller Collembola reared at 5 °C lost water at a faster rate (Leinaas *et al*. 2009). This indicates that even under a gradual temperature change, allowing for adaptation for decreased moisture loss in Collembola, smaller bodied species are more likely to be detrimentally affected. Collembola have also demonstrated the ability to empty their gut contents in order to decrease water loss during drought and freezing, but this comes with the tradeoff of decreased metabolic rates and halted reproduction (Testerink 1983). This indicates that increased variation in future precipitation regimes is likely to have negative effects on collembolan populations, even if adaptation or physiological strategies improve survival.

The only effect of CO_2 was higher Shannon-Wiener diversity under ambient CO_2 conditions compared to elevated CO_2; however, there were no significant differences in richness or evenness between CO_2 treatments, so this is likely due to cumulative increases in both richness and evenness in ambient CO_2 chambers. The effects of increased atmospheric CO_2 on soil fauna are thought to be indirect, through effects on the plant community, and therefore, the root systems microbes rely on. Previous studies have shown either weak or neutral effects of CO_2 enrichment on soil fauna (Niklaus *et al*. 2003; Bardgett 2005; Kardol *et al*. 2011). For example, in a six-year CO_2 enrichment study in a nutrient poor Swedish grassland, there were no changes in N availability or fungal:bacterial ratios indicating a lack of direct effect on nutrient cycling and decomposition rates (Niklaus *et al*. 2003). Increased CO_2 may decrease soil aggregate size due to increases in soil moisture caused by decreased evapotranspiration, but this has not been observed to cause changes in mesofaunal communities over time periods studied (Blankinship *et al*. 2011). Further research would be required on varied CO_2 levels for longer periods to determine if there are eventual shifts in microbial community composition that may have bottom-up effects on higher trophic positions. However, there is evidence that the limited effects of CO_2 on soil communities diminish over time, whereas temperature and moisture effects become stronger (Blankinship *et al*. 2011), so the focus should continue to be on the intersection of these two key variables in the future.

2.4.3 Biotic interactions as mechanisms for Collembola community change

Direct effects of climate on Collembola communities are only likely to be observable for relatively strong, sudden effects (Blankinship *et al*. 2011), as demonstrated here in the precipitation treatments. Indirect effects, such as top-down or bottom-up effects, are more likely to affect and alter Collembola communities but the ability to observe and mechanistically disentangle indirect effects is challenging due to their mediation through other taxa trophically linked to Collembola. For instance, it is possible that large-bodied, drought-sensitive predators, predominantly soil mites, are moisture-limited, and therefore, increased in abundance with increased watering, causing a top-down trophic

cascade and decreasing the abundance of Collembola. Previous studies have demonstrated top-down control by predators on collembolan abundances. For example, Schneider and Maraun (2009) found that smaller, less sclerotized groups of mites in the suborder Oribatida were more susceptible to predation, and that Collembola in the family Entomobryidae were not significantly affected by increased predator density. This same relationship may hold true for smaller edaphic species of Collembola, and may explain the increase in relative abundance of species in this study. I would also expect the largest loss of collembolan density to be in the most abundant species strictly due to their availability to predators, which would also explain the increase in species evenness at higher moisture levels (Schneider & Maraun 2009).

A previous study by Cole and colleagues (2005) found that N and lime addition to improve soil fertility increased soil microarthropod density, but did not change diversity, which indicates that soil fauna communities are primarily bottom-up controlled and that competition and predation are not dominant factors in determining species composition. Similar results were found by Ponsard and colleagues (2000) who found that densities of detrivorous and predatory soil fauna increased under higher litter deposition and that seasonal changes were determined by litter deposition, not predation, providing more evidence for the dominance of bottom-up control in soil food webs. This relates to a classic model by Hunter and Price (1992), who hypothesized that increased abiotic and biotic heterogeneity increases the strength of bottom-up control. Because of the high species diversity and physical heterogeneity in soil, soil fauna food webs are thought to favour bottom-up control (see Polis & Strong 1996). The cumulative results of these studies provide evidence for bottom-up control of collembolan community structure, which lends credence to the hypothesis that the observed community responses in this study may be due to fungal:bacterial ratio shifts.

2.4.4   Consequences of Collembola community change

It is difficult to tell what the functional ramifications of these shifts in collembolan density and community composition would be because different community compositions have not been explicitly tied to functional outcomes or process rates due to difficulties selectively removing certain soil groups and the immense complexity of soil

food webs (Nielsen *et al*. 2011; Sylvain & Wall 2011). Filser (2002) suggests a conceptual model whereby under lower moisture conditions, fungi would be more prominent, leading to a switch to fungivory, dominance of fungivorous species, and increased N mobilization as fungal hyphae are broken down. The inverse is suggested for high moisture conditions, where bacteria are expected to dominate, leading to rapid consumption of labile substrates and N immobilization in bacteria and their collembolan consumers (Filser 2002). However, this is a simplification which disregards the strong effects of community composition on N cycles in soil (Nielsen *et al*. 2011).

To predict the functional ramifications of these community shifts, we can examine the BSS generated for these communities. Because the distribution of collembolan body sizes is relatively consistent at higher watering levels, we can expect that trophic transfer efficiency will remain similar through this trophic level. The dominance of bottom-up control in soil systems (Cole *et al*. 2005) leads to the hypothesis that there will be similar effects in other groups and trophic positions. The general loss of abundance at all body sizes paired with changes in community composition indicates that collembolan traits are an important predictor of their response to new conditions (*sensu* Mouillot *et al*. 2013). Although Collembola of all sizes appear similarly affected by moisture saturated soil conditions, this could have long-term ramifications for community structure following disturbance, as larger-bodied species have smaller populations and may take longer to recover – in effect, their lower abundances leave them more vulnerable to extinction even if all size classes are affected equally.

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Chapter 3

3 Bottom-up effects of resource availability on collembolan communities

3.1 Introduction

It is very likely that nitrogen (N) is the primary factor limiting carbon (C) uptake by terrestrial plants in temperate forests (IPCC 2013). One of the predicted outcomes of anthropogenic climate change is an increase in atmospheric N deposition by 90-190 Tg per year by 2100 due to human activity (agriculture and industry) and biological feedbacks (IPCC 2013). Coupled with atmospheric N deposition, there is also a high likelihood that increased global surface temperatures will lead to increased decomposition rates, which would in turn increase N mineralization in soils worldwide (IPCC 2013). These increased decomposition rates may also increase the amount of readily available (labile) C in soils through increased root exudation by living plants, and increased breakdown of difficult to digest (recalcitrant) litter substances, such as lignin. Increased temperatures may also interact with increased CO_2 levels to increase plant growth rates which may also increase plant C uptake, and therefore, C availability in terrestrial systems (Eisenhauer *et al*. 2012).

Changes in nutrient availability could have major ramifications for soil communities, which are thought to be dominated by bottom-up forces, for the simple reason that litter continuously decomposes rather than accumulating (Hairston *et al*. 1960; Chen & Wise 1999; Maraun *et al*. 2001). Changes in C and N availability are also expected to affect the C:N ratios of litter, which is a major determinant of decomposition rates and soil microbial community structure (Bardgett & Wardle 2003). Boreal forest systems generally have high C:N ratios due to low nutrient loading and plant productivity, with variation depending on wildfire and logging regimes (Paré *et al*. 2011). In a recent meta-analysis, it was found that fungal:bacterial ratios in soil communities on a global scale can be consistently predicted by soil and litter C:N ratios, with higher C content favouring a higher proportion of fungi (Fierer *et al*. 2009). A shift in fungal:bacterial ratios will have cascading effects at higher trophic levels because primary soil consumers

rely on bacterial and fungal food sources (Moore & Hunt 1988; Laakso *et al*. 2000; Fierer *et al*. 2009). Previous studies have shown through ^{13}C isotope analysis that C absorbed by plants as CO_2 enters the soil food web in the form of root exudates and gets transferred up trophic positions to predatory mites (Ruf *et al*. 2006). Therefore, it is important to understand how soil communities will be affected by higher atmospheric N deposition and litter loading to understand how decomposition rates, C sequestration, and other soil functions may change (Maraun *et al*. 2001; Cole *et al*. 2005; Salamon *et al*. 2006; Cole *et al*. 2008).

There are many important linkages between Collembola and nutrient cycling in soils: Collembola increase N mineralization, soil respiration, leaching of dissolved organic C, and plant growth (Setälä & Trofymow 1996; Filser 2002; Addison *et al*. 2003; Nielsen *et al*. 2011). The effects of Collembola on nutrient cycling are mediated by grazing on lower trophic positions, whereby Collembola increase fungal growth and microbial respiration, and also may prey on nematodes (Klironomos *et al*. 1992; Filser 2002; Sylvain & Wall 2011). All of these effects are, in turn, modulated by interactions between Collembola and their environment (chiefly temperature and moisture) and other soil biota (through competition and predation). Collembola are, therefore, an excellent model for determining the bottom-up effects of nutrient addition on intermediate soil consumers because they both affect and respond to changing resource availability, and because of their trophic links to both primary decomposers and predators. Further, the species diversity of Collembola can reciprocally influence the diversity of plant communities through their effects on nutrient cycling, with higher plant species and functional diversity supporting higher collembolan diversity (Sabais *et al*. 2011, Eisenhauer *et al*. 2012).

3.1.1   Press versus pulse dynamics

It is important to assess how soil biodiversity will respond both to short-term and long-term inputs of nutrients because effects may differ depending on the frequency and intensity of nutrient changes. Although the terminology of short-term *pulses* and long-term *presses* has more commonly been applied to perturbation and disturbance studies (Freedman *et al*. 2014), this framework may also be applied to the effects of nutrient

inputs on food webs (*sensu* Scheu 2002). A short-term burst of nutrients, or pulse, might occur if there is sudden die-off of certain herbaceous plant species or downstream washing of agricultural fertilizers. This may increase microbial biomass in the short term, especially of bacteria which are thought to take advantage of labile substrates more readily than fungi (Wardle *et al*. 2004). However, although experiments have demonstrated bottom-up effects on soil fauna as a result of increased microbial biomass (Jones *et al*. 1998; Rillig *et al*. 2009), in several previous studies increases in plant growth in response to nutrient addition have failed to cause increases in microarthropod abundance (Boxman *et al*. 1998; Cole *et al*. 2008). The variable response of microarthropod grazer abundance to resource availability highlights the tenuous linkages between microbial biomass and the community structure of organisms at higher trophic levels. Therefore, it is thought that the effects of microbial biomass on microarthropod abundance and diversity only appear after a lag period following the addition of labile substrates.

A long-term, chronic change in nutrient availability, or press, may be expected if there is increased availability of nutrients decomposed from recalcitrant litters such as tree trunks, or if there is an ongoing increase in atmospheric N deposition. A press of nutrients is more likely to result in long-term increased plant growth, especially in the case of N addition, which may lead to an increase in litter deposition and larger nutrient pools in soils (Magill *et al*. 2000). However, the duration of these effects increases the probability of competitive exclusion as formerly nutrient-limited species of soil fauna are able to out-compete specialist feeders and species adapted to poorer conditions (e.g., differences in nutrient retention efficiency shown by Larsen and colleagues (2009)). Murphy and colleagues (2012), in one of the few studies compare the effects of pulses and presses of nutrients on plant and soil communities simultaneously, demonstrated loss of arthropods following a N pulse but a sustained increase during a N press. They also demonstrated ongoing changes to predator-prey ratios in response to the nutrient press, indicating the effects of nutrient addition are dependent on trophic position and nutrient input regime (Murphy *et al*. 2012).

3.1.2   BSS responses to nutrient availability

Previous studies on the body size distribution of soil fauna have demonstrated the responsiveness of soil community body size spectrum (BSS) slopes to nutrient availability. For example, in an analysis by Mulder and colleagues (2011) of Dutch soil community body size distributions incorporating bacteria, nematodes, mites, collembolans, enchytraeids, and earthworms, forest communities had significantly shallower BSS slopes than grasslands or fields, indicating a relatively higher prevalence of large-bodied organisms. In that study, forest communities were associated with several measures of soil fertility, and forest species compositions were negatively correlated with increased P and microbial C availability (Mulder *et al*. 2011). Another study by Mulder (2010) on Dutch pastures, grasslands, and heathlands found that N availability explained 35.74% of body size distribution shifts, with a higher relative proportion of small-bodied organisms (nematodes) and loss of collembolans and enchytraeids under decreased nutrient availability. A third example comes from a study by Mulder and Elser (2009), who found that increased C:N ratios explained steeper BSS slopes for soil animal communities (i.e., higher available C relative to N increased the relative abundance of small species). It should be noted that P ratios were also a strong explanatory variable in these studies, with higher proportions of available P increasing dominance of large-bodied species (Mulder & Elser 2009; Mulder 2010). These studies provide compelling evidence that body size distributions may be partially structured by soil nutrient availability and stoichiometric nutrient ratios, but research is required in systems other than Dutch grasslands.

3.1.3   Objectives and predictions

To assess and compare the effects of short- and long-term C and N availability on soil microarthropods I conducted an experiment in a black spruce (*Picea mariana* (Mill.)) forest near Chicoutimi, Quebec. I applied C, N, or a combination of the two at concentrations relevant to future climate predictions. I repeated this full-factorial cross using aqueous solutions of nutrients to simulate rapidly accessible labile inputs (pulse) or solid additions of nutrients to simulate a delayed, chronic increase in nutrient availability (press). Fauna were collected during setup to assess initial community status, after four

months to assess short-term responses, and after one year to account for seasonality and assess long-term responses. Although previous experiments have tested the effects of aqueous nutrients (Maraun *et al*. 2001; Salamon *et al*. 2006), solid fertilizer (Cole *et al*. 2005), or whole food additions (Chen & Wise 1999), very few studies (Murphy *et al*. 2012) have simultaneously tested and compared the effects of pulse and press fertilization types at biologically relevant levels on soil fauna communities from a single location.

My overall objective was to examine the effect of press and pulse nutrient addition on Collembola abundance, species richness, diversity, community composition, and body size distribution over short (4 month) and long-term (12 month) temporal scales. I predicted that the addition of N in general would increase collembolan abundance and richness because it is thought to be limiting in forest systems at a global scale (Galloway *et al*. 2004; IPCC 2013). Increased N availability may lead to increased plant growth, increased root exudation, and increased fungal and bacterial growth, therefore causing a trophic cascade up to the level of mesofauna. I predicted that collembolan abundance would be further increased when C was included alongside N addition due to increased microbial activity caused by C supplementation. I also predicted that the collembolan community would respond more strongly to the aqueous pulse of nutrient inputs in the short term, demonstrating higher relative increases in abundance and richness after four months, but that the community would return to its initial state after one year. I predicted that the press of nutrients would increase collembolan abundance and richness after one year, signaling long-term effects of increased nutrient availability.

In terms of body size, I predicted that nutrient supplementation of either type would lead to a steeper BSS slope as smaller-bodied organisms took advantage of increased microbial and fungal availability. I hypothesized that smaller bodied organisms would respond more quickly because of their ability to access bacteria and fungi in smaller pore spaces, their postulated preference for microbial feeding, and their larger initial population densities. I predicted that one year after treatment body size distributions would become more even. In the case of nutrient pulses, I predicted that this would be due to a decrease in the abundance of small species from their previously elevated levels as the nutrient pulse was exhausted. In the case of nutrient presses, I predicted that this

shallower BSS slope would be due to an increase in the relative abundance of large-bodied species, as they took advantage of longer-term positive effects on plant growth, and therefore, litter amount and quality.

3.2   Methods

3.2.1   Site description

The nutrient provisioning experiment was conducted in the Forêt d'Enseignement et de Recherche Simoncouche (48° 23'N, 71° 25'W; 350 m asl), administered by the University of Quebec at Chicoutimi (UQAC). The area is dominated by black spruce forest (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) which grew after a 1922 forest fire (Rossi *et al*. 2013). It has a mor-type humus soil on drained glacial till with a 10 cm deep organic horizon (Rossi *et al*. 2013). The forest floor is dominated by the feathermosses *Hylocomium splendens* (Hedw.) Schimp. and *Pleurozium schreberi* (Brid.) Mitt. with the haircap moss *Polytrichum commune* (Hedw.) occasionally present. Snow cover up to 150 cm generally lasts from November to May (Rossi *et al*. 2013). The mean annual temperature at the research site from 2002-2008 was 1.9 °C and increased to an average 13.3 °C over the growing season (May-September) (Rossi *et al*. 2011).

3.2.2   Experimental setup

On June 10th and 11th, 2013, experimental plots were designated in a 200 m^2 area of relatively flat continuous feathermoss forest floor. Forty 25 cm $\times$ 25 cm experimental plots were established every 2 m in a 5 $\times$ 8 plot grid pattern, and each was marked with a small central flag and GPS coordinates. Photographs of each plot were taken. This layout was used to ensure nutrient effects were independent while minimizing plot variability. Deviation from this pattern and spacing was sometimes necessary due to topographic or debris considerations (e.g., boulders or large fallen logs). A 5 cm diameter, 10 cm deep soil core was extracted from each plot using a PVC soil corer, and sealed in its corer for analysis to assess initial community structure pre-treatment (T0). Cores were kept chilled before being returned to the lab for fauna extraction.

Plots were treated with C and N additions in a full-factorial design under either a pulse or press nutrient addition regime. Treatments received an equivalent nutrient supplement of 5.6 g per m^2 yr^{-1} N, 6.4 g per m^2 yr^{-1} C, both nutrients, or neither as a control. For the pulse nutrient treatments, plots were treated with aqueous nutrient addition in 20 ml MilliQ water. Control plots received only the 20 ml water, N treatments received a 5% aqueous solution of dissolved ammonium nitrate, and C treatments received a 5% aqueous solution of glucose. For plots where both N and C were applied, the solution was 5% ammonium nitrate and 5% glucose in 20 ml water. For press nutrient treatments, solid fertilizers were used. Control treatments received no amendment, N treatments received 1 g nitrogenous fertilizer (Scott's Turf Builder, 30:0:3 N:P:K ratio), C treatments received 1 g crushed glucose tablets (Dex 4 brand), and combination treatments received 1 g of each fertilizer. Five replications were used per treatment (total 40).

3.2.3   Sampling

On September 16th and 17th, 2013, a single 5 cm diameter by 10 cm deep soil core was removed from each plot to assess the short-term community response (4 months post-application, T4). On May 30th, 2014, a final set of soil cores were extracted from each plot (12 months post-application, T12) to assess long-term community response. At each time point, each plot was photographed to help demonstrate differences in plant cover over time. For T4 and T12 sampling events, soil cores were kept cool at 4 °C pending soil fauna extraction within 72 hours of sampling using Tullgren funnel extractions over 48 h into 75% EtOH. At the final sampling period, an additional 25 g from each plot was removed with a trowel to perform further nutrient and soil property analyses.

Collembola were sorted, identified to species level using the keys of Christiansen and Bellinger (1998), and enumerated using a 10-50 × magnification dissecting scope (Nikon SMZ745T) and a 400 × magnification compound light microscope (Eclipse Ni-U). Soils were sufficiently dried to a constant weight during Tullgren funnel extraction, and dry weights were recorded to allow for standardization. Collembolan abundance and richness in each soil core was divided by soil weight to standardize, generating individual and species density values. These standardized values were used in all subsequent analyses. Shannon-Wiener diversity (H') was calculated for each soil core community as per

Equation (3). Pielou's evenness (J') was also calculated for each soil core, using Equation (4). The average body size of each species was found by measuring the length of 10 representative specimens using the compound light microscope and applying the body mass estimates derived by Edwards (1967) (see Equation 5).

3.2.4   Data analysis

A nested repeated measures ANOVA was performed with sampling time acting as the repeated component, and full-factorial N and C treatments applied within pulse or press addition types. Standardized abundance and standardized richness were log-transformed to meet assumptions. A strong seasonal effect was observed in these preliminary analyses, so the standardized abundance, standardized richness, diversity, and evenness of T0 samples was subtracted from the values of T4 and T12 samples from the same plot to calculate the change in community structure between the initial community and post-treatment. These initial-corrected values were then used in a second set of repeated measures ANOVAs to determine the change in abundance, richness, diversity, and evenness between the fall of 2013 and spring of 2014 compared to initial levels in response to nutrients provided and pulse or press addition. These analyses were conducted in Statistica 7 (StatSoft Inc., 2004).

Standardized species abundance data was used to construct Bray-Curtis percent similarity matrices for all samples at all sample times, and separately for samples within each time point. These similarity matrices were used to generate NMDS plots to observe changes in Collembola community composition throughout the experiment, and within each time point. A subsequent ANOSIM was used to determine differences in community composition between times (where applicable), nutrient addition, and addition (pulse versus press) types. These analyses were completed using Primer (PRIMER-E Ltd., 2001).
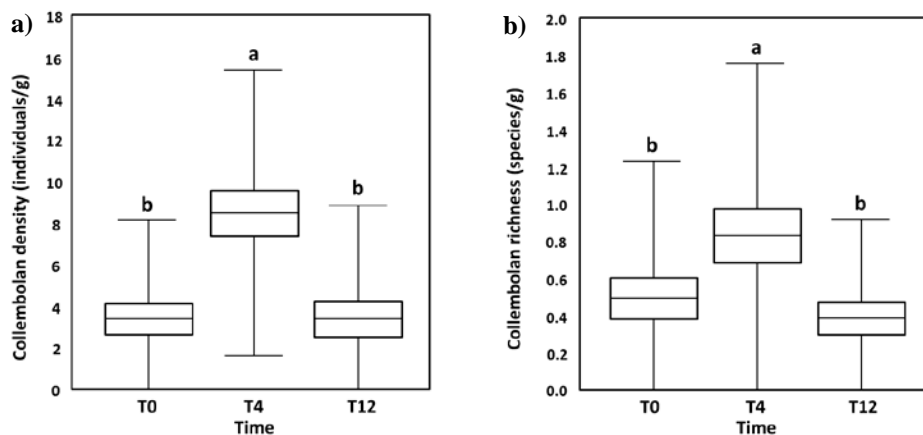
Fig. 3.1. Seasonal effects on collembolan density and richness. Samples had significantly higher collembolan density ($F_{2,96} = 16.992$, $p < 0.001$) and richness ($F_{2,96} = 11.491$, $p < 0.001$) from T4 compared to T0 and T4. Different letters indicate significant differences between treatments. Box plot mid lines represent the mean, box limits are ±SE, whiskers are ±SD.

Body size spectra were generated separately for both press and pulse treatments with species abundances pooled by nutrient addition. These spectra were generated by plotting the log-transformed average mass of each collembolan species against the log-transformed abundance of each species and then plotting a regression line against the 75th quartile of most abundant species. A homoscedasticity of slopes test was used to check for differences in body size distribution evenness between nutrient treatments. These analyses were performed in R using the 'quantreg' package (R Foundation for Statistical Computing, 2013).

3.3   Results

A total of 7,595 Collembola individuals in 26 species were identified and sorted. Species richness and collembolan density had a strong seasonal trend whereby T4 samples had significantly greater collembolan density ($F_{2,96} = 16.992$, $p < 0.001$; Figure 3.1a) and species richness ($F_{2,96} = 11.491$, $p < 0.001$; Figure 3.1b) values than samples collected in the spring for both T0 and T12 sample times, which were not significantly different from each other. Analysis of T0 samples alone revealed that before treatment, plots randomly designated for pulse treatments had significantly higher collembolan density ($F_{1,32} =$
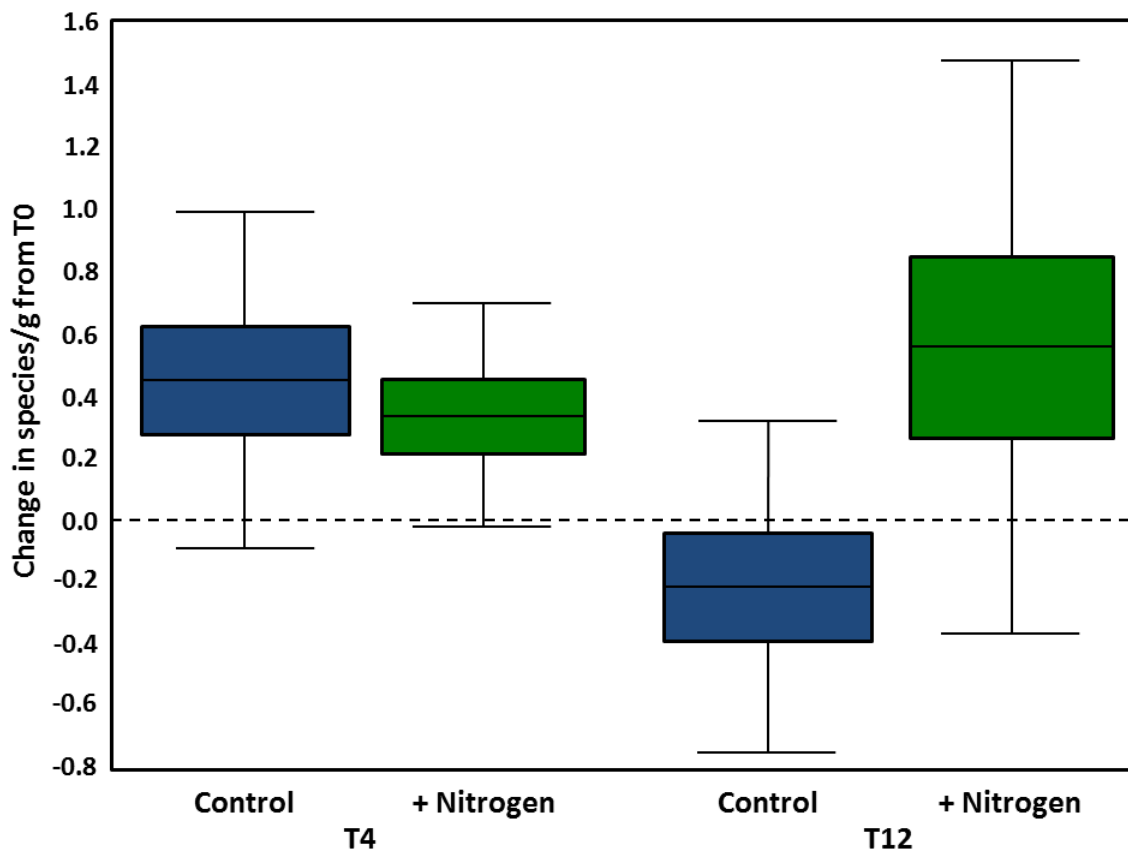
Fig. 3.2. The effects of 4g N per m^2 yr^{-1} aqueous pulse addition on collembolan species richness per gram dry weight of soil, standardized by initial community richness at experimental setup. Note the increase richness in all T4 samples compared to T0, indicating a seasonal effect. Note also that N addition prevented the loss of species at T12 compared to T4 (repeated measures ANOVA, $F_{1,16}$ = 6.828, p = 0.019). Box plot mid lines represent the mean, box limits are ±SE, whiskers are ±SD.

5.120, p = 0.030) and richness ($F_{1,32}$ = 7.780, p = 0.009) than plots randomly designated for press treatments. Therefore, to correct for starting differences between plots and focus on short-term versus long-term shifts from a seasonal pattern, T4 and T12 samples were standardized by subtracting the abundance, richness, diversity, and evenness of T0 communities, to show change in these values compared to before nutrient addition.

To correct for initial differences in plots designated for pulse and press treatments, and to more clearly demonstrate different effects between these treatments, data were analysed separately for pulse and press treatment types. For pulse treatments alone, the only effect
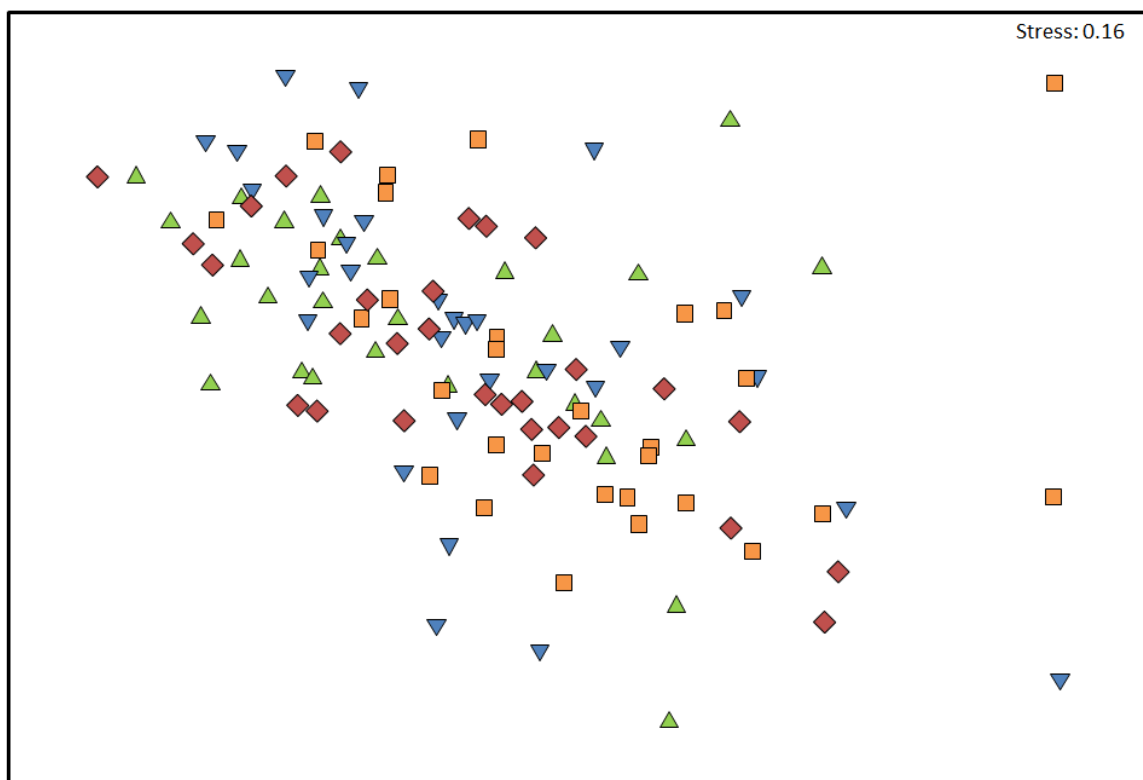
Fig. 3.3. Non-metric dimensional scaling (NMDS) based on Bray-Curtis % similarity of species abundances in response to nutrient additions after one year (△ = Control treatment, ▨ = Carbon addition, ▽ = Nitrogen addition, ◆ = both C and N addition). Each point represents one community and the distance between points represents the degree of similarity, in terms of species and abundances. There are significant differences in composition between N addition treatments and C addition treatments (R = 0.158, p = 0.026), as well as between N addition treatments and Control treatments (R = 0.346, p < 0.001). Combination treatments are significantly different in composition from Control treatments (R = 0.243, p = 0.005).

on collembolan density was significantly lower densities in T12 compared to T4 ($F_{1,16}$ = 11.005, p = 0.004). There was a significant difference in richness between T4 and T12 pulse treatments with N addition ($F_{1,16}$ = 6.828, p = 0.019; Figure 3.2), but although richness was highest richness in T12 N addition treatments, groups could not be distinguished pairwise with Tukey's test. Pulse N addition treatments demonstrated significantly higher evenness in T12 compared to T4 ($F_{1,16}$ = 4.806, p = 0.043). The only significant effects in press treatments when separated were decreased collembolan density ($F_{1,16}$ = 6.522, p = 0.021) and richness ($F_{1,16}$ = 5.069, p = 0.039) in T12 compared to T4. There seemed to be a trend of higher richness in press N addition samples without

C added, but this effect was not found to be significant. The only effect of other nutrient types was a significant effect on evenness in the interaction between time and C addition, whereby T12 plots with C added had a higher evenness than T4 ($F_{1,16} = 5.045$, p = 0.039); however, once again, groups were not significantly different when compared pairwise with Tukey's test.

In terms of community composition, all T0 samples were relatively homogeneous with the exception of compositional differences in plots designated for press and pulse treatments (ANOSIM, global R = 0.091, p = 0.016). In T4 samples, the only significant differences in community composition were between Control and N addition samples (ANOSIM, R = 0.144, p = 0.024). In T12 samples, significant differences were observed between N addition treatments and C (R = 0.158, p = 0.026) and Control treatments (R = 0.346, p < 0.001; Figure 3.3). In T12, Combination treatments were significantly different from C plots (R = 0.243, p = 0.005; Figure 3.3). There were no significant differences in community composition between pulse and press treatments in T4 or T12.
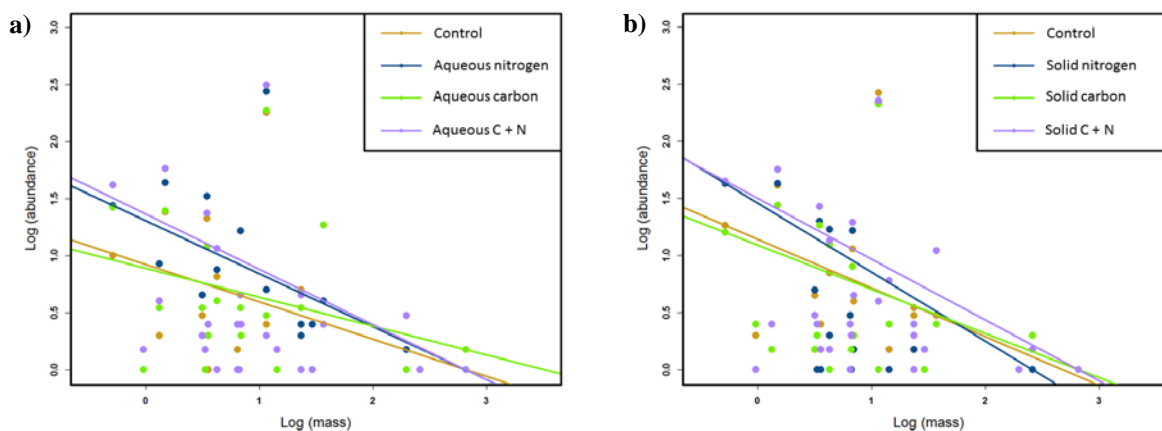
Fig. 3.4. Body size spectrum (BSS) for Collembola in response to aqueous (a) and solid (b) nutrient treatments. Each point represents the log-transformed abundance of a collembolan species of a certain log-transformed mass. Lines are 75th quartile regressions of the most abundant species. The slopes of the regression lines are not significantly different according to ANCOVA. However, it appears that treatments that included nitrogen had a relatively greater abundance of smaller-bodied species, as indicated by the steeper slope and higher intercept at smaller sizes.

Body size spectra were generated separately for each addition type with abundances pooled by nutrient type. For both aqueous and solid treatments, there were no significant differences between slopes according to ANCOVA tests (Figure 3.4). However, for both addition types, there appeared to be a relatively greater abundance of smaller-bodied species (as indicated by a higher intercept) under N and C+N nutrient addition.

3.4 Discussion

3.4.1 Nutrient addition on Collembola communities

Concurrent and concomitant with changes in climate are the anticipated changes in resource and nutrient dynamics in boreal forest soil systems. Which nutrients and how these nutrients will enter the soil system are anticipated to dictate whether nutrient addition will have a positive (van der Wal *et al*. 2009), negative (Boxman *et al*. 1998; Xiankai *et al*. 2008; Xu *et al*. 2009) or neutral effect (Cole *et al*. 2008) on soil biodiversity. Generally, it is thought that highly productive ecosystems will sustain a greater proportion of fast-growing plants, which in turn will support higher soil bacterial biomass and a greater proportion of earthworms with a loss of microarthropods (Wardle *et al*. 2004). In this study, despite nutrient additions equivalent to 56 kg ha yr^{-1}, nutrient addition in the form of labile C and biologically available N had only minor effects on the collembolan community. A study by Maraun and colleagues (2001) using aqueous nutrient addition added as much as 2.8 g m^2 yr^{-1} glucose and 102 g m^2 yr^{-1} ammonium nitrate (simulating five times greater natural litterfall input) split between treatments every two weeks without seeing direct effects of N addition on Collembola. In that experiment, it was found that C addition significantly reduced collembolan density unless phosphorus was also added, but this was theorized to be due to the detrimental effects of increased earthworm densities in response to C changing soil structure (Maraun *et al*. 2001).

The addition of N mimicking 2050 atmospheric deposition rates kept collembolan richness at 12 months post-application comparable to 4 month post-application, although T12 samples were not significantly different in richness with or without N. This contrasts with previous studies which found a loss of abundance and richness in response to N

Plate 2. Moss plot at experimental setup (left) and four months after aqueous addition of 5.6 g m^{-2} yr^{-1} ammonium nitrate (right). Aqueous N addition, whether combined with aqueous carbon addition or not, caused significant burning of the moss carpet, which may have had further effects on belowground microbial communities.

inputs equivalent to 25 kg N ha^{-1} yr^{-1} (Xu *et al*. 2009). In a review of nutrient addition experiments, Xiankai and colleagues (2008) hypothesized that N deposition will actually cause global soil biodiversity loss. However, an experiment by van der Wal and colleagues (2009) found that a long term (40 year) addition of multiple nutrients, including 160 kg N ha^{-1} yr^{-1}, resulted in a positive impact on collembolan biomass when paired with P (31 or 52 kg ha^{-1} yr^{-1}) and K (291 or 332 kg ha^{-1} yr^{-1}) addition. The current study seems to provide evidence for neutral or slightly positive bottom-up effects of N addition on Collembola. The short term pulse of N addition seemed to have a stronger effect than the N press because the interaction between nutrients and time appeared significant for species richness, although the effect was limited to a minor increase in richness. Aqueous addition of nitrogen, both individually and in combination with C, caused significant burning of the moss carpet, which may have upset microbial community composition (Plate 2). This burning effect persisted one year after treatment.

Body size spectra revealed slight positive effects of N addition on smaller bodied species (Figures 3.4 and 3.5). This may be compared to previous findings by Mulder (2010), where N availability partially explained collembolan abundance. In that study, large soil fauna were particularly affected by lack of nutrients, leading to steeper BSS slopes (Mulder 2010). It appears that in my study, smaller species which would typically be associated with euedaphic, fungivorous or bacterivorous life history strategies were more

affected by N addition, which is a preliminary indication that N addition positively affected the microbial community, either directly or through positive effects on plant growth which increased root exudate availability and litter quality for microbes. These positive effects may then have been transferred to these smaller species through a bottom-up trophic cascade.

3.4.2   Mechanisms for indirect effects of nutrients

Any effects of nutrient addition on collembolan communities would have to be indirect, through changes in microbial biomass or plant growth. One explanation is that an increase in microbial growth and availability in these plots over winter caused an increase in available resources for Collembola, and facilitated individual survival, and therefore, maintained species richness for T12 sampling. Microbial effects and microbial biomass have been indicated as a dominant factor in Collembola abundance (Filser *et al*. 2002). However, an increase in microbial biomass would lead to a general increase in the abundance of many species, and not just Collembola. For example, long-term Ca and N (120 kg N ha^{-1} yr^{-1}) additions were found to increase microarthropod densities and the proportion of predators, but did not affect overall microarthropod diversity (Cole *et al*. 2005).

It is difficult to predict which species will benefit from resource addition due to the complexity of soil food webs caused by the prevalence of omnivory (Ruess *et al*. 2005) and diet switching (Ladygina *et al*. 2008), the heterogeneity of soil structure, and the mobility of predators (Chen & Wise 1999). Fountain and colleagues (2008) found that only two of 17 collembolan species and one of 44 spider species were affected by N addition (240 kg ha^{-1} yr^{-1}) and liming in a Scottish soil. This is thought to indicate that in fertile soils where nutrients are transferred to higher trophic positions predation pressure is able to prevent competitive exclusion, and therefore, maintain grazer diversity (Cole *et al*. 2005). It is known that Collembola differ in their efficiency of resource retention, metabolic rates, and investment in reproduction, which are thought to be adaptations to different resource availabilities (Larsen *et al*. 2009). This may partially explain the increase in abundance of specific species following nutrient addition. In my study, it appears that small-bodied species were more positively affected than larger-bodied

species. Differences in T12 community composition between N added plots and Control and C plots were driven by higher abundances of the dominant *Folsomia penicula* and *Onychiurus* (*A.*) *absaloni*, a relatively high prevalence of the Entomobryid *Argenia cyanura*, and increased prevalence of a Neanurinae species from the genus *Hypogastrura*. Unfortunately, the sample plots in this experiment were too small to capture enough apex soil predators (spiders and centipedes) to draw definitive conclusions on predator densities or potential energy transfer to higher trophic positions.

Another potential explanation for the small effect on collembolan richness one year after nutrient addition is indirect effects through the plant community. Increased moss growth under N deposition could lead to deeper moss mats and increased vascular plant root growth, which would in turn lead to higher microbial biomass and potentially buffer soil temperature over winter. It was found in a long-term nutrient addition study that collembolan density increased in parallel with plant biomass in response to NPK addition, leading to the hypothesis that microarthropod abundances are positively related to plant biomass (van der Wal *et al*. 2009). However, in a study by Cole and colleagues (2008), experimental additions of N fertilizer up to 24 g per m^2 yr^{-1} over a 2-year period increased plant biomass but did not affect collembolan abundances in undisturbed conditions. Although increased root growth could give a competitive advantage to species which rely on saprotrophic microbes, it is not known if root growth or microbial biomass truly increased. Conversely, burning of the moss mat could cause a short term burst of nutrients as fungi and bacteria decompose the plant material, which would have bottom-up effects on Collembola. The stronger positive effect of N addition on small species typically thought to be euedaphic and microbiovorous may indicate this was the case. Temperature buffering could be predicted to cause increased collembolan abundance for all species, with greater advantage given to species which are more cold-sensitive, but it is not known if those species which were higher in abundance in N treatments exhibit these characteristics. Further research could examine their feeding characteristics and temperature optima.

The effects of C addition were less than for N addition. Treatments with C addition were meant to mimic increased availability of root exudates, which is predicted under

increased atmospheric CO_2 and may lead to increased C uptake by soil microbes (Ruf *et al*. 2006). The only significant effect of C addition was higher evenness in T12 samples under press conditions, which was comparable to T4 samples but not significantly different from T12 samples without C addition. The addition of 6.4 g C per m^2 is not likely to alter C:N ratios sufficiently to affect fungal:bacterial ratios in this system, given the slope of the global relationship (Fierer *et al*. 2009). This could be a further indication that this system is not C-limited and that N availability is a more important determinant of microbial growth. Although bacteria are thought to respond more to C limitation on a global scale (Bardgett & Wardle 2003), it is thought that bacteria require more N than fungi for each unit C of biomass growth (Bardgett & McAlister 1999; Kuijper *et al*. 2005; Fierer *et al*. 2009), which may explain the lack of effect with C alone. Another possible explanation is that changes in fungal:bacterial ratios did occur, but Collembola switched diets as they are well documented to do in response to food quality (Klironomos *et al*. 1992; Ruess *et al*. 2005; Chamberlain *et al*. 2006; Ladygina *et al*. 2008), temperature, and age (Haubert *et al*. 2007). This diet switching may have provided a small advantage to less common species, leading to slightly increased evenness.

3.4.3   Seasonality of Collembola Communities

Deviations from regular seasonal patterns are of chief interest in determining the effects of nutrient additions. The data show a considerable seasonal effect, with abundance and richness of Collembola peaking in fall (T4) samples. There was also a seasonal shift in community composition where four species in the family Sminthuridae and one species in the family Isotomidae were more abundant in the fall and did not appear (or only a single individual appeared) in spring (T12) samples. This parallels findings by Chagnon and colleagues (2000) who found evidence of seasonal abundance changes in eight epigeic species from Quebec sugar maple forests but no evidence of seasonality in euedaphic species. However, a study by Rochefort and colleagues (2006) found no evidence of seasonal shifts in abundance during one year of May to October sampling in an urban Quebec City lawn. The findings of this study also contrast with Hijii (1987) who found that soil microarthropod abundances were similar between seasons in a Japanese cedar plantation. Seasonality may play a larger role in more northerly forests where

winters are more severe and productivity is generally lower, leading to greater costs of overwintering and a more pronounced recovery of faunal abundances during the growing season. Therefore, the most compelling result of this experiment is that collembolan density and richness in the spring one year after treatment were comparable to densities the preceding fall in plots supplemented with aqueous nitrogen fertilizer, whereas plots without N addition contained lower collembolan density and richness, and the overall trend indicated a significant decrease in collembolan density and richness from T4 to T12. However, there were no significant differences between nutrient treatments in the fall. This indicates that the primary effect of long-term N addition in the boreal forest will be more rapid rejuvenation of the collembolan community following winter, but only at relatively high levels of rapidly accessible N.

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4 Interactions between environmental effects on soils

4.1 Consequences of global environmental change on soil biodiversity

As stressed by Kardol and colleagues (2011), it is important to study the multiple effects of GEC factors on soil communities simultaneously, as they will actually apply to soils, to discover interactions between environmental factors. My research has sought to do exactly this, and echoes previous findings that interactions between environmental variables can be more important than direct effects. The most notable example was the effect of air temperature directly and indirectly through changes in moisture on soil biodiversity. Many studies have reinforced that the effects of increased temperature on soil fauna are expressed by increased evaporation from soil, and therefore, drying (Blankinship *et al*. 2011; Kardol *et al*. 2011; Makkonen *et al*. 2011). Blankinship and colleagues (2011) especially stressed that the effects of temperature on soil mesofauna were mostly explained by resulting changes in soil drying and that only cold, dry forest communities demonstrated significant direct responses to warming.

Temperature is also expected to interact with nutrient dynamics through increased decomposition and plant growth rates: for example, a model by Xu-Ri and colleagues (2012) suggests that warming could increase N_2O emissions from natural systems by 1 Tg yr^{-1} per approximate 1 °C increase in temperature. Their model also indicated that rising CO_2 is most likely to interact with this pattern through its effect on warming (Xu-Ri *et al*. 2012). Zaehle (2013) modeled a more conservative increase in N_2O emissions of 0.5 Tg N yr^{-1} per 1 °C of warming and reiterated the importance of N limitation in global C sequestration. Atmospheric CO_2 levels, while not directly affecting soil communities that are adapted to high CO_2 levels in soil, can also interact with nutrient cycles. Eisenhauer and colleagues (2012) found that increased atmospheric CO_2 in a long-term field experiment had a positive effect on soil moisture and net primary productivity that resulted in increased detritivorous microarthropod densities which counteracted the negative effect of N fertilization.

Although temperature, precipitation, and CO_2 may interact with nutrient levels to alter the community composition of soil fauna, significant functional redundancy in soils may buffer the system against changes in process rates. For example, Hunt and Wall (2002) developed a model of soil biodiversity incorporating 15 functional groups and successively removed them from the model to check for effects on the other functional groups. They found that only the removal of six of the 15 functional groups modeled affected the abundance of another group (Hunt & Wall 2002). Their model also predicted that only the loss of bacteria, saprotrophic fungi, and root-feeding nematodes would have major effects on ecosystem function (specifically decomposition). The functional redundancy of soil fauna has been supported by empirical studies, such as by Salminen and colleagues (2009), who found that exclusion of nematodes and enchytraeids changed bacterial community composition but did not affect decomposition rates or soil C and N content. While Wall and colleagues (2008) found that richness was a predictor of decomposition rates across geographic regions in a global litter bag study, the functional redundancy of soil fauna and the ability of fauna such as Collembola to switch diets (Ruess *et al*. 2007) makes predicting the effects of species loss at local scales problematic.

I found that increased precipitation severity decreased the density and richness of Collembola, which contrasts with previous studies that have found decreased collembolan abundance under drought regimes (Pflug & Wolters 2001, Kardol *et al*. 2011, Makkonen *et al*. 2011). This also contrasts with the collembolan susceptibility to desiccation noted by Tsiafouli and colleagues (2005) and others, suggesting that Collembola communities in general have an optima soil moisture and are sensitive to physical disturbances in soil systems. I also observed a shift in community composition towards species with epigeic characteristics, suggesting surface-dwelling collembolans are most responsive to climate changes. These results may be compared with the findings of Makkonen and colleagues (2011) who found similar shifts in epigeic species in response to the loss of soil moisture. However, collembolan community responses appeared to affect all size classes similarly. I posit that increasingly severe precipitation events, as well as severe drought act as a disturbance in the surface soil system, either by altering fungal:bacterial ratios or in the case of intense and frequent precipitation, by

decreasing the amount of habitable air-filled pore space. I am not aware of any previous studies framing the effects of severe precipitation on soil communities as a disturbance. However, my research provides evidence that moisture-related disruptions to soil microbial communities and pore composition can override positive effects of increased soil moisture. My research also provides evidence that variation in soil moisture content (wetting and drying cycles) may be a more important factor in determining mesofaunal response than moisture content alone. Other microarthropods may respond differently to increasingly intense precipitation – I refer primarily to mites, which do respond to changes in soil moisture but are thought to be more tolerant to moisture changes than Collembola due to their globular body shape and thicker cuticle (Harte *et al*. 1996). But moisture-tolerant mites may not be able to tolerate loss of pore space or shifts in microbial community composition, so the structural and biotic consequences of more severe precipitation regimes may detrimentally affect soil fauna formerly thought to be tolerant to moisture fluctuations. The effects of GEC on habitable soil pore space are poorly studied and require further research.

The addition of N had a slight positive effect on collembolan density and richness, especially in smaller-bodied species, in contrast to reviews (Wardle *et al*. 2004; Xiankai *et al*. 2008) and studies (Boxman *et al*. 1998; Xu *et al*. 2009) that have found negative effects of N fertilization on mesofauna. Positive effects of N treatments on abundance and richness were apparent despite a lower level of N addition (56 kg ha^{-1} yr^{-1}) and a shorter period of study (one year) than many other experiments used (e.g., 160 kg ha^{-1} yr^{-1} over 40 years to show positive effects in van der Wal and colleagues (2009)). This may be caused by initial N availability in the system as it is known that boreal forests are generally N-limited (Galloway *et al*. 2004) and positive effects of N addition on mesofauna have been observed in nutrient-poor systems (Cole *et al*. 2008). Under aqueous N fertilization 'burning' of the moss carpet was observed without negative consequences for collembolan density and richness. This seemingly counter-intuitive result (i.e., loss of substrate resulting in increased diversity) may be by the death and decomposition of these mosses providing a temporary increase in fungal abundance, which subsequently bolstered their collembolan consumers, specifically the small euedaphic species most able to enter small pore spaces where fungal hyphae had

penetrated. This increase in collembolan abundances and richness would therefore be time-delayed as fungi spread to take advantage of decaying moss, but would also be temporary, and I would expect this shift to disappear as moss decay and fungal activity decreased. In order to determine if fungal (bottom-up) factors and ascertain whether more fertility-adapted plants could then colonize the former moss mat and support a collembolan community, longer-term monitoring would be required.

4.2   Soil theory as it applies to general ecology

Research in soil ecology has not yielded theories that can satisfactorily explain the immense complexity of the soil system (see Lavelle 2009). However, work in this exceptionally interconnected component of the environment is valuable for the theoretical perspective it can give on systems where certain key assumptions or patterns are not present. For example, the Intermediate Disturbance Hypothesis (IDH) does not apply well to soil systems: increasing disturbance leads to decreases in soil biodiversity in almost all cases, especially in response to agricultural processes, rather than reaching an optimum at intermediate levels (see Bardgett *et al*. 2005). My research suggests that this could be predominantly due to changes in physical soil structure caused by disturbance. For example, decreases in habitable pore space caused by more intense precipitation detrimentally affected all size classes of Collembola, rather than giving an advantage to the smaller species still able to navigate the soil environment. This suggests that disturbances and other top-down effects are likely to have a universally detrimental effect on biodiversity if they decrease habitable spaces, such as what occurs under land use change resulting in soil compaction or removal. My research also highlighted differential responses between species whereby certain globular, pigmented species (Family Sminthuridae) were more successful under increased precipitation and temperature regimes. This is a potential avenue for further use of trait-based research to better define which traits are most associated with tolerance to disturbances. An extension of the experiment detailed in Chapter 2 could be to return all mesocosms to normal conditions and track which species traits and body sizes are most associated with recovery following disturbance.

Trophic relationships have been difficult to quantify in soil food webs because of the high prevalence of omnivory, diet switching, and intraguild predation, combined with internal trophic feedbacks caused by turnover and decomposition of predatory mesofauna and macrofauna by their former prey (Scheu 2002). However, several studies have highlighted that soil communities do respond to bottom-up control (Cole *et al*. 2005) and that responses of soil fauna to bottom-up effects differ depending on age (Fountain *et al*. 2008), trophic position (Maraun *et al*. 2001), and relationship to the plant community (Eisenhauer *et al*. 2012; Murphy *et al*. 2012). These studies showing variable effects highlight the need for trait-based approaches that assess the relative importance of basal resources to different trophic positions in the soil food web.

My research indicates maintained richness after one year in response to short-term N addition and maintained evenness after one year in response to C addition, compared to seasonal patterns. This demonstrates that these differential effects of bottom-up control may apply to boreal forest soil communities. On a broader scale, this complexity of trophic relationships in soil encourages broader thinking about trophic relationships in all systems because of the coupling between production and decomposition processes. Strong linkages have been demonstrated between aboveground and belowground processes (Wardle *et al*. 2004), whereby litter qualities (such as C:N ratios) can have strong effects on soil communities (Bardgett 2005), and plant diversity can be reciprocally positively affected by soil diversity (Eisenhauer *et al*. 2012; Sabais *et al*. 2011). Further research is needed to quantify the effects of certain traits and trait diversities on the rate of ecosystem processes, and work could be done to search for relationships between above and belowground organisms rather than treating the systems separately.

4.3   Diversity and seasonality of boreal forest Collembola

It should be noted that seasonality has not been a major factor in other collembolan studies (Rochefort *et al*. 2006) but appeared to be present in my nutrient-addition study, with populations in the fall demonstrating higher collembolan density and species richness compared to the spring. It is possible that this was due to an interaction with nutrient availability, as decomposition increases in the warm summer months creating

more fungal availability for Collembola, or due to increased precipitation over the summer which increased soil moisture content to support more species. However, there is also a strong possibility that decreased collembolan density and richness was the result of vertical migration by epigeic species which may appear to change community composition but does not truly change total abundance (Chagnon *et al*. 2000; Krab *et al*. 2010). Broader sampling with deeper soil cores, several varieties of collection, and some measurement of egg or juvenile presence would be required to thoroughly examine species-specific responses to seasonality in this community. However, for any of these explanations for seasonal variation, these patterns are likely to become more pronounced under future GEC conditions as temperature and precipitation extremes become more common, either leading to more seasonal fluctuation in abundance or a greater prevalence of vertical migration.

4.4 Concluding remarks

In this thesis, I demonstrated that collembolan diversity and community composition respond to the increases in temperature, atmospheric CO_2, precipitation, and nutrient deposition predicted for the next century of GEC. In a laboratory mesocosm study I demonstrated that warmer temperatures with more intense rainfall are likely to lead to decreased abundances and richness in collembolan communities, due to severe precipitation events acting as a direct disturbance. In a year-long field manipulation, I found that pulse events of N input are likely to increase abundance and richness, at least in the short term. Long-term effects will depend on changes in physical soil structure and chemical properties, as well as the effects of these factors on other soil biota and the aboveground plant community. In these experiments I found that GEC caused different effects on community composition depending on collembolan life strategies and body sizes. The potential consequences for a shift in epigeic species, with larger body size and more detritivorous feeding, may alter rates of mechanical breakup of litter leading to changes in ecosystem-level processes such as decomposition and nutrient cycles. Understanding soil biodiversity is intricately linked to our understanding of how ecosystems will change under future environmental conditions. This represents a step

forward in understanding the future of the living world beneath our feet, which all terrestrial life springs from and to which it must return.

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Appendices

Appendix A. Densities of Collembola (individuals per 100 g) from soil/moss mesocosms subjected to fully crossed temperature, CO_2, and moisture conditions (D = Drought, C = Control, S = Saturated) for 18 weeks.

Species	Initial	11.5 °C 430 ppm D	C	S	750 ppm D	C	S	15.5 °C 430 ppm D	C	S	750 ppm D	C	S	19.5 °C 430 ppm D	C	S	750 ppm D	C	S
Entomobryidae																			
Corynothrix borealis Tullberg, 1876	6.35 ± 0.31	9.15 ± 1.63	-	-	3.91 ± 0.45	-	-	5.29 ± 1.13	13.24 ± 5.08	-	5.34 ± 0.91	3.61	-	2.87	4.99 ± 0.51	-	7.81 ± 0.50	7.65 ± 1.06	-
Hypogastruridae																			
Hypogastrura (H.) sp. 1	2.03 ±0.16	11.72	7.11 ± 0.72	-	-	-	-	4.07	32.97 ± 12.13	-	3.08 ± 0.11	3.61	-	14.33	15.28 ± 3.07	2.62	-	4.07	-
Neanurinae sp. 1	97.33 ± 4.74	52.48 ± 9.90	157.86 ± 28.74	16.87 ± 4.31	35.06 ± 10.18	20.53 ± 2.50	53.61 ± 17.87	110.75 ± 25.44	95.00 ± 21.04	12.29 ± 3.59	46.78 ± 6.42	90.77 ± 12.05	17.16 ± 1.23	59.18 ± 19.91	101.53 ± 12.60	8.31 ± 2.70	68.98 ± 13.07	188.94 ± 29.85	37.61 ± 8.31
Oudemansia sp. 1	97.33 ± 4.74	46.90 ± 6.95	21.08 ± 3.81	14.36 ± 2.31	87.75 ± 20.11	93.94 ± 16.59	20.76 ± 6.32	221.12 ± 86.03	42.11 ± 13.10	59.73 ± 16.57	19.73 ± 3.75	46.44 ± 6.73	50.51 ± 21.48	356.90 ± 142.28	98.12 ± 34.48	24.36 ± 4.14	12.17 ± 1.88	45.52 ± 6.74	313.78 ± 14.48
Paranura colorata Mills, 1934	2.36 ± 0.32	5.79 ± 0.99	6.43 ± 1.79	4.32	-	3.68	7.70 ± 1.47	5.02 ± 0.60	15.48 ± 5.73	15.82	5.93 ± 0.73	3.83 ± 0.10	-	3.05 ± 0.19	7.00 ± 1.58	-	6.32	3.66 ± 0.18	28.48
Isotomidae																			
Argenia cyanura Fjellberg, 1986	18.44 ± 0.73	100.69 ± 18.96	50.59 ± 5.04	19.65 ± 2.38	48.79 ± 9.02	30.63 ± 3.55	9.80 ± 1.88	20.33 ± 2.59	19.12 ± 2.51	18.80 ± 7.35	22.33 ± 3.68	63.63 ± 11.69	18.48 ± 2.10	43.11 ± 5.73	350.58 ± 86.34	171.61 ± 58.76	6.58	200.70 ± 35.28	33.99 ± 6.07
Isotoma (D.) uniens Christiansen & Bellinger 1980	3.77 ± 0.56	5.05	8.98 ± 3.01	4.04	-	22.23 ± 9.06	-	-	17.54	2.68	3.51	-	-	-	-	-	-	3.31	-

Isotomid sp. 1	2.48 ± 0.29	-	-	-	4.80	-	-	15.09	-	22.70 ± 10.08	-	-	-	-	-	-	16.45	-	-
Isotomiella minor (Schäffer), 1896	19.52 ± 0.94	40.89 ± 7.66	98.63 ± 22.38	31.69 ± 8.33	42.60 ± 10.23	40.87 ± 12.54	9.86 ± 2.20	205.66 ± 63.46	56.29 ± 15.34	22.79 ± 5.13	48.75 ± 5.20	150.06 ± 34.46	6.09 ± 0.53	114.69 ± 23.11	132.70 ± 25.09	9.00 ± 3.19	247.14 ± 62.48	89.73 ± 15.61	20.18 ± 4.73
Folsomia penicula Bagnall, 1939	730.36 ± 19.64	2045.28 ± 207.4	705.89 ± 106.95	97.60 ± 12.41	932.88 ± 73.00	1438.71 ± 286.09	253.39 ± 47.28	1037.83 ± 81.30	587.95 ± 97.08	75.37 ± 22.22	1571.30 ± 99.12	1068.52 ± 130.71	22.96 ± 6.58	1844.39 ± 360.67	774.48 ± 160.78	79.51 ± 17.28	1656.91 ± 136.91	753.32 ± 96.26	51.19 ± 6.41
Onychiuridae																			
Onychiurus (A.) absoloni (Börner), 1981	45.31 ± 2.51	150.33 ± 25.55	20.63 ± 4.00	78.08 ± 6.03	287.23 ± 86.72	358.61 ± 41.41	138.85 ± 24.64	293.51 ± 77.54	320.58 ± 150.19	59.57 ± 13.89	170.30 ± 37.17	696.34 ± 65.57	45.29 ± 6.61	102.52 ± 14.61	98.58 ± 19.37	239.98 ± 33.96	259.37 ± 57.45	625.57 ± 100.16	176.61 ± 26.98
Onychiurus sp. 1	-	70.25 ± 9.41	308.83 ± 78.16	4.32	-	21.34 ± 2.12	-	98.37 ± 25.99	44.89 ± 7.25	3.57	-	32.49	-	41.10 ± 8.15	134.53 ± 17.99	7.69	38.22	73.45	-
Tullbergia (M.) iowensis Mills, 1932	247.85 ± 11.58	91.82 ± 14.84	234.88 ± 35.66	18.44 ± 2.81	54.02 ± 16.88	29.47 ± 1.88	39.91 ± 6.23	143.69 ± 18.68	74.01 ± 12.23	19.12 ± 4.87	24.90 ± 1.97	40.60 ± 2.35	18.86 ± 2.96	358.57 ± 88.62	178.28 ± 26.79	13.63 ± 1.57	83.93 ± 13.86	17.67 ± 2.67	8.19 ± 1.22
Willemia sp. 1	49.61 ± 1.64	14.50 ± 3.19	19.22 ± 2.48	-	-	-	-	-	13.43 ± 2.37	-	-	-	-	11.46	18.66 ± 4.87	10.47	-	-	-
Willemia sp. 2	-	-	-	-	21.65 ± 5.39	7.88 ± 0.17	14.30 ± 3.45	-	-	-	11.54 ± 1.59	27.65 ± 2.36	5.56 ± 1.00	-	-	-	11.30 ± 0.72	14.30 ± 2.66	10.80 ± 0.65
Paronellidae																			
Salina sp. 1	0.70 ± 0.93	3.49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sminthuridae																			
Arrhopalites obtusus Zeppelini & Christiansen, 2003	-	7.09 ± 0.85	10.91 ± 0.59	6.64 ± 0.32	3.01	-	-	-	11.43 ± 2.35	-	7.76 ± 2.31	27.34	-	20.06	46.91 ± 5.87	5.84 ± 0.55	32.37	23.48 ± 1.87	16.04 ± 4.15
Arrhopalites sp. 1	-	-	-	3.64	4.02 ± 0.39	3.91 ± 0.07	-	4.76 ± 0.66	-	2.55	15.70 ± 4.34	3.61	-	6.04 ± 0.83	-	15.54 ± 6.02	3.60	13.07 ± 2.87	35.19 ± 14.95

Collophura quadrioculata (Denis), 1933	-	-	11.36	10.92	5.43 ± 0.66	14.04 ± 3.25	4.06	14.20 ± 2.64	27.11 ± 4.90	8.59 ± 2.22	53.91 ± 12.19	46.24 ± 8.92	3.34 ± 0.11	-	-	157.97 ± 72.55	27.03 ± 8.99	62.24 ± 13.06	210.96 ± 82.03
Sminthurus (S.) medialis Mills, 1934	-	17.38 ± 2.73	11.36	6.04 ± 1.16	10.11 ± 1.09	8.21	5.37 ± 0.46	4.07	8.06	3.06 ± 0.26	30.33 ± 6.53	2- ± 4.03	-	2.99 ± 0.11	14.32 ± 5.24	10.65 ± 2.48	6.58 ± 0.81	54.06 ± 17.70	236.03 ± 102.61
Sminthurides (S.) violaceus Reuter, 1881	0.74 ± 0.07	41.69 ± 9.10	28.13 ± 5.63	14.55 ± 1.18	9.60	11.45 ± 3.70	-	9.73 ± 0.80	8.48 ± 0.61	2.68	25.96 ± 6.88	32.57 ± 8.96	-	8.00 ± 0.62	276.72 ± 37.09	2.92	9.71 ± 0.08	75.55 ± 11.86	39.89 ± 10.94
Tomocerinae																			
Tomocerus (P.) flavescens Tullberg, 1871	9.06 ± 0.47	20.03 ± 8.27	4.64 ± 0.18	3.64	9.60	8.21	-	4.60 ± 0.51	4.94 ± 0.45	-	7.17	10.93 ± 1.66	2.65	-	20.58	-	9.87	-	14.08
Tomocerus sp. 1	0.54 ± 0.07	-	-	-	-	-	-	7.55	-	-	-	-	-	-	4.57	-	-	-	-

Appendix B. Densities of Collembola (individuals per 100 g) from experimental plots in a black spruce forest treated with fully crossed aqueous or solid N, C, C + N nutrient additions or control (Con) conditions initially (T0), after four months (T4), and after twelve months (T12).

	T0								T4								T12							
	Aqueous				Solid				Aqueous				Solid				Aqueous				Solid			
Species	Con	N	C	N+C	Con	N	C	N+C	Con	N	C	N+C	Con	N	C	N+C	Con	N	C	N+C	Con	N	C	N+C
Entomobyridae																								
Corynothrix borealis Tullberg, 1876	-	-	-	-	-	-	-	-	11.71 ± 3.00	0.81 ± 0.32	5.46 ± 1.47	9.86 ± 3.54	4.76 ± 1.17	1.65 ± 0.66	1.81 ± 0.70	0.80 ± 0.32	-	4.99 ± 2.00	0.37 ± 0.15	1.36 ± 0.54	0.61 ± 0.25	-	0.69 ± 0.28	0.45 ± 0.18
Hypogastruridae																								
Hypogastrura (*H.*) sp. 1	2.65 ± 1.06	1.25 ± 0.50	-		5.94 ± 2.38	-	0.77 ± 0.31	9.26 ± 3.70	4.12 ± 1.48	-	56.28 ± 14.24	0.84 ± 0.34	24.32 ± 7.39	-	11.73 ± 4.69	29.99 ± 12.00	0.47 ± 0.19	16.14 ± 4.16	0.92 ± 0.37	2.06 ± 0.77	-	6.57 ± 1.85	-	-
Oudemansia sp. 1	15.80 ± 3.09	6.69 ± 1.04	3.25 ± 0.82	3.85 ± 1.27	14.86 ± 5.94	7.11 ± 1.44	0.77 ± 0.31	2.94 ± 0.58	6.53 ± 1.63	37.44 ± 5.38	5.45 ± 1.34	6.15 ± 2.11	21.95 ± 5.51	55.08 ± 16.17	12.24 ± 1.81	29.43 ± 7.18	5.71 ± 1.08	24.00 ± 7.69	0.34 ± 0.13	3.30 ± 0.83	4.19 ± 1.36	18.97 ± 5.45	-	7.35 ± 1.67
Neanurinae sp. 1	-	-	-	-	-	-	-	-	11.92 ± 3.27	12.05 ± 4.82	4.38 ± 1.10	-	6.20 ± 1.59	-	-	5.72 ± 2.02	-	14.96 ± 5.99	0.67 ± 0.27	2.57 ± 0.97	1.38 ± 0.33	13.49 ± 3.46	0.27 ± 0.11	0.90 ± 0.36
Paranura colorata Mills, 1934	2.65 ± 1.06	0.87 ± 0.35	-	2.57 ± 0.44	7.59 ± 1.27	-	-	0.78 ± 0.31	-	1.77 ± 0.71	-	-	-	-	-	2.70 ± 0.68	0.93 ± 0.37	0.28 ± 0.11	0.75 ± 0.19	5.13 ± 1.94	3.20 ± 0.84	1.83 ± 0.73	0.75 ± 0.19	1.76 ± 0.70
Isotomidae																								
Argenia cyanura Fjellberg, 1986	-	3.76 ± 1.50	-	1.41 ± 0.35	55.18 ± 16.45	13.54 ± 3.34	10.25 ± 2.52	48.29 ± 13.98	42.49 ± 12.16	15.65 ± 3.09	1.89 ± 0.76	14.36 ± 2.95	1.21 ± 0.48	6.22 ± 1.05	21.74 ± 5.79	40.13 ± 11.67	2.13 ± 0.85	22.31 ± 5.91	1.28 ± 0.15	16.67 ± 4.50	3.50 ± 0.97	30.30 ± 5.62	8.53 ± 2.64	12.14 ± 3.41
Folsomia penicula Bagnall, 1939	437.08 ± 91.40	170.91 ± 56.51	66.80 ± 12.73	93.10 ± 14.26	363.38 ± 24.38	109.12 ± 14.21	101.09 ± 9.52	270.97 ± 49.47	839.67 ± 92.37	674.41 ± 86.96	408.65 ± 50.77	451.86 ± 105.38	842.10 ± 110.51	234.17 ± 37.31	569.61 ± 104.95	417.06 ± 41.39	75.03 ± 5.00	322.26 ± 68.73	35.15 ± 4.24	355.94 ± 73.54	229.61 ± 51.94	568.61 ± 143.04	62.12 ± 2.89	88.68 ± 13.05
Folsomina sp. 1	2.87 ± 0.75	0.29 ± 0.12	12.54 ± 3.09	-	-	-	2.72 ± 1.09	21.63 ± 7.20	7.88 ± 2.98	5.54 ± 1.50	7.37 ± 2.95	0.95 ± 0.38	-	-	-	6.22 ± 1.01	-	8.93 ± 2.08	0.52 ± 0.14	-	-	-	-	-

Isotomid sp. 1	-	0.29 ± 0.12	-	1.51 ± 0.42	2.97 ± 1.19	-	-	-	-	-	-	-	-	3.31 ± 1.32	-	1.50 ± 0.60	3.97 ± 1.59	0.74 ± 0.30	-	-	-	-	-	-
Isotomid sp. 2	-	1.04 ± 0.42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isotomid sp. 3	4.06 ± 1.62	-	-	-	2.97 ± 1.19	-	-	-	-	-	-	-	-	-	1.17 ± 0.31	1.50 ± 0.60	-	-	-	-	-	-	-	-
Isotomid sp. 4	-	-	-	-	-	0.93 ± 0.37	-	1.56 ± 0.62	-	-	-	-	-	1.65 ± 0.66	-	-	-	-	-	-	-	-	-	-
Isotomid sp. 5	-	-	-	-	-	-	-	-	3.63 ± 1.45	-	-	1.11 ± 0.44	-	-	-	4.50 ± 1.80	-	-	-	-	-	8.94 ± 2.75	-	-
Isotomid sp. 6	-	-	-	-	-	-	-	-	30.87 ± 10.38	9.04 ± 3.62	-	-	24.24 ± 7.35	-	6.49 ± 1.46	3.37 ± 1.16	-	-	-	-	-	-	-	-
Isotomiella minor (Schäffer), 1896	48.14 ± 8.66	13.04 ± 2.47	20.13 ± 6.02	21.50 ± 4.78	64.99 ± 6.88	22.40 ± 3.52	9.29 ± 2.12	169.73 ± 52.76	94.52 ± 14.28	58.01 ± 9.95	11.46 ± 2.67	35.88 ± 7.83	167.08 ± 51.67	34.91 ± 4.03	61.52 ± 12.50	74.83 ± 14.72	10.90 ± 2.57	46.31 ± 15.10	3.27 ± 0.48	18.49 ± 2.78	10.81 ± 3.44	17.57 ± 1.28	5.22 ± 1.25	11.72 ± 1.55
Onychiuridae																								
Onychiurus (A.) absoloni (Börner), 1981	23.60 ± 4.31	2.62 ± 0.53	9.78 ± 3.32	10.70 ± 1.90	33.47 ± 5.37	23.72 ± 3.12	21.12 ± 4.08	84.29 ± 21.37	147.66 ± 27.14	85.69 ± 25.61	38.50 ± 6.62	32.61 ± 6.73	102.24 ± 5.71	66.10 ± 8.23	45.19 ± 7.94	207.48 ± 44.15	4.74 ± 0.73	52.17 ± 13.24	6.45 ± 0.99	90.26 ± 25.46	13.10 ± 1.16	69.86 ± 12.47	11.73 ± 2.61	24.44 ± 2.85
Tullbergia (M.) iowensis Mills, 1932	3.13 ± 1.25	6.35 ± 1.41	9.59 ± 3.59	6.11 ± 1.77	43.98 ± 5.83	5.96 ± 1.58	4.98 ± 0.95	95.47 ± 36.75	31.47 ± 8.11	80.33 ± 19.72	44.55 ± 4.89	43.96 ± 9.57	43.53 ± 5.51	34.91 ± 3.89	36.57 ± 10.05	71.68 ± 11.53	9.38 ± 1.70	50.98 ± 13.90	6.85 ± 1.14	26.78 ± 5.40	6.75 ± 0.94	96.72 ± 22.58	4.32 ± 0.75	38.53 ± 8.97
Willemia sp.	2.09 ± 0.84	0.42 ± 0.17	3.13 ± 0.89	2.17 ± 0.87				0.78 ± 0.31	2.76 ± 1.11	21.94 ± 4.12	7.49 ± 1.47	7.22 ± 2.89	6.96 ± 1.87	3.29 ± 1.32	1.81 ± 0.72	5.02 ± 1.12	0.47 ± 0.19	14.46 ± 5.66	-	1.47 ± 0.53	-	0.64 ± 0.26	-	-
Paronellidae																								
Salina sp. 1	-	2.60 ± 1.04	-	-	5.71 ± 1.73	1.23 ± 0.49	1.49 ± 0.47	0.65 ± 0.26	-	-	-	-	-	-	-	-	-	4.99 ± 2.00	-	0.22 ± 0.09	0.15 ± 0.06	-	1.06 ± 0.42	7.53 ± 1.03
Sminthuridae																								
Arrhopalites obtusus	-	0.29 ±	2.27 ±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.28 ±	2.21 ±

Zeppelini & Christiansen, 2003		0.12	0.91																			0.11	0.68
Arrhopalites sp. 1	4.06 ± 1.62	2.03 ± 0.51	-	-	4.04 ± 1.16	0.44 ± 0.17	-	9.91 ± 3.65	-	-	-	-	3.31 ± 1.32	-	-	1.32 ± 0.53	-	-	2.45 ± 0.98	0.61 ± 0.25	3.65 ± 1.46	1.63 ± 0.41	-
Collophura quadrioculata (Denis), 1933	4.06 ± 1.62	0.29 ± 0.12	0.85 ± 0.34	-	-	-	-	-	-	-	-	-	-	-	-	0.47 ± 0.19	-	-	1.95 ± 0.78	-	-	0.55 ± 0.13	3.70 ± 1.39
Sminthurus (S.) *medialis* Mills, 1934	-	0.29 ± 0.12	2.27 ± 0.91	-	2.97 ± 1.19	-	-	-	-	-	-	-	3.28 ± 0.80	-	-	-	-	-	-	-	-	-	1.76 ± 0.70
Sminthurides (S.) *violaceus* Reuter, 1881	-	-	-	2.76 ± 0.84	-	-	2.72 ± 1.09	9.26 ± 3.70	-	-	1.15 ± 0.46	-	-	-	-	-	7.35 ± 2.82	0.43 ± 0.17	7.36 ± 2.94	0.81 ± 0.15	-	0.47 ± 0.19	1.76 ± 0.70
Tomoceridae																							
Tomocerus (P.) *flavescens* Tullberg, 1871	1.04 ± 0.42	-	-	-	-	1.92 ± 0.77	-	0.73 ± 0.29	-	-	1.15 ± 0.46	-	-	-	-	-	-	-	-	-	-	-	-
Tomocerus sp. 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.28 ± 0.11	3.55 ± 1.42	-	0.56 ± 0.14	-	-	-	-

Curriculum Vitae

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