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Graduate Program in Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

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INTERACTIONS AMONG BIOTIC AND ABIOTIC CONTROLS OF CARBON DYNAMICS IN A GLOBAL CHANGE FIELD EXPERIMENT

(Thesis format: Integrated Article)

by

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Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

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Abstract

Climate warming and increased atmospheric nitrogen deposition may substantially influence biosphere C cycling over the next century by altering ecosystem processes such as productivity and decomposition. Field studies are commonly used to explore plant responses to global change, although the underlying mechanisms can be difficult to isolate owing to the lack of control of factors such as plant-animal interactions. Ultimately, indirect effects via herbivore and detritivore responses may feedback to influence plant responses to the experimental treatments. The goal of this thesis was to explore interactions among biotic and abiotic drivers of carbon dynamics within the context of experimental warming and nitrogen addition in the field.

Evidence from a herbivore exclusion experiment revealed that mollusc effects on net primary productivity were more pronounced in warmed plots than in ambient temperature plots, likely as a result of temperature-related increases in mollusc metabolic activity and plant consumption rate. Furthermore, the effects of rodent exclusion on grass biomass were significantly greater in N-fertilized plots than non-fertilized plots. Feeding experiments suggested that this finding is likely attributed to increased grass palatability in response to N addition.

Results from a litter decomposition experiment indicated that warming impeded the contribution of detritivores to carbon turnover, though this effect was transient. Increased precipitation over the course of the experiment may have promoted recovery of the detritivore community, and could also account for the significant detritivore effects observed following one year of incubation. Reciprocal litter transplants between the treatments plots and untreated areas of the field indicated that the observed responses from the main experiment were unlikely to be accounted for by the individual effects of litter quality or microenvironment alone.

Overall, the influence of global change factors such as warming and increased atmospheric nitrogen deposition on carbon-related processes such as productivity can be substantially modified by indirect effects on herbivore dynamics, with consumer-specific treatment effects suggesting that this relationship is complex and can depend on both diet quality and microclimate. Though detritivores were relatively insensitive to warming and N addition as decomposition progressed, they likely play an important role in the overall magnitude of ecosystem C turnover.

Keywords

Global change, warming, N deposition, field experiment, *Poa pratensis*, *Bromus inermis*, plant, biomass, herbivore, rodent, *Microtus pennsylvanicus*, *Deroceras reticulatum*, soil, moisture, litter, detritivore, decomposition, mass, nitrogen, tissue quality, plot, microenvironment, community, ecosystem

Co-Authorship Statement

A version of Chapter 2 was published in the journal *Oikos* with Hugh Henry as co-author. Dr. Henry contributed to the writing of the manuscript.

A version of Chapter 3 was published in the journal *Oecologia* with Hugh Henry as a co-author. Dr. Henry contributed to the study design and data analysis, provided equipment and funding, and contributed editorial comments to the manuscript.

A version of Chapter 4 has been submitted for publication in the *Journal of Mammalogy* with Hugh Henry as co-author. Dr. Henry contributed to the study design and data analysis, provided equipment and funding, and contributed editorial comments to the manuscript.

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List of Abbreviations

C: Carbon

CH_4: Methane

CO_2: Carbon Dioxide

DOC: Dissolved Organic Carbon

DIC: Dissolved Inorganic Carbon

EEA: Extracellular Enzyme Activity

N: Nitrogen

N_2O: Nitrous Oxide

SOM: Soil Organic Matter

VOC: Volatile Organic Compounds

Chapter 1

1 General Introduction

Terrestrial ecosystem dynamics are largely regulated by the biogeochemical cycling of carbon (Schimel 1995). The net effects of carbon sequestration and respiration at the ecosystem level result from complex multi-trophic interactions within the animal-plant-soil continuum (Fig. 1.1). The factors often assumed to be most central to these interactions are climate and plant quality (Swift et al. 1979, Forchhammer et al. 2005, Stiling and Moon 2005), which in turn are subject to drivers of global change such as warming and nitrogen deposition (IPCC 2007, Xia and Wan 2008). Although previous research has also addressed the consequences of environmental change for animal (Bale et al. 2002), and soil (Mosier 1998) dynamics, it remains unclear how biotic and abiotic controls of carbon dynamics interact within the context of global change field experiments.

1.1 Plant-ecosystem carbon relations

1.1.1 Carbon regulation of plant fitness and community structure

Plant-C relations exist as a series of carbon gains and losses resulting from photosynthesis and the combination of intrinsic plant carbon requirements for processes such as maintenance, growth and reproduction, coupled with the influence of interacting environmental factors including climate, soil nutrient availability, herbivores and other biota (Pearcy et al. 1987). Carbon acquired through photosynthetic assimilation may be subsequently respired in response to plant energy demand, stored as sugars, structural carbohydrates, and other C-based compounds, utilized for phytomass synthesis, or exported from the plant as root exudates and volatile organic compounds (Garnier 1991, Kesselmeier and Staudt 1999, Kuzyakov and Domanski 2000). Ultimately, allocation patterns are suggested to maximize plant fitness (DeAngelis et al. 2012), such as carbon reinvestment in the development of new leaf tissue for photosynthesis, as well as C allocation to root growth for structural support, and nutrient and water acquisition. From a fitness perspective, plant survival and reproductive output are highly correlated with net productivity gains (Bazzaz et al. 1987), while the outcome of plant competitive interactions contributes substantially to plant community structure (Goldberg and Barton 1992). Extrapolating to the ecosystem level, interspecific variation in plant community carbon relations has a significant impact on broad-scale patterns of biosphere carbon cycling and sequestration (Chapin 2003).

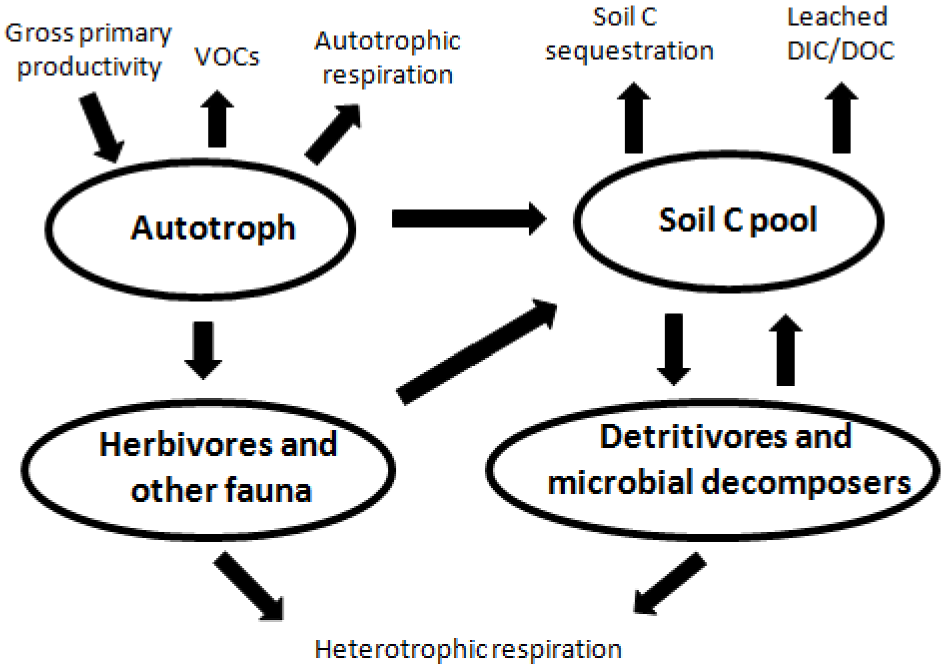

Figure 1.1. Major ecological processes involved in the transfer of carbon within a terrestrial ecosystem. Modified from Chapin et al. (2009). *VOC* – Volatile Organic Compounds, *DIC* – Dissolved Inorganic Compounds, *DOC* – Dissolved Organic Compounds

1.1.2 Heterotrophic influences on plant carbon

As the result of millions of years of coevolution, plant-carbon dynamics have been substantially influenced by interactions with heterotrophic organisms (Berenbaum 1983, Thompson 2009), with consequences ranging from individual plant behaviours (Karban 2008) to the structuring of entire plant communities (Becerra 2007). For instance, mycorrhizal fungi stimulate costly plant production and the release of C-based root exudates into the soil (Kuzyakov and Domanski 2000) although, in exchange, the fungi serve to greatly enhance plant acquisition of water and nutrients from the soil (Narula et al. 2009). Likewise, pollinators are often rewarded with nectar or other carbon-based resources, and the chemical attraction of insects to host plants is often attributed to the odour of carbon volatiles (Raguso 2004, Kessler and Baldwin 2011). In contrast to these mutualistic associations, phytophagy confers costly shifts in C allocation to plant maintenance (Holland et al. 1996) as well as the production of defense compounds (Arnold and Schultz 2002), often resulting in an overall reduction in plant fitness (Louda 1983, Whitham and Mopper 1985). However, low levels of herbivory may also benefit host plants by the stimulation of nutrient cycling and plant growth (Belovsky and Slade 2000).

While global estimates of herbivory indicate that much less plant tissue is consumed than what is available (Roy et al. 2001), indirect herbivore effects contribute substantially to ecosystem carbon dynamics. Regardless of the tissue quantity removed, folivores often prefer young, highly nutritious plant leaves (Hanley et al. 1995), and concentrating feeding activity on seedlings not only increases the likelihood of plant mortality, but even sub-lethal consumption preempts future production of photosynthetic plant tissue (Marquis 1992, Peters 2007). Poor quality resources generally avoided by herbivores are often characterized by low tissue digestibility, and by low decomposability due to their reduced N content and high lignin concentration (Meentemeyer 1978, Mattson 1980). Conversely, palatable plants generally exhibit high litter turnover rates (Grime et al. 1996). Together, these relationships suggest that the effects of herbivore diet preference may lead to plant community dominance by low quality, slow decomposing plant species, the consequences of which may strongly influence ecosystem C cycling (Pastor and Naiman 1992). Other important determinants of plant palatability include carbon-based defenses such as phenolics (Bennett and Wallsgrove 1994), the evolution of which has been greatly influenced by the history of interactions with primary consumers (Roy et al. 2001).

1.1.3 Plant carbon as detritus

Soil C sequestration plays a crucial role in biosphere C dynamics, and more than 80% of terrestrial carbon is stored in the form of highly stable soil organic matter (SOM; IPCC 2007), which consists of plant, animal, and microbial residues in various stages of decay. At an estimated 1500 Pg (1Pg = 10^{15} g) of carbon, the terrestrial stock accounts for approximately twice the atmospheric C pool (Trumbore 2000). Of all carbon sources, senesced plant material represents the largest contribution to SOM formation (Schimel 1995, Kalbitz et al. 2000), suggesting that litter turnover is an integral component of ecosystem carbon storage. Furthermore, over short time scales the majority of soil-respired carbon is derived from young SOM pools (Trumbore 2000), largely because soil heterotrophs are highly sensitive to new litter inputs (Bowden et al. 1993). Plant decomposition dynamics can also influence ecosystem productivity, because litter decay is associated with nitrogen mobilization (McGill and Cole 1981), while physical and chemical properties of the litter layer may suppress the establishment of new seedlings (Bosy and Reader 1995) and influence overall plant community structure (Facelli and Pickett 1991).

The decomposition of plant material is predominantly controlled by climate, tissue chemistry, and biotic activity (Swift et al. 1979, Aerts 1997). Assuming substrate supply and enzyme activity are not limiting, the rate of litter decomposition is positively correlated with temperature (Meentemeyer 1978), while plant secondary metabolites such as tannins promote carbon sequestration by forming recalcitrant products during the process of decay (Horner et al. 1988). Of the interacting biota, saprophytic microorganisms exhibit the greatest influence over litter turnover, because they are able to produce enzymes which break down components of plant material, such as lignin, that are resistant to decomposition (Masai et al. 2007). In turn, limits to decomposer activity and hence carbon cycling include litter quality, as well as soil temperature and moisture (Schlesinger and Andrews 2000). In addition to microbes, soil fauna also contribute significantly to litter breakdown, both directly through the consumption and fractionation of detritus, and indirectly through litter-soil mixing as well as through the consumption of microbial saprobes (Swift et al. 1979, Wall et al. 2008). In general, detritivores play an important role in plant decomposition and their removal has significant consequences for litter turnover and nutrient cycling (Seastedt 1984, Wall et al. 2008). As with their microbial counterparts, soil fauna are sensitive to environmental conditions such as soil temperature and moisture (Didden 1993, Simpson et al. 2012), with strong regional and seasonal variation in detritivore community composition (Levings and Windsor 1996, Ekschmitt et al. 2003).

1.2 Climate warming and ecosystem carbon cycling

1.2.1 Warming and plant community dynamics

Anthropogenic global change is expected to dramatically alter terrestrial ecosystem dynamics over the next century (Fig. 1.2). As a consequence of increasing atmospheric CO_2 concentration, global mean surface air temperatures are expected to increase 1.4 to 5°C by 2100 (Houghton et al. 2001). Already climate warming is causing shifts in plant phenology and range distributions at a global scale (Walther et al. 2002), which may have significant consequences for ecosystem structure and productivity (Van der Putten 2012). At the individual plant level, warming can enhance the production of proteins associated with plant development and phytomass synthesis (Swarbreck et al. 2011), as well as influence reproductive effort (Liu et al. 2012), root mortality (Wan et al. 2004), and leaf chemistry (Sardans et al. 2012). Likewise, elevated levels of evapotranspiration in response to increasing temperatures can decrease plant water use efficiency, as well as increase overall plant water stress (Allen et al. 2003), which in turn can influence biodiversity (Thomas et al. 2004) and ecosystem productivity (Ciais et al. 2005). Furthermore, increased variability in precipitation patterns (both timing and amount) will be an important component of climate change (Weltzin et al. 2003), suggesting that in some regions the negative effects of warming on plant water relations may be further exacerbated by interactions with drought (Luo et al. 2008). Ultimately, interspecific variation in plant responses to warming will contribute substantially to shifts in plant community species composition (e.g. Hoeppner and Dukes 2012), and overall ecosystem dynamics may be particularly sensitive to changes at the functional group level. For example, effects of warming on photosynthetic carbon assimilation differ between C3 and C4 plants species (Sage and Kubien 2007), thus ecosystem consequences may be further pronounced due to shifts in their relative persistence under climate change (Collatz et al. 1998).

Warming may further alter the temporal dynamics of ecosystem carbon cycling by influencing processes associated with plant phenology (Menzel et al. 2006). One of the primary concerns related to climate change is the possible asynchrony in species interactions (Tylianakis et al. 2008, Yang and Rudolf 2010), the potential consequences of which include plant species extinctions resulting from failed plant-pollinator associations (Memmott et al. 2007). Alternatively, advancement of the growing season may function to alleviate herbivory pressure from consumers such as migrating caribou, which are unable to track temporal shifts in resource availability (Post and Forchhammer 2008). Other phenological consequences of warming include changes to the overall length of the growing season, primarily due to the importance of temperature cues for plant cold acclimation and de-acclimation processes in fall and spring, respectively (Kalberer et al. 2006). In some regions, earlier cold de-acclimation in response to warming may result in greater ecosystem productivity (Rammig et al. 2010). However, the influence of warming on acclimation dynamics may vary as some plant species are more sensitive to changes in photoperiod (Kalberer et al. 2006), suggesting that interspecific differences in acclimation responses to changing temperatures may lead to shifts in ecosystem structure. Warming may also influence plant phenology indirectly due to earlier snow melt, which can also impact plant production (Myneni et al. 1997). However, seasonal temperature effects are likely to vary regionally, because ecosystem responses to warming over winter may negatively impact plant communities in colder climates (Kreyling 2010). Winter warming can increase the occurrence of transient mid-winter thaw events that remove the protective thermal insulation provided by snow cover, resulting in plant freezing damage and reduced productivity (Bokhorst et al. 2011).

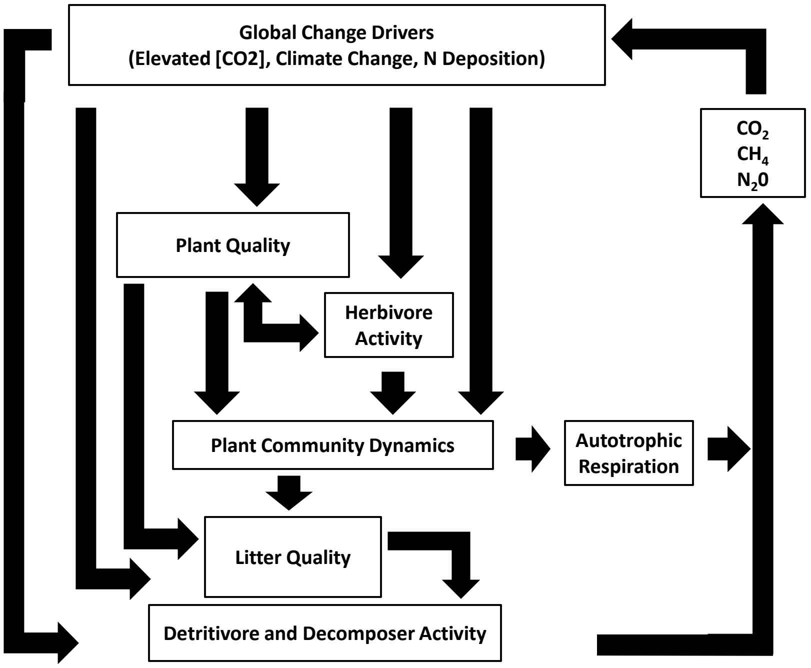

Figure 1.2. Overview of ecosystem parameters subject to the effects of global change. Modified from Ostle et al. (2009).

1.2.2 Global warming and C turnover

Temperature is an important component of both plant litter decomposition and SOM cycling dynamics (Post et al. 1982, Coûteaux et al. 1995), and consequently global warming may significantly increase ecosystem carbon respiration (Jones et al. 2005, Wu et al. 2011). One of the main concerns regarding rising temperatures is the possibility of a carbon-cycle climate feedback, whereby increased respiration further exacerbates the magnitude of climate warming (Fig. 1.2; Cox et al. 2000). This is particularly pertinent in northern ecosystems, where the melting of permafrost may release substantial quantities of carbon in various forms, causing a runaway greenhouse effect (Serreze and Francis 2006). Even in milder climates, where extensive soil carbon stocks form relatively stable C pools that exhibit turnover rates on the scale of centuries to millennia (Trumbore et al. 1990), this older, more recalcitrant carbon may be strongly affected by warming, because the temperature sensitivity of C turnover increases in response to declining substrate quality (Davidson and Janssens 2006, Conant et al. 2008). However, C efflux responses to warming might only be transient, and soil respiration thermal acclimation has been reported (Bradford et al. 2010), likely due to physiological changes such as reduced carbon use efficiency in the decomposer community (Tucker et al. 2013). Furthermore, the magnitude of the respiration response may depend on interactions of warming with other drivers of global change such as drought, which can negate the effects of increased temperature on soil C efflux (Schindlbacher et al. 2012).

Ecosystem processes such as soil respiration and biogeochemical nutrient cycling are largely determined by microbial decomposition of plant litter (Aerts 1997). Warming may significantly influence litter turnover (Shaw and Harte 2001) and microbial responses to increased temperature are, therefore, an important component of predicting future changes in carbon dynamics. Community responses to increased temperature include changes to microbial biomass C and N (Belay-Tedla et al. 2009, Bell et al. 2010), as well as community composition (Zhang et al. 2005). Functionally, climate change may have a significant influence on microbial extracellular enzyme activity, or EEA (Henry 2012). German *et al.* (2012) found that the reaction rates of numerous extracellular enzymes directly responsible for the breakdown of SOM including cellobiohydrolase, beta-glucosidase, beta-xylosidase, α-glucosidase, and N-acetyl-beta-d-glucosaminidase, varied in response to warming, although other field studies have reported no *in situ* temperature effects on either oxidative or hydrolytic EEA (Bell et al. 2010, Kardol et al. 2010). Regardless, functional shifts in enzyme activity in response to direct warming effects may result from the temperature sensitivity of enzymatic reaction rates (Aerts 2006), as well as from changes in microbial enzyme production (Cusack et al. 2010). Indirectly, decomposition dynamics may vary with respect to the thermal kinetics of available carbon substrates, because the temperature sensitivities of the latter are related to substrate molecular complexity (Davidson and Janssens 2006). Furthermore, initial litter substrate properties including overall lability may be related to changes in tissue chemistry in response to warming during the plant growth phase (Sardans et al. 2012).

1.3 Nitrogen deposition and ecosystem carbon cycling

1.3.1 Plants and N availability

Nitrogen limitation contributes substantially to the restriction of plant growth and reproduction on a global scale (White 1993). Although nitrogen represents the most dominant component of the atmosphere, it exists predominantly (>99%) in the form of an inert dinitrogen gas, thus remaining inaccessible to most biota (Sprent 1987). The limited quantities of atmospheric nitrogen that get naturally incorporated into the biosphere result predominantly from the activities of N-fixing microorganisms (Schlesinger 1991), although both lightning (Soderland and Rosswall 1982) and rock weathering (Morford et al. 2011) also contribute. In turn, nitrogen recycling through the ecosystem depends on microbial mineralization of N-bound substrates (Booth et al. 2005), or alternatively direct uptake of dissolved organic N by plants (Näsholm et al. 2009). Plant nitrogen demand is primarily related to the requirements of photosynthetic machinery such as Ribulose-1,5-bisphosphate carboxylase oxygenase, or Rubisco, the enzyme most directly responsible for the carboxylation process in plant CO_2 fixation (Chapin et al. 1987). At the level of an individual leaf, rates of carbon assimilation are positively related to nitrogen content, as is leaf chlorophyll content (Evans 1983). Nitrogen availability also has important implications for both plant vegetative and reproductive development (Nightingale 1948).

Atmospheric nitrogen deposition is predicted to increase substantially over the next century in many regions (Galloway et al. 2004). Owing to fertilizer use, N-emissions from internal combustion engines, and the cultivation of leguminous crops, anthropogenic nitrogen fixation currently exceeds N fixation by all natural sources combined (Vitousek 1994), and this manipulation of the nitrogen cycle will have significant consequences for ecosystem dynamics (Vitousek et al. 1997). Nitrogen fertilization can influence plant tissue chemistry (Johnson et al. 2001), transpiration rate (Bowman et al. 1995), N use efficiency (Heskel et al. 2012), and patterns of resource allocation (Tilman and Wedin 1991). Likewise, increased N availability can increase both photosynthetic activity and biomass accumulation (Brix 1971, Baddeley et al. 1994, Xia and Wan 2008), which extrapolated to the level of plant communities often leads to increased ecosystem productivity (Lebauer and Treseder 2008). However, productivity gains may prove to be only short-term, because chronic N enrichment has the capacity to shift plant growth limitation from nitrogen to phosphorus supply (Phoenix et al. 2003). Additionally, plant responses to nitrogen availability can vary by species, with interspecific differences in N effects leading to changes in plant competitive interactions and species diversity (Liira et al. 2012). As with consequences of global warming, effects of N deposition can manifest at the level of functional groups, and C3 plants may gain competitive superiority over C4 plants (Tilman and Lehman 2001) owing to the decline in the advantage of high nitrogen use efficiency that benefits C4 plants under N-limiting conditions (Tilman 1990). While nitrogen addition may simply shift community dominance to a small number of N-demanding species (Silvertown 1980), more extreme whole-ecosystem consequences include the conversion of nutrient-poor heathlands to relatively nutrient-rich grasslands (Aerts and Berendse 1988). Furthermore, N deposition can increase ecosystem invasibility by exotic flora (Dukes and Mooney 1999), the establishment of which can substantially alter ecosystem structure and function (Levine et al. 2003, Hooper et al. 2005). The ultimate consequences of these changes to species composition and ecosystem biodiversity include dramatic alterations to both hydrological and nutrient (C and N) cycling dynamics (Liao et al. 2008, Ehrenfeld 2010), as well as overall ecosystem productivity (Hooper et al. 2005).

1.3.2 Effects of N deposition on plant decomposition

Shifts in ecosystem litter decomposition dynamics will also result from both direct and indirect effects of atmospheric N deposition (Manning et al. 2008). After climate effects, substrate quality represents the most influential component of plant litter decay (Swift et al. 1979, Coûteaux et al. 1995). Initial litter C/N and lignin/N content are suggested to influence plant decomposition rates, although the relative importance of each component may vary as decay progresses (Berg 1986, Taylor et al. 1989). Nitrogen fertilization can significantly alter these ratios (Baron et al. 2000), as well as overall plant N content (Xia and Wan 2008), although the resultant rates of litter turnover may increase, decrease, or remain unchanged (Knorr et al. 2005). The direction of this response likely varies with respect to the stage of litter decomposition, because N addition can impede the turnover of lignin-dense litter fractions typical of advanced substrate decay (Fog 1988).

Plant decomposition is highly dependent on soil microorganisms (Swift et al. 1979), the activities of which may be strongly regulated by N availability (Craine et al. 2007) suggesting that changes in microbial dynamics are a critical component of ecosystem responses to N deposition. In general, microbes respond positively to increased substrate N concentration, resulting in increased litter turnover (Melillo et al. 1982, Hobbie 2005). However, N deposition also imposes direct effects on decomposers, shifting soil parameters such as microbial biomass and community composition (Compton et al. 2004, Frey et al. 2004, Treseder 2008). Functionally, N addition can increase the production and activity of microbial extracellular enzymes, such as cellulases, which are responsible for the breakdown of labile carbon litter fractions (Zeglin et al. 2007). Conversely, N fertilization impedes the production of oxidases, which function to decompose resistant substrate components such as lignin (Sinsabaugh et al. 2002). Together, these relationships may represent the mechanisms responsible for the variable effects of nitrogen enrichment on patterns of decomposition (Carreiro et al. 2000).

From an ecosystem perspective, N deposition can have broadscale effects on C respiration and soil nutrient dynamics (Phoenix et al. 2012). Although nitrogen enrichment may increase soil respiration under certain scenarios (Gallardo and

Schlesinger 1994), it is likely to decrease C efflux for most ecosystems (Janssens et al. 2010, Ramirez et al. 2012), partially due to the stabilization of old soil carbon pools in response to N addition (Neff et al. 2002). Conversely, N mineralization tends to increase under nitrogen addition, with concomitant decreases in overall soil C/N (Nave et al. 2009). Likewise, increased N availability can increase efflux of nitrogen gases such as N_2O (Magill et al. 1997). Indirectly, nitrogen deposition can influence decomposition dynamics through modification of plant community structure, thus altering the quality of litter entering the detrital system (Suding et al. 2005, Vinton and Goergen 2006).

1.4 Herbivore and detritivore responses to global change

1.4.1 Global Warming

Historical records suggest that global warming can substantially alter plant-herbivore dynamics (Wilf and Labandeira 1999). Although herbivores may respond to increased temperature indirectly through changes in plant phenology (Dewar and Watt 1992, van Asch and Visser 2007), nutrient stoichiometry (Tan et al. 1978, Thorvaldsson et al. 2007) or secondary defense chemistry (Zvereva and Kozlov 2006), direct effects of global warming are likely to have equally important consequences for primary consumers, particularly insects (Fig. 1.2; Bale et al. 2002, Robinet and Roques 2010). Temperature has a strong influence on insect life history traits, including growth rate (Tauber et al. 1986), diapause (Hodek and Hodkova 1988), and voltinism (Altermatt 2010). Many studies have reported increased insect abundances in response to increased temperature (reviewed in Bale *et al.* 2002), although Adler et al. (2007) reported negative effects of warming on the population growth rate of aphids. Likewise, among other invertebrates, moisture-sensitive terrestrial molluscs are susceptible to desiccation stress (Carne-Cavagnaro et al. 2006). Ultimately, the nature of these relationships depends on species-specific thermal optima (Rouault et al. 2006). General patterns of invertebrate herbivore fitness responses to warming suggest positive effects increase with latitude, and are related to temperature regulation of ectothermic metabolism (Deutsch et al. 2008).

Ecosystem consequences of herbivore responses to warming may include significant shifts in both plant community structure (Woodward 1992) and food-web dynamics

(Petchey et al. 1999). These changes may be related to greater feeding pressure resulting from increases in herbivore population densities (Cannon 1998, Coley 1998), the effects of which may be further amplified by a decreased occurrence of herbivore pathogen infection (Stireman et al. 2005). Shifts in plant/herbivore dynamics are likely to be the most extreme in polar regions, where climate warming is predicted to be more substantial than in tropical and temperate zones (Hodkinson et al. 1998). Likewise, warming may disproportionately benefit insect species inhabiting colder climates in general, because increased temperatures could mitigate the high overwintering mortality typically experienced in these regions, although this effect may be offset by the loss of protective snow cover (Bale and Hayward 2010). Negative consequences of warmer temperatures on overwintering insects include the depletion of both energy and water stores (Williams et al. 2012). Likewise, warming over summer may stress insects by exposing them to temperatures above their upper thermal limits (Calosi et al. 2008). Assuming overall net effects of warming on insect fitness are positive however, geographic range expansion of insect pests may occur as a result of increased overwintering survival and increased population densities coupled with increased invasibility of regions that have traditionally been too cool for successful pest establishment (Wallner 1987, Battisti et al. 2005). Moreover, the risk of pest outbreaks resulting from these novel plant-herbivore interactions is of major concern in both natural (Ayres and Lombardero 2000) and agricultural (Cannon 1998) ecosystems, and may have significant ecological and economic consequences (Ayres and Lombardero 2000).

Detritivore responses will also contribute to shifts in ecosystem dynamics under global warming (Lavelle et al. 1997). Increased temperatures can impose severe climatic stress on soil organisms (Pritchard 2011), reducing detritivore abundance, biomass and speices richness (Briones et al. 1997, Xu et al. 2012). Soil microclimate has an important influence on detritivore responses, and negative effects of warming are most pronounced in xeric systems (Blankinship et al. 2011). Interannual climate variation may also play a role – e.g. Harte *et al.* (1996) reported that warming increased mesofaunal biomass and diversity in moist soils, but the opposite occurred when soils were dry. Because wet soils are generally able to support large populations of soil fauna (Wardle 2002), important interactions are likely to occur between changes in global temperatures and precipitation patterns. Where moisture is not limiting, increases in both the abundance and overall activity of detritivores in response to warming may significantly influence ecosystem carbon cycling as a result of changes in litter processing dynamics (Cole et al. 2002). Like herbivores, detritivore responses to warming in cold biomes may be of particular importance to ecosystem dynamics, because litter turnover within these regions is typically a slow process (Aerts 2006). Furthermore, increased temperatures may expand detritivore ranges, which in turn can have important consequences for broadscale patterns of decomposition and C cycling (Van Geffen et al. 2011).

1.4.2 Nitrogen Deposition

Herbivore feeding dynamics are largely determined by the quality of plant resources (Moran and Hamilton 1980, Owen-Smith and Novellie 1982, Raupp and Denno 1983). Although optimal foraging theory suggests that energy intake is the most influential factor governing forage selection (Macarthur and Pianka 1966, Charnov 1976), herbivores may be equally sensitive to differences in plant N content (White 1993). Herbivore nitrogen demand is driven primarily by a stoichiometric mismatch between primary producers and their respective consumers, because phytomass is largely composed of N-free structural carbohydrates such as cellulose, resulting in much larger C/N ratios (e.g. 20:1 in herbaceous angiosperms) relative to those of animals such as mammals and insects (7:1 and 4:1, respectively; Reiners 1986). Herbivore nitrogen requirements are further exaggerated during breeding periods (Awmack and Leather 2002), and the inability to synthesize essential amino acids necessitates their dependence on plant-based sources (Dadd 1973).

Anthropogenic nitrogen deposition has the potential to strongly influence plant-herbivore interactions on a global scale (Fig. 1.2; Tylianakis et al. 2008). For instance, nitrogen fertilization can significantly increase diet palatability to herbivores (Hartley et al. 1995, Strengbom et al. 2003). Positive effects of high N diets include increased herbivore body mass, reduced development time, and increased reproductive output (White 1978, Cameron and Eshelman 1996). Although herbivore responses to increased N availability are often attributed to the effects of higher nutrient content, they may also reflect reductions in C-based plant defenses (Coley et al. 1985, Throop and Lerdau 2004). In addition to dietary implications, nitrogen enrichment can influence resource patch use by herbivores, including increased activity in response to increased vegetation N content (Bakker et al. 2005), or conversely decreased activity should fertilization reduce patch biodiversity (Hall et al. 1991). Likewise, an increase in patch biomass in response to N addition can influence habitat use, because some herbivores depend on dense vegetation for refuge from predation (Mossman 1955).

Detritivores contribute substantially to the processes of plant decomposition (Swift et al. 1979) and terrestrial C cycling (Osler and Sommerkorn 2007), so their responses to N deposition therefore have large consequences for ecosystem carbon turnover (Fig. 1.2; Blankinship et al. 2011). Litter quality is an important component of detritivore dynamics, particularly with respect to tissue N content (Hendriksen 1990, Hättenschwiler and Bretscher 2001). Increased leaf nitrogen concentration in response to N fertilization can be retained post-senescence (Henry et al. 2005), indicating that changes in plant chemistry under N deposition are likely to have important consequences for detritivore activity. Additionally, the influence of nitrogen enrichment on the soil microenvironment can have direct consequences for soil fauna, including the negative effects of salt desiccation (Lohm et al. 1977) and ammonium toxicity (Wei et al. 2012). Although organisms occupying lower trophic positions may not respond as strongly to nitrogen input as those in higher positions (Murphy et al. 2012), possibly due to factors such as the dilution of N effects on resource quality by nitrogen resorption during senescence, overall N effects on detritivore communities can include changes in both abundance and species richness (Haddad et al. 2000, Wimp et al. 2010).

1.5 Global change and carbon dynamics in grass-dominated systems

Grass-dominated ecosystems are of great ecological importance in both temperate and tropical regions (Cramer et al. 2008), and they account for more than 25% of Earth’s vegetated surface area (Ramankutty and Foley 1999). Furthermore, grasslands contain approximately 12% of terrestrial SOM (Schlesinger 1977) and 30% of the terrestrial soil carbon stock (Anderson 1991), representing an important component of global carbon storage (Scurlock and Hall 1998, Conant et al. 2001). However, these highly productive systems also exhibit pronounced rates of biomass turnover (Gill and Jackson 2000) and C respiration (Verburg et al. 2004), suggesting that grassland processes contribute to both sink and source dynamics of global C cycling.

Anthropogenic global change may contribute substantially to shifts in both the structure and function of grass-dominated ecosystems (Stevens et al. 2004, Lin et al. 2013). Factors such as climate warming and increased N deposition have influential effects on decomposition (Frey et al. 2004, Bontti et al. 2009), plant competition and community structure (Zavaleta *et al.* 2003, De Boeck *et al.* 2008), plant-animal relations (Moise & Henry 2012), and ecosystem carbon cycling (Schimel et al. 1990, Zeng et al. 2009). The importance of these responses may be further magnified because there is a predicted geographic expansion of grass systems under future environmental scenarios (Emanuel et al. 1985, Lin et al. 2013). Field experiments are commonly used to explore the effects of global change factors on ecosystems, because they allow for the interpretation of ecosystem responses within the context of realistic ecological assemblages and natural environmental variability (Carpenter 1996). However, a major drawback inherent to this methodology includes potential artifacts imposed by treatment infrastructure, such as influences on humidity and airflow when using warming cloches (Kennedy 1994), or unrealistic drying effects associated with the employment of soil heating cables (Shaver et al. 2000). Furthermore, the addition of nutrients to simulate increased mineralization under climate warming is common, yet likely inaccurate and ultimately misleading due to unrealistic consequences for factors such as mycorrhizal associations (Woodward 1992).

Global change manipulations in field experiments can have important implications for plant-animal interactions (Tylianakis et al. 2008). In plot-level field studies, the indirect effects of experimental treatments on plants, mediated through herbivore responses, can represent the most significant component of overall plant responses (Peters et al. 2006). Warming effects on plant community structure can vary dramatically depending on the intensity of grazing pressure (Post and Pedersen 2008, Olofsson et al. 2009). Likewise, manipulation of the detritivore community can significantly influence plant decomposition responses to global change (Coûteaux *et al.* 1991, Rouifed *et al.* 2010), and the negative effects of ammonium toxicity (Wei et al. 2012), salt desiccation (Lohm et al. 1977), and temperature stress (Pendall et al. 2008) on soil fauna may diminish their overall role in litter fragmentation.

Herbivores such as molluscs and small mammals can have a strong influence on plant dynamics in grass-dominated systems (Ostfeld et al. 1997, Strauss et al. 2009), but the cryptic nature of their feeding habits (e.g. they are nocturnal, obscured by the plant canopy, and they selectively feed on seedlings) suggests their influence within the context of global change field studies may be difficult to detect without explicit quantification. More importantly, owing to the small spatial scale inherent to many of these studies, exaggerated levels of herbivory may result from concentrated herbivore activities within the treatment plots that contain the highest quality resources (Moise and Henry 2010). In addition, although many detritivores operate at smaller spatial scales than herbivores, and are therefore less likely to actively choose between treatment plots, potential interactions between global change drivers and the effects of soil fauna on litter turnover remain largely unexplored in decomposition experiments (Wall et al. 2008). Overall, these relationships suggest that there is a critical need to better understand the interactive effects of biotic and abiotic drivers of plant dynamics in the context of global change field experiments in order to improve the accuracy of predicted shifts in ecosystem carbon dynamics under future environmental scenarios.

1.6 Major study species

The field study components of this thesis were conducted in a temperate old field located in London, Ontario, Canada. This site was formerly an agricultural field, and it was sown with grasses and left unmanaged (no plowing or mowing for almost 30 years). The dominant plant species include the graminoids *Poa pratensis* L. and *Bromus inermis* Leyss., while the forbs *Cirsium arvense* L., *Lotus corniculatus* L., *Solidago altissima* L., *Daucus carota* L, and *Asclepias syriaca* L. are also present in patches. A plot-level global change field manipulation was initiated at this location in 2006. My field experiments were conducted in the context of the year-round warming and nitrogen addition treatment plots that were part of this experiment. Feeding experiments were performed in the Duckhouse animal facility located at Western University. Experimental grass diets used in these assays consisted of *P. pratensis and B. inermis* grown at the field site.

Results from small mammal live trapping revealed that the meadow vole (*Microtus pennsylvanicus*) was the most common rodent herbivore at our field site, although the short-tailed shrew and deer mouse were also present. Meadow voles contribute significantly to the structure of grassland plant communities (Howe et al. 2006). Likewise, variation in resource quality has important consequences for meadow vole diet preferences (Bergeron and Jodoin 1987) as well as habitat use (Oatway and Morris 2007), suggesting that they are likely to exhibit sensitivity to shifts in plant community dynamics under global change. Evidence from pitfall trapping indicated that the grey field slug (*Deroceras reticulatum* Müller) was the most abundant molluscan herbivore. Like meadow voles, *D. reticulatum* also responds to variation in plant quality (Dirzo and Harper 1982), and is an important herbivore in both natural (Strauss et al. 2009) and agroecosystems (South 1992). Furthermore, shifts in patterns of herbivory by *D. reticulatum* may contribute significantly to changes in plant community composition (Cleland et al. 2006).

1.7 Dissertation structure

My dissertation consists of four chapters, each developed and prepared as manuscripts for publication. These manuscripts are thematically linked by the overarching hypothesis that interactive effects between animals and drivers of global change (warming and nitrogen deposition) influence carbon-centric ecosystem processes such as primary productivity and plant decomposition. Chapters 2 and 3 have been published, while Chapters 4 and 5 are currently under review for publication. In Chapter 2, I present a theoretical consideration of potential shortcomings associated with the lack of control inherent in field experiments. Specifically, I discuss how herbivore activities within the context of global change experiments may represent a significant artifact, confounding the interpretation of observed treatment responses.

In Chapter 3, I investigated the effects of herbivore exclusion on plant community responses to the interactive effects of warming and nitrogen addition. Because herbivores are sensitive to changes in diet and habitat quality, shifts in their feeding activities in response to treatment manipulations may feed back to influence overall plant responses to drivers of global change. However, interactions between these factors remain largely unexplored in the context of plot-level field studies, thus the mechanisms governing treatment effects remain difficult to isolate. Understanding the contribution of herbivores to these relationships will be critical for predicting ecosystem changes under future environmental scenarios. For this study, I examined the effects of rodent and mollusc exclusion on the aboveground net primary productivity of grasses and forbs across the growing season.

In Chapter 4, I explored the consequences of N enrichment on diet and habitat choice in the meadow vole, *M. pennsylvanicus*. Evidence from Chapter 3 revealed that rodent herbivores responded positively to N fertilization, and the experiments described in Chapter 4 were conceived in order to explore potential underlying mechanisms, such as changes in grass palatability and plot microenvironment. Moreover, there is currently little information on meadow vole responses to nitrogen manipulation of grasses, despite the importance of grasses to vole diet and foraging dynamics. For this study, I quantified both relative meadow vole activity (measured indirectly as number of tracks) across global change treatment plots, as well as the relative consumption of fertilized and non-fertilized grasses in a cafeteria-style feeding experiment.

In Chapter 5, I investigated the interactive effects of detritivores, warming, and N addition on grass litter decomposition. Despite the possibility for detritivores to contribute substantially to litter turnover dynamics, studies investigating decomposition responses to global change focus primarily on microbial responses. Factors such as warming and N deposition may impose both direct and indirect effects on soil fauna, resulting in changes to the patterns and magnitude of faunal litter processing. For this study I prepared two separate types of grass litter samples, one allowing for detritivore access and the other restricting it, and incubated them in the global change treatment plots. Additional litter transplantation trials were established in order to investigate the mechanisms (i.e. plant quality, plot microenvironment) governing litter mass loss responses in the main experiment.

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Chapter 2

2 Like moths to a streetlamp: exaggerated animal densities in plot-level global change field experiments[1]

2.1 Global change field experiments at the plot level

Climate change, elevated atmospheric carbon dioxide concentrations and increased atmospheric N deposition are all environmental factors predicted to have large effects on ecosystems over the next century at a global scale. Global change experiments in the field have generally focused on the responses of plants (e.g. Harte and Shaw 1995, Shaver and Chapin 1995, Dukes et al. 2005), the main primary producers and most influential structural component of most terrestrial ecosystems. Although many valuable mechanistic data regarding plant responses to global change also have been obtained under controlled growth conditions, the use of field experiments is necessary to observe these responses in the context of natural plant assemblages, established soil microbial communities, background climate variability and associations with animals such as pollinators and herbivores (Carpenter 1996). Unfortunately, the tradeoff of added realism is that the mechanisms responsible for changes in productivity and plant species composition in response to experimental treatments can be difficult to identify, given the potential contributions of numerous indirect effects to the overall treatment effect.

Various techniques have been used to explore the effects of warming in field experiments (e.g. open top chambers, overhead heaters, passive night time heating curtains, fluid filled pipes or heated wires; see review by Shen and Harte 2000), and likewise there have been

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Citation: Moise ERD and Henry HAL. 2010. Like moths to a streetlamp: exaggerated animal densities in plot-level global change field experiments. Oikos 119:791-795 a range of manipulations to simulate changes in precipitation (sprinklers, drip irrigation, rain gutters, snow removal; Frampton et al. 2000, Hardy et al. 2001), elevated atmospheric CO_2 (e.g. open top chambers, free-air exchange rings; Hall et al. 2005, Peters et al. 2007) and increased atmospheric nitrogen deposition (aqueous N addition or addition of slow release pellets or salts; Lindberg and Persson 2004, Cleland et al. 2006). The development of newer techniques has often been prompted by a desire to avoid artifacts inherent in the older techniques, as in free-air exchange rings for CO_2 delivery, which were designed to avoid the unwanted effects of open top chambers on other environmental factors such as wind, humidity and temperature (Miglietta et al. 2001). However, the common trait of these techniques, both new and old, is that they are typically applied at the plot level. While statistical significance can be achieved through replication, there is a concern that small-scale studies that attempt to extrapolate plot-level results to ecosystem-level responses often lead to inaccurate interpretation, since such localized manipulations can easily ignore relationships present at the community or ecosystem level (Kareiva and Anderson 1988, Schindler 1998, Englund and Cooper 2003).

2.2 Like moths to a street lamp

In addition to the introduction of potentially confounding microclimate artifacts, a concern about experimental infrastructure in global change experiments has been that it can physically restrict the access of influential herbivores or pollinators, whose activities may interact with the experimental treatments. Examples include the blocking of caribou and muskox feeding by warming infrastructure (Post and Pedersen 2008) and the exclusion of specialist butterfly pollinators from open top CO_2 chambers as a result of the updraft of air (C. Field pers. comm.). However, free access to experimental plots does not ensure that global change treatments feature realistic densities of herbivores, detritivores or pollinators. On the contrary, much like moths swarm around streetlamps, animals that prefer the local conditions in treated plots may congregate at artificially high densities, or conversely, those that are repelled by the treatments may choose to avoid them. Although these behavioural preferences may be interpreted as realistic community-level responses to global change treatments (i.e. animals that prefer heated plots might be expected to flourish in a warmer climate, or the opposite might be expected for animals that shun heated plots), the animal densities in experimental plots may nonetheless grossly exaggerate their future densities in response to global change. As an illustration of this point, I conducted a warming experiment and was intrigued to discover a turkey nesting under an overhead heater in a one square meter area plot. If other turkeys would have found the other heated plots to be equally inviting, then by extrapolation I could have arrived at the erroneous conclusion that the density of turkeys under future climate conditions would be one per square meter! Clearly, the turkey was merely responding to the localized treatment effect, and in order to survive it needed to be subsidized by resources beyond the area of the experimental plot. Likewise, animals repelled by a localized treatment can simply choose to remain in the matrix surrounding experimental plots, whereas in a future world they would not be privileged with such a choice; rather, they would be forced to tolerate the new conditions, re-locate over a long distance or perish. While turkeys nesting under heaters are easy to detect, other animals might be far less conspicuous, particularly if they are small or if their use of plots varies diurnally (e.g. nocturnal feeders) or seasonally (e.g. under the snowpack). Selective herbivory on newly germinating plants early in the growing season may also go unnoticed but have a large and disproportionate effect on the relative species abundance of adult plants later in the season (Hanley et al. 1995). Here, I describe how these potential animal congregation and avoidance artifacts may have been overlooked in the interpretation of results from many plot-level global change field experiments. I also provide suggestions for how to best interpret the results of these experiments and how to isolate the effects of these artifacts from the more meaningful treatment effects.

2.3 Valid projection or artifact?

The manner and extent to which animals respond either directly or indirectly to experimental treatments likely vary among global change factors (Fig. 2.1). For example, while nitrogen fertilization can directly decrease the abundance of sensitive species such as gastropods through direct toxicity (Huhta et al. 1983) or salt-induced desiccation

(Lohm et al. 1977), these direct effects are likely transient. However, with respect to indirect effects, both nitrogen addition and elevated CO_2 can alter plant nutritional quality or species composition (Hartley et al. 2000, Zavaleta et al. 2003), and when these treatments are applied at the plot level, herbivores may choose between the more (or less) palatable plants in the plots and those in the surrounding area. Conversely, herbivores would not be given such a choice in a future environment, since these changes in vegetation quality would take place at a landscape scale. When nitrogen or CO_2 additions increase plant biomass (Owensby et al. 1994), increased shading by leaf canopy can also provide a cooler microclimate than the surrounding area at the soil surface. In contrast to nitrogen addition and elevated CO_2, both warming and simulated precipitation changes almost always have direct and persistent effects on plot microclimate, and they can also have indirect effects on animals by altering plant species composition and the nutritional quality of the plants within a plot (Peñuelas et al. 2008). The direct effects of warming on microclimate suitability can vary diurnally and seasonally. For example, while warming may deter a given herbivore from a plot by exacerbating desiccation and heat stress at noon on a hot summer day (Sjursen et al. 2005, Carne-Cavagnaro et al. 2006), the same herbivore may prefer warmed plots on cool nights or in the spring or fall. Extreme levels of animal congregation can also occur in early spring when snow melts earlier in warmed plots than in the surrounding area (Roy et al. 2004).

There are numerous examples of global change field experiments where changes in animal abundance in response to treatments at the plot level have been used to infer changes in animal densities under future environmental conditions (Table 2.1a) or where direct measures, such as leaf damage, have been used to quantify herbivory (Table 2.1b). In both cases, these studies have focused primarily on small invertebrate herbivores such as insects or gastropods. The extent to which these studies are vulnerable to density artifacts likely depends on the incidence of animal immigration and emigration. The studies that are most vulnerable to density artifacts are those where field manipulations present no physical barriers to animal movement (e.g. Haddad et al 2000, Sternberg 2000, Roy et al. 2004 and Adler et al. 2007). As suggested by Roy et al. (2004), these small-scale field manipulations may serve only to present elaborate choice experiments. In contrast, Agrofleece sheets applied to cloches by Strathdee et al. (1993) likely restricted most animal movement; however, the behaviour of some animals may be disrupted by confinement to a small plot (Underwood 1986). Frampton et al. (2000) also attempted to reduce animal dispersal by erecting 20 cm high polythene barriers around each plot, but they may have only restricted the movement of some flightless arthropods, and would be less effective against flying organisms such as Diptera and some Hymenoptera and Hemiptera, which were all present. Similarly, open top chambers used by Stiling et al. (2002) and Hall et al. (2005) were 3 m high, but Lepidoptera were the major herbivores present in both studies and thus would have been able to fly over the barriers. Others, such as Miles et al. (1997) and Whittaker and Tribe (1998), who used cloches as passive solar heaters, and Richardson et al. (2002), who used open top chambers, intentionally designed their plot edge barriers to allow the passage of animals. Overall, both measures of animal densities and herbivore damage in field plots can potentially be criticized for their vulnerabilities to the congregation and avoidance artifacts described above. However, these artifacts can also explain important changes in plant productivity and species composition in global change studies that neglect to measure herbivore effects, and these effects may instead be mistakenly attributed to direct plant responses (Fig. 2.1; Peters et al. 2006).

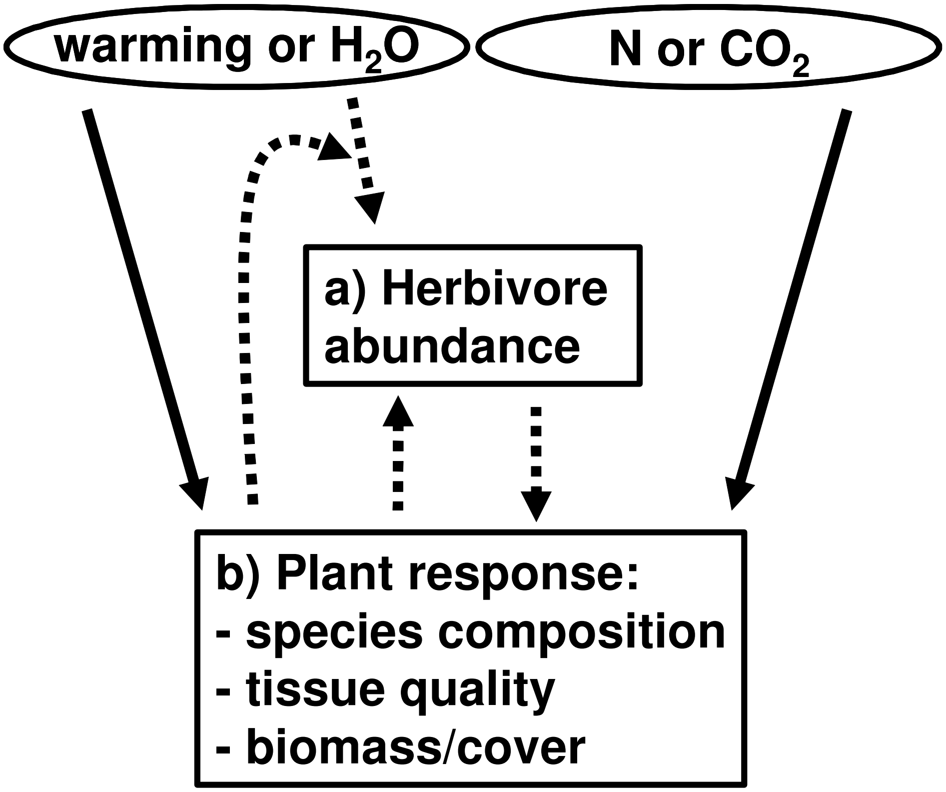

Figure 2.1. Potential mechanisms whereby global change treatments (ovals) both directly and indirectly affect a) herbivore abundances, and b) plant responses, in experimental field plots. The dotted arrows denote effects that potentially lead to exaggerated responses resulting from animals congregating in or being repelled by the plots.

Table 2.1. Global change studies that have measured herbivore responses at the plot-level in the field.

Study	Manipulated variable	Herbivore response
a) *changes in herbivore abundance*		
Strathdee *et al*. (1993)	Temperature	Increased abundance of the arctic aphid *Acyrthosiphon svalbardicum*
Miles *et al.* (1997)	Temperature	Increased density of the heather psyllid *Strophingia ericae*
Whittaker and Tribe (1998)	Temperature	Increased density of the spittlebug *Neophilaenus lineatus*
Frampton *et al.* (2000)	Precipitation	Increased abundance of farmland arthropods in response to water
Haddad *et al.* (2000)	Nitrogen	Increased insect abundance
Sternberg (2000)	Temperature, precipitation	Species specific effects on gastropod abundance
Adler *et al.* (2007)	Temperature	Decreased abundance of the aphid *Obtusicauda coweni*
b) *changes in herbivore damage*		
Richardson *et al*. (2002)	Temperature, nutrients	Species specific effects on insect abundance and plant biomass removal
Stiling *et al.* (2002)	CO_2	Decreased herbivore densities and leaf consumption
Roy *et al.* (2004)	Temperature	Increased plant damage
Hall *et al.* (2005)	CO_2	Decreased herbivory damage

2.4 Suggested improvements for the interpretation of global change treatments in the field

Although field plots may harbor unrealistically high or low densities of animals as the result of experimental treatments, qualitatively, the preference by an animal for a specific set of environmental conditions may indicate the future success of this species under those conditions. In addition, despite the potential risks of misinterpreting animal responses to plot-level global change treatments they, in some cases, provide useful mechanistic information (Table 2.2). For animals that operate at a small spatial scale, useful quantitative responses may be obtained. For example, changes in soil microarthropods, nematodes or soil microorganisms in response to global change treatments (e.g. Kennedy 1994, Treonis et al. 2002, Yergeau and Kowalchuk 2008) may provide meaningful quantitative data if their movements in and out of plots are relatively limited. Likewise, for animals that remain sessile, even over a single life stage or season, plot level global change experiments may be appropriate for studying phenological or physiological responses; examples include warming effects on gall flies overwintering in goldenrod stems (Irwin and Lee 2003) and the time to first hatching in insect eggs (Masters et al. 1998).

If it is suspected that the densities of herbivores are exaggerated in response to global change treatments, direct plant responses can be isolated through the use of exclosed subplots within the main experimental plots (Peters et al. 2006). Similarly, enclosures such as mesh bags containing insects (Adler et al. 2007) may be used to explore interactions among the experimental treatments and herbivore feeding, once again, provided that confinement does not strongly influence herbivore behaviour (Underwood 1986). Changes in food preferences within plots in response to global change treatments, relative to preferences in control plots (Peters 2000), can also provide meaningful predictive data. Finally, the results from plot level global change experiments can be integrated with gradient studies or time series analyses conducted at the landscape scale (Dollery et al. 2006, Wiedermann et al. 2009).

Table 2.2. Suggested improvements for the interpretation of results from global change experiments conducted at the plot level in the field.

Suggested improvement	Reference
Restrict extrapolation of animal responses to sessile organisms or life or stages, or organisms that operate at an extremely small spatial scale (e.g. bacteria).	Masters et al. 1998
Embed animal exclosures or enclosures in plots.	Peters et al. 2006
Quantify changes in herbivore feeding preferences in response to treatments.	Lindroth et al. 1997
Integrate with gradient studies or time series analyses at the landscape scale.	Dollery et al. 2006 Wiedermann et al. 2009

2.5 Conclusions

Given that global change experiments conducted in the field often operate over multiple years and require large investments of time and resources, it is understandable why researchers often attempt to explore all possible community and ecosystem responses. However, I contend that these experiments are best-suited for exploring the responses of sessile organisms, and interactions among plants and animals must be carefully considered in the interpretation of these experiments. Moving forward, it is crucial that we bear in mind the potential for unintentional, or hidden effects (Huston 1997), of plot level manipulations. Although my examples have dealt primarily with potential artifacts caused by herbivores, congregation in plots or avoidance of plots by influential detritivores or pollinators could also feed back on plant growth. Overall, despite the valuable mechanistic data that can be obtained from plot-level field experiments, it is clear that their results must also be interpreted in the context of complementary studies conducted at the landscape level.

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Chapter 3

3 Interactions of herbivore exclusion with warming and N addition in a grass-dominated temperate old field[2]

3.1 Introduction

Climate warming and increased atmospheric nitrogen deposition over the next century are both expected to exert strong bottom-up effects on primary producers and ecosystems (Woodward 1992, Cleland et al. 2007). Warming can influence primary production by extending the plant growing season (Zhou et al. 2001) or by altering photosynthesis (Hobbie and Chapin 1998) and soil nutrient dynamics (Koch et al. 2007), and nitrogen addition increases plant production in most terrestrial systems (LeBauer and Treseder 2008). Although less frequently examined in the context of global change field experiments, the ability of top-down factors to regulate primary producers is also well-established (Huntly 1991, Borer et al. 2005). Feeding by large mammalian herbivores is often both conspicuous and dramatic (Milchunas and Lauenroth 1993), while the actions of small, inconspicuous herbivores are much less obvious (MacDougall and Wilson 2007). For example, small rodents and molluscs can substantially alter plant community biomass and species composition (Hanley et al. 1995a, Howe et al. 2006). Understanding the role of small herbivores may, therefore, be critical for understanding plant responses in the context of global change field experiments (Moise and Henry 2010).

In a broad context, the simultaneous influences of top-down and bottom-up on plant productivity have led to investigations of the relative importance, as well as potential

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Citation: Moise ERD and Henry HAL. 2010. Like moths to a streetlamp: exaggerated animal densities in plot-level global change field experiments. Oikos 119:791-795 interaction, of these factors (Power 1992, Turkington 2009). How might these interactions be expected to occur, however, in the context of warming and increased N availability? Changes in plant species composition, tissue quality and chemical defenses in response to warming (Peñuelas et al. 2008) and N addition (Ayres 1993, Hartley et al. 1995, Somers et al. 2008), can affect herbivore feeding. Likewise, changes in aboveground cover in response to these factors may provide shelter to small herbivores that are vulnerable to predation when exposed (Mossman 1955), as well as alter soil surface temperatures by modifying soil shading (Hutchison and Henry 2010). Warming can also directly influence herbivores; while the densities of insect herbivores often increase in response to warming (Miles et al. 1997, Whittaker and Tribe 1998), warming can negatively affect moisture-sensitive animals such as land molluscs (Carne-Cavagnaro et al. 2006). Some animals even respond directly to nitrogen application (Lohm et al. 1977, Huhta et al. 1983), but more often herbivores are attracted to plants fertilized with nitrogen, resulting in increased grazing pressure (Hartley and Mitchell 2005).

Despite the potential for interactions between top-down and bottom-up effects on plants, a recent meta-analysis by Gruner et al. (2008) found little support for interactions between herbivory and nutrient addition. However, this analysis of 191 studies was limited to only 15 studies in terrestrial systems that generally did not exhibit significant herbivore removal effects, thus limiting the ability to detect interactions between top-down and bottom-up effects. Therefore, the importance of interactions between herbivory and bottom-up effects for plants in terrestrial systems remains an open question. Gruner et al. (2008) also suggested that research on this topic may move forward by simultaneously examining multiple producer resources and by considering the combined effects of multiple herbivore taxa.

I used rodent and mollusc exclosures in the plots of a warming and nitrogen addition field experiment to test the hypothesis that herbivore effects interact with plant biomass responses to these treatments. Based on the assumption that nitrogen addition improves plant tissue quality, I predicted that the effects of rodent and mollusc exclusion would be higher in the N-fertilized plots than in control plots. Additionally, based on the assumption that molluscs would avoid heat-induced desiccation, I predicted that the effects of mollusc exclusion would be lower in heated plots than in ambient temperature plots.

3.2 Methods

3.2.1 Study site

I conducted this experiment in a temperate old field located in London, Ontario, Canada (43^{o} 01’ 45” N 81^{o} 12’ 50” W, elevation 264 m). This site is a former agricultural field that has not been plowed or mowed for more than 25 years. The mean annual temperature for the site is 7.5 °C, with a low monthly mean of -6.3 °C (January) and a high monthly mean of 20.5 °C (July), and a mean annual precipitation of 818 mm (Canadian Climate Normals 1971–2000, Environment Canada, National Climate Data and Information Archive). The dominant plant species are the grasses *Poa pratensis* L. and *Bromus inermis* Leyss., with the forbs *Cirsium arvense* L. and *Lotus corniculatus* L. present in patches (Hutchison and Henry 2010). Common herbivores present at the site (based on Longworth and pitfall trapping) include the meadow vole, *Microtus pennsylvanicus*, and the grey field slug *Deroceras reticulatum*, although other rodent, rabbit, snail and insect herbivores are also present.

3.2.2 Nitrogen addition and warming experiment

I administered warming and N addition treatments to 1 m^2 circular plots arranged in a factorial block design, with two temperature levels (warmed and ambient) crossed with two nitrogen treatments (N addition and control), divided among 10 blocks (n=10 for each treatment combination, for a total of 40 plots; see Turner and Henry (2009) for a description of the full experiment). Warming of approximately 2-3 °C at the soil surface was attained using 150 W ceramic infrared heaters (Zoo-Med Laboratories, San Luis Obispo, CA, USA), which mimic solar heating, but without the production of photosynthetically active radiation (Shen and Harte 2000). Warming of the plots began in late November 2006. Each year, a pulse of 2 g m^{-2} of N was added as aqueous ammonium nitrate in spring to simulate nitrogen inputs from snow melt, and an additional 4 g m^{-2} y^{-1} of N as slow release ammonium nitrate pellets in early summer to simulate continuous N deposition throughout the summer. This rate of addition is consistent with rates of atmospheric nitrogen deposition expected in this region by 2050 (Galloway and Cowling 2002).

3.2.3 Exclosure design and implementation

I constructed rodent exclosures out of steel mesh cylinders (30 cm tall, 20 cm diameter, mesh size 0.5 cm) fastened to wooden stakes, and mollusc exclosures out of sewer-grade PVC pipe (20 cm internal diameter, 10.5 cm height) with copper mollusc-repellant tape applied to the exterior top 5 cm. In early April 2009, I placed one rodent exclosure and one mollusc exclosure in each of the 40 global change plots, with the bases of the rodent exclosures resting flush against the soil, and the mollusc exclosures driven 5 cm into the soil. For biomass sampling, I placed permanent 12 cm diameter sampling rings on the surface of the ground in each of the exclosures, as well as a control sampling ring in a non-exclosed area of each plot. All exclosures were inspected weekly to ensure they had not been breached by the target organisms. I also set up 6 blocks of mollusc exclosures without copper tape outside of the treatment plots to test for the effect of the PVC pipe alone relative to unexclosed areas.

3.2.4 Sampling regime

I estimated aboveground plant biomass non-destructively in the sampling rings five times between June and November 2009 (8-10 June, 20-22 July, 24-26 August, 28-30 September, 8-10 November) using allometric equations for converting plant shoot height into dry weight. To develop the allometric equations, for each of the five sampling periods I destructively sampled shoots outside of the plots from the four species that occurred in the sampling rings (the grasses *P. pratensis* and *B. inermis*, and the forbs *C. arvense* and *L. corniculatus*), and dried the tissue at 60 °C for 72 hours prior to weighing. The two dominant grasses account for approximately 96% of the total biomass at our site, and the two dominant forb species account for >90% of the total forb biomass (Hutchison and Henry 2010). I counted individual leaves for *P. pratensis* and whole tillers for *B. inermis*, and included both green shoots and the current year's senesced shoots. Thus, my biomass estimates were of cumulative biomass production minus losses to herbivory or other factors.

3.2.5 Data analysis

I calculated rodent and mollusc exclosure effects for each plot by subtracting the cumulative biomass from the control sampling ring from that of the rodent and mollusc exclosures, respectively. I then used repeated measures ANOVA to test for differences in exclosure effects between the warming and N addition treatments, with warming, N addition and their interaction term as between-subjects factors, and date and all interactions between date and warming and N addition as within-subjects factors (plot was included as a random factor nested in warming and N addition). With respect to covariance structure, the analysis produced very similar results regardless of whether the model assumed unstructured covariance or whether AR or Toeplitz covariance structures were used to account for dependence between time points (the latter two increased the significance of the P-values slightly). When ANOVA results were significant ($P < 0.05$), I used a Tukey's test to identify the significantly different treatment combinations. I performed the analyses using the Fit model platform in JMP 4.0 (SAS Institute; SPSS was used to test different covariance structures). I used exclosure effects on both aboveground grass biomass and total aboveground biomass (grasses and forbs) as dependent variables, and also analyzed exclosure effects on *B. inermis* biomass as a percentage of total grass biomass. The presence of forbs in the sampling rings was too intermittent to justify an analysis of forb biomass alone. I analyzed grass shoot abundance and height for a representative month (July) in a similar manner as for biomass, but without repeated measures.

3.3 Results

3.3.1 Responses of exclosure effects to N addition

Across sampling dates, grass biomass was consistently highest in nitrogen-fertilized rodent exclosures, but grass biomass did not increase with nitrogen addition in non-exclosed areas (Fig. 3.1a). The effect of rodent exclusion on grasses (in Fig. 3.1a, the difference between a filled or open bar in the ‘rodent exclosure’ column and the corresponding bar in the ‘non-exclosed’ column – statistical results presented in Table 3.1) was more than double in fertilized plots than in non-fertilized plots (P=0.003), whereas the effect of mollusc exclusion did not increase significantly for N-fertilized grasses (P=0.509; Fig. 3.1a). Grass shoot abundance generally increased inside of rodent exclosures, whereas shoot height was most sensitive to nitrogen addition (Fig. 3.2). Rodent exclusion increased *B. inermis* biomass as a percentage of total grass biomass by 7% in non-fertilized plots, whereas this percentage increase was significantly higher (27%) in N-fertilized plots (P<0.001). There were no significant treatment effects on the response of grass species composition to mollusc exclusion. Total biomass in non-exclosed areas was higher in non-fertilized plots than in N-fertilized plots (Fig. 3.1b). The effect of rodent exclusion on total biomass was significantly higher in fertilized plots than in non-fertilized plots (P<0.001), but there was a significant date × nitrogen interaction (P=0.014) caused by an increased response of the rodent exclusion effect to N in July and August (Fig. 3.1b).

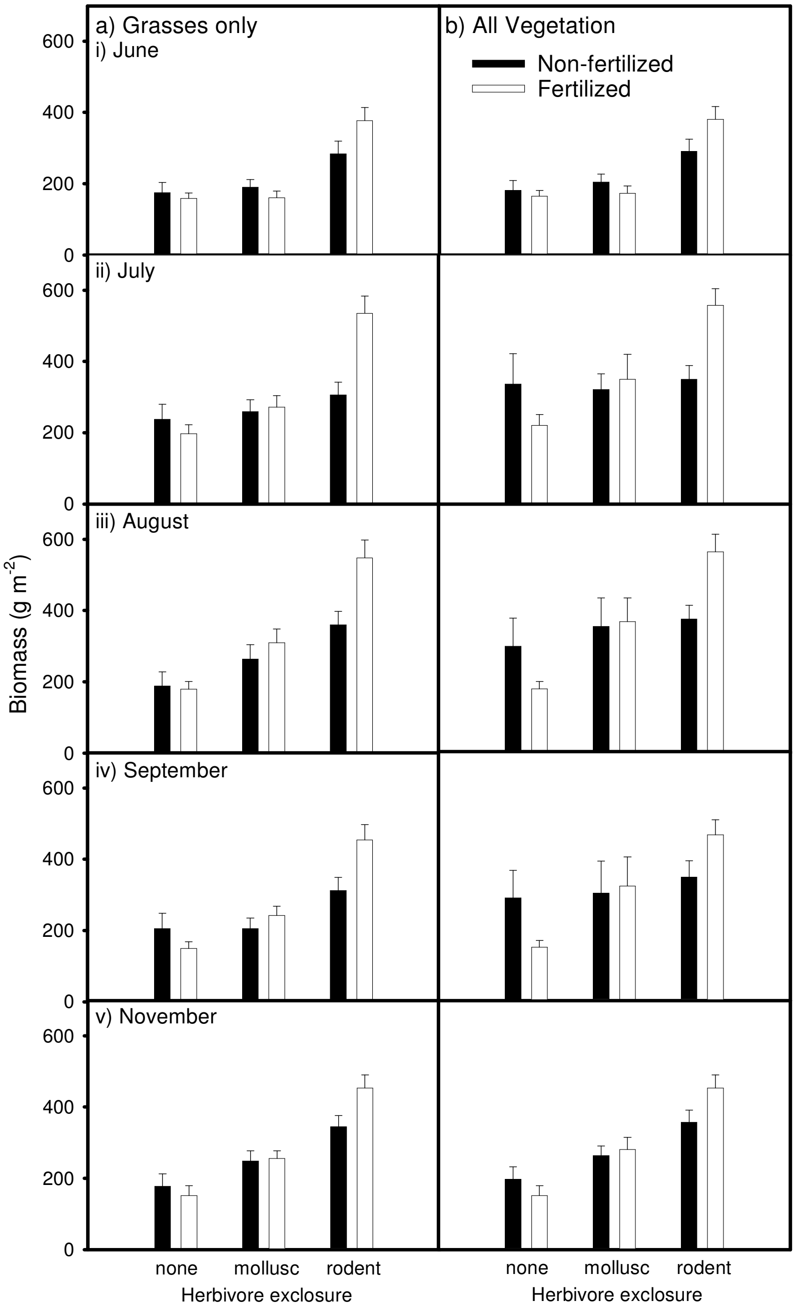

Figure 3.1. Cumulative aboveground biomass of a) grasses and b) all vegetation in fertilized (open bars) and non-fertilized (filled bars) plots in non-exclosed areas, mollusc exclosures and rodent exclosures, pooled over warming treatments (n=20). Corresponding statistical results for treatment effects on exclosure effects (with exclosure effects estimated as the biomass difference between exclosed and non-exclosed areas within a fertilization treatment) are presented in Table 3.1.

Table 3.1. Summary of ANOVA F and P-values for effects of treatment and date on estimates of rodent and mollusc effects (the biomass difference between exclosed and non-exclosed areas within a given global change treatment) on grasses alone and total aboveground biomass in a grass-dominated old field. Degrees of freedom are displayed in parentheses after the effects.

	Rodent exclusion				Mollusc exclusion			
	Grasses		Total		Grasses		Total	
Effect	F	*P*	F	*P*	F	*P*	F	*P*
between-subjects								
$W_{(1,33)}$	3.45	0.072	5.28	0.028*	5.51	0.025*	1.07	0.308
$N_{(1,33)}$	10.09	0.003**	14.59	<0.001***	0.44	0.51	1.60	0.214
$W \times N_{(1,33)}$	0.12	0.736	0.62	0.436	0.13	0.717	1.30	0.262
within-subjects								
$D_{(4,138)}$	6.92	<0.001***	2.26	0.066	5.75	<0.001***	2.62	0.038*
$W \times D_{(4,138)}$	0.56	0.689	1.29	0.276	1.28	0.279	0.33	0.859
$N \times D_{(4,138)}$	2.46	0.048*	3.25	0.014*	0.51	0.726	1.11	0.355
$W \times N \times D_{(4,138)}$	0.93	0.446	1.22	0.304	0.66	0.618	1.14	0.342

W - warming; N - Nitrogen; D - Date.

Asterisks denote significance (*0.05-0.01,**0.01-0.001,***<0.001).

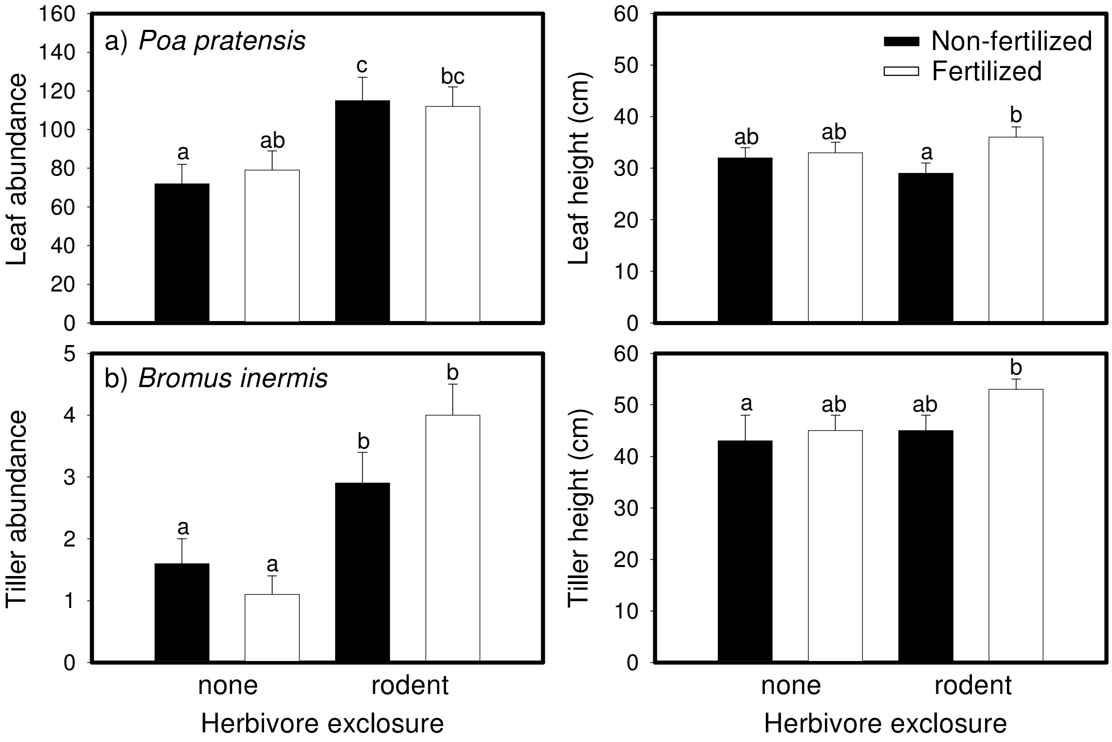

Figure 3.2. Mean abundances and heights of a) *P. pratensis* leaves and b) *B. inermis* tillers in non-exclosed and rodent exclosed subplots receiving no fertilization (filled bars) and N fertilization (open bars). Error bars denote standard error, and different lower case letters within a panel indicate a statistical difference between bars based on a Tukey's HSD test ($P<0.05$).

3.3.2 Responses of exclosure effects to warming

In non-exclosed areas, both grass and total biomass were higher in ambient temperature plots than in warmed plots (Fig. 3.3a, b). The effect of mollusc exclusion on grass biomass was higher in warmed plots than in ambient temperature plots (P=0.025), and grass abundance was generally higher inside mollusc exclosures than in non-exclosed areas (Fig. 3.4). However, there was no significant difference in plant biomass between control PVC rings that lacked copper tape and non-exclosed areas (P=0.28). A significant influence of date on the effect of mollusc exclusion resulted from there being no substantial exclusion effect on the first sampling date, but a significant effect thereafter (Fig. 3.3a, b). Although warming did not significantly influence the effect of rodent exclusion on grass biomass, for total biomass there was a significant increase in the effect of rodent exclusion in warmed plots (P=0.028). The effect of mollusc exclusion on total biomass did not respond significantly to warming (P=0.308), and for all analyses, there were no significant interactions between nitrogen and warming with respect to exclosure effects on plant biomass.

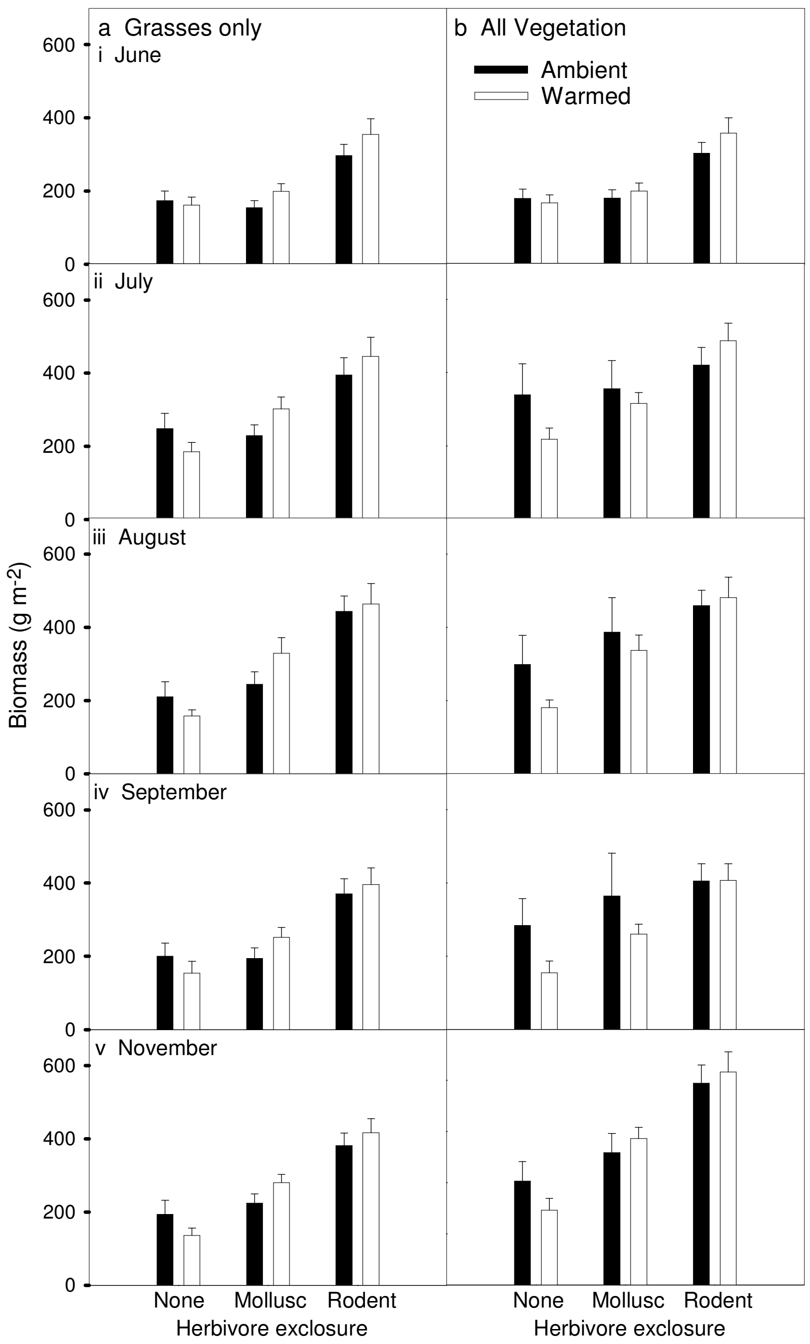

Figure 3.3. Cumulative aboveground biomass of a) grasses and b) all vegetation in ambient temperature plots (filled bars) and warmed plots (open bars) in non-exclosed areas, mollusc exclosures and rodent exclosures, pooled over nitrogen treatments (n=20). Corresponding statistical results for treatment effects on exclosure effects (with exclosure effects estimated as the biomass difference between exclosed and non-exclosed areas within a warming treatment) presented in Table 3.1.

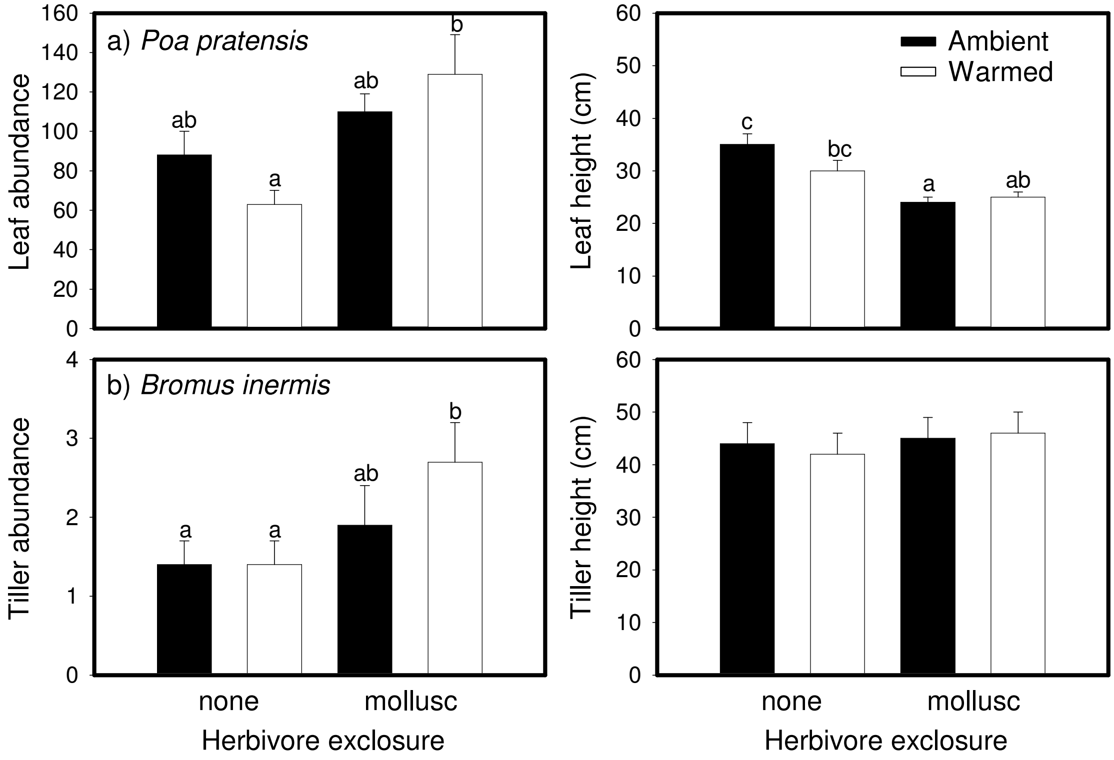

Figure 3.4. Mean abundances and heights of a) *P. pratensis* leaves and b) *B. inermis* tillers in non-exclosed and mollusc exclosed subplots in ambient temperature plots (filled bars) and in warmed plots (open bars). Error bars denote standard error, and different lower case letters within a panel indicate a statistical difference between bars based on a Tukey's HSD test ($P<0.05$).

3.4 Discussion

3.4.1 Herbivores and global change field studies

My results demonstrated that the exclusion of herbivores can interact substantially with warming and N addition to influence aboveground plant biomass. In addition, multiple types of herbivores can contribute to this effect simultaneously, with variation between herbivore taxa in how they respond to different treatments and how they affect different plant functional groups. Accounting for losses of biomass to herbivory is one of the main challenges of estimating net primary production (Fahey and Knapp 2007), and my results suggest that herbivores can differentially influence estimates of production between treatment and control plots.

Throughout my experiment, non-exclosed areas featured an intact upper canopy of undamaged grass shoots, such that the effects of herbivory were relatively inconspicuous upon casual examination of the plots. While I made no attempt to explicitly distinguish herbivory from other herbivore-related effects such as trampling, upon close examination of the shoot bases under the thick litter layer, substantial evidence of herbivory was observed. Such inconspicuous feeding is consistent with the dominant herbivores at my site. Meadow voles construct tunnels in the litter under tall grass swards to avoid avian predators (Mossman 1955), and harvest the fresh and highly nutritious re-growth of grass shoots within these tunnels. Thus, unlike other grazers such as cattle or geese that can clip down the entire grass sward to a uniform low height, feeding by voles can be mostly hidden and highly localized. At my site, the reduction in shoot abundance in non-exclosed areas relative to rodent exclosed areas was consistent with localized feeding. Slugs, another dominant herbivore at the site, forage nocturnally (Newell 1966, Lyth 1983), and the grey field slug concentrates feeding on seedlings (Byers and Bierlein 1982, Hanley et al. 1995b). This feeding strategy can be difficult to detect, and the removal of seedlings can be more destructive than an equivalent amount of feeding on larger plants, because of the pre-emption of future growth (Marquis 1992, Peters 2007). Although my non-destructive sampling technique, which accounted only for differences in leaf and tiller abundance and length, was potentially vulnerable to biases caused by chewing along leaf margins and the resulting reduction in leaf thickness in non-exclosed areas, there was no evidence of such feeding damage at my site. Besides feeding, other herbivore activities that could have influenced plant biomass outside the exclosures include tunneling into the grass and litter layer, and urine and fecal inputs. However, concerning the latter, I would have expected increased nutrient inputs from urine and feces to increase plant biomass, rather than reduce it.

3.4.2 The influence of nitrogen on exclosure effects

The effects of rodent exclusion, but not those of mollusc exclusion, increased in response to nitrogen addition. This finding is consistent with vole feeding trials that have demonstrated an increased palatability of nitrogen-enriched plant tissue (Hartley et al. 1995, Lantova and Lanta 2008), but not with those that have shown increased consumption of nitrogen-enriched plants by slugs (Gebauer 2002). Nevertheless, the insensitivity of slug plant consumption to nitrogen addition has been previously observed (Cleland et al. 2006). Vole abundance increased in a nitrogen addition field experiment conducted by Treberg et al. (2010), although herbivore effects on plant biomass were not quantified, and the authors hypothesized that the voles might be directly attracted to the fertilizer pellets. In addition to changes in plant tissue quality, N fertilization could result in a denser canopy cover, which can protect small mammals from avian predators (Birney et al. 1976), and vole density has correlated previously with increased plant cover (Mossman 1955, Hall et al. 1991). Over the longer term, changes in plant species composition caused by fertilization can also alter vole abundance, possibly in the opposite direction than expected (Boonstra and Krebs 2006).

Although there was no appropriate cage control available for the rodent exclosures to rule out potential direct effects of the cages on plant growth, as described above, careful observation at ground level confirmed the presence of substantial vole feeding in non-exclosed areas and an absence of vole feeding in rodent exclosed areas (this observation was also consistent with the shoot abundance data). Likewise, the large N fertilization effect inside rodent exclosures was consistent with the strong N limitation on plant biomass production at my site (Turner and Henry 2009). The lack of an N addition effect on plant biomass outside the rodent exclosures was consistent with more intense herbivore activity in the N addition plots, and by definition could not be explained by direct cage effects or other associated artifacts. Finally, with my analysis of differences in exclosure effects among N treatments, any direct effects of the rodent exclosures on plant growth would have effectively cancelled out. While an exclosure diameter of 20 cm could be perceived as relatively small, the grasses grew mostly vertically, and the subplots were sufficiently large to accommodate as many as 100 individual grass shoots each. Because the grasses at my site are clonal, I could not exclude the possibility that resources were shared underground between exclosed and non-exclosed tillers. However, any such resource sharing would only have functioned to dampen the observed exclosure effects.

The effect of rodent exclusion was moderate in June and increased through July, after which the effect remained stable. This pattern likely reflects an emphasis on shoot feeding during the active growth phase of the plants, with the latter slowing dramatically in mid-July with decreased soil moisture and seed set (Hutchison and Henry 2010). Trends in exclusion effects on grasses in response to nitrogen addition were similar to trends in exclusion effects on total biomass, which can be explained by grasses making up approximately 96% of the total aboveground biomass at my site. However, while the presence of herbivores appeared to equalize aboveground grass biomass in non-exclosed areas between fertilized and non-fertilized plots, total aboveground biomass decreased in non-exclosed areas in fertilized plots.

3.4.3 The influence of warming on exclosure effects

Contrary to my hypothesis, warming increased the effects of mollusc exclusion on grass biomass. Nevertheless, a more thorough examination of the timing of slug feeding and their temperature optima provides an explanation for this effect. In particular, although warming can cause water stress for slugs during the day, as noctural feeders (Newell 1966, Lyth 1983), nighttime temperatures may be more relevant for slug feeding. Feeding by the grey field slug increases with increasing temperature until a peak of 14 °C (Wareing and Bailey 1985). In comparison, the average minimum nightly temperatures at our site over May, June, July, August, September, and October were 6.7, 12.2, 13.2, 14.6, 10.5, and 3.8 °C, respectively (Climate Data Online, Environment Canada, National

Climate Data and Information Archive). Given that the heaters warm the soil surface by approximately 2-3 °C in my experiment, the warming would typically move the slugs closer to their optimal feeding temperature at night. While warming possibly influenced grass tissue quality and hence palatability, increased grass biomass production in response to warming can increase plant C:N ratios and decrease grass leaf N, P, and K (Tan et al. 1978, Shen et al. 2009), and the effect of warming is generally one of decreasing grass digestibility (Thorvaldsson et al. 2007). Outside of the plots, the lack of a significant difference in plant biomass between PVC rings lacking copper tape and non-exclosed areas indicated that mollusc exclusion, and not the presence of the PVC rings alone, was responsible for the exclosure effects. There was no substantial influence of warming on mollusc exclusion effects for the first sampling date, but likewise, the influence of warming on mollusc exclusion ceased to increase over the second half of the summer, which was consistent with the reduced production of new grass shoots at this time.

While for grasses there was a significant increase in the effect of mollusc exclusion with warming and no effect of warming on the effect of rodent exclusion, the opposite occurred for total biomass. Nevertheless, a single forb species, the legume *Lotus corniculatus*, was mostly responsible for the difference between grass biomass and total biomass, and this species was distributed patchily at the site, and not present in many of the experimental blocks. I also made no effort to quantify the effects of herbivore exclusion on belowground plant biomass in my experiment. In previous years, root biomass decreased with warming and did not respond to nitrogen (Hutchison and Henry 2010). However, given the rapid root turnover of the dominant grasses at my site (Stewart and Frank 2008), and problems associated with sampling fine root biomass, it can be difficult to estimate cumulative root biomass from multiple estimates of standing root biomass.

3.4.4 Conclusions

My results demonstrated that interactions between top-down and bottom-up factors can have a substantial influence on plant biomass in a grass-dominated system. In addition, they revealed taxon-specific variation in herbivore effects between warming and N addition treatments. Although the potential for small herbivores such as voles and slugs to exert strong top-down effects on plant biomass is well-established in the literature, the litter layer can conceal much of their activity and damage in grass dominated-systems. Their influence on plant responses to global change factors in field experiments may therefore go undetected if it is not explicitly quantified or controlled for. The responses of herbivores in my experiment may have reflected their preferences with respect to small scale spatial variation in food quality and environmental conditions among the plots and the surrounding area. Thus, caution should be exercised in interpreting my results as a projection of the magnitude of top-down effects on plant biomass under future global change scenarios. Specifically, drivers of global change will act at much larger spatial scales than in the experimental plots, and at these larger scales the responses of other factors (e.g. predators, pathogens) to global change may have important top-down effects on herbivore populations. Furthermore, in plot-level field experiments herbivores may concentrate their activities in the preferred treatment plots, whereas future environmental changes at larger spatial scales would not provide herbivores with such a choice (Moise and Henry 2010). When consumer exclusion is not practical, monitoring of plot usage by herbivores (using traps, direct counts, dropping counts or tracking tunnels) or the scoring of herbivore damage may provide useful insights into the relative strength of top-down and bottom-up processes within the context of these experiments. Future studies performed at the landscape scales may better address how top-down effects will contribute to the regulation of herbivore populations in response to global change.

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Chapter 4

4 Nitrogen effects on diet choice and habitat use by the meadow vole, *Microtus pennsylvanicus*

4.1 Introduction

Resource quality is one of the most important components of plant-animal interactions (Stiling and Moon 2005). Although traditional foraging theory suggests that herbivore feeding dynamics are governed primarily by energy requirements (Charnov 1976, Belovsky 1986), plant nitrogen availability may also play a critical role (White 1993). Nitrogen acquisition is an important component of the feeding dynamics of herbivores (Mattson 1980), particularly because they depend on plants as a source of essential amino acids (Dadd 1973). Likewise, plant N concentration is positively related to important forage nutritional components such as protein content, dry matter digestibility, and digestible energy (Mårell et al. 2002). In addition to direct dietary implications, plant quality can have important ecosystem-level consequences, because herbivore density (Grant et al. 1977) and community composition (De Sassi et al. 2012), as well as foraging dynamics (Day and Detling 1990, Throop and Lerdau 2004) are often related to N availability.

Rodent herbivores are strongly influenced by variation in resource quality (Schetter et al. 1998, Galende and Raffaele 2012). In general, herbivore feeding responses to increased N availability can be attributed to increased tissue nitrogen content (Hartley et al. 1995, Ball et al. 2000, Throop and Lerdau 2004), although changes in the concentration of secondary defense compounds may also contribute to fertilization effects (Rousi et al. 1993). Implications of high protein diets include increased body mass and earlier sexual maturation (Cameron and Eshelman 1996), as well as increased reproductive success (White 1978). Evidence from diet choice experiments suggests that voles respond to the fertilization of shrubs and tree seedlings (Hartley et al. 1995, Strengbom et al. 2003). Overall, however, graminoids represent a much larger component of the vole diet than woody species (Thompson 1965). Although N addition can significantly influence grass palatability in other species of small mammalian herbivores such as hares (Paupério and Alves 2008, Somers et al. 2008), and the effects of variation in other components of grass tissue quality such as silica content have been examined in voles (Massey and Hartley 2006), surprisingly, very little is known regarding vole feeding responses to changes in grass tissue nitrogen. It is likely that voles would be sensitive to changes in grass tissue nitrogen, given that plant N concentration is correlated with meadow vole feeding preferences among plant species (Bergeron and Jodoin 1987, Marquis and Batzli 1989).

In addition to its effects on diet preference, plant tissue quality can influence herbivore habitat use (Batzli and Lesieutre 1991). For instance, meadow voles can discriminate between resource patches of varying quality at relatively small spatial scales, although under conditions of high density this relationship may be replaced by habitat selection at the landscape-level (Oatway and Morris 2007). Nitrogen addition can increase patch visitation by herbivores (Bakker et al. 2005), or alternatively reduce patch use when fertilization leads to a loss of plant diversity or a decline in the quality of preferred forage species (Hall et al. 1991). Furthermore, increases in plant biomass in response to fertilization can influence the use of resource patches for refuge, because voles rely on overhead vegetative cover as a means of protection from avian predators (Mossman 1955). Despite the importance of patch structure to herbivore protection, plant nutritive quality may be more important than sward height in determining plant-herbivore interactions (Somers et al. 2012), highlighting the need to distinguish between N effects on diet choice and microhabitat preference. Likewise, because rodent herbivores contribute substantially to plant community composition (Ostfeld et al. 1997, Howe et al. 2006), understanding these relationships can have important implications for overall ecosystem structure.

Temporal variation in N availability and demand may further complicate the influence of fertilization on plant-herbivore interactions. Consumer dietary nitrogen demands are particularly high during reproduction (Mattson 1980, Awmack and Leather 2002), suggesting that the effects of N addition may vary in relation to breeding periods. Likewise, the importance of grasses in the vole diet varies seasonally (Lindroth and Batzli 1984, Haken and Batzli 1996), and temporal variation in grass responses to N addition (Wherley et al. 2009, Larsen et al. 2012) may influence the relative palatability of grasses collected from the field for use in diet experiments. Ideally, plant material collected from the field for feeding experiments should be routinely clipped in order to mimic the high quality re-growth preferred by hares and voles, but even in these cases, foliar protein content can vary significantly over time (Somers et al. 2012).

The objective of this study was to determine the effects of N addition on grass palatability and resource patch selection by the meadow vole, *Microtus pennsylvanicus*, in a temperate old-field using a combination of feeding trials and tracking tunnel observations. Based on the assumption that N addition would increase plant tissue N content, I predicted N addition would increase both grass palatability and microhabitat preference in the field. I explored temporal variation in N effects on herbivore dynamics by repeating the feeding trials and tracking tunnel observations multiple times across the plant growing season. I predicted that vole responses to grass N addition would be highest from early- to mid- growing season, when the grasses grow most actively.

4.2 Methods

4.2.1 Study site and forage preparation

The study site was located in a temperate old field in London, ON, Canada (43°1′46″ N, 81°12′52″ W). Previously used for agriculture, this site was sown with the perennial grasses Kentucky bluegrass (*Poa pratensis* L.) and smooth brome (*Bromus inermis* Leyss), and was not mowed or plowed in more than 25 years. Other vegetation at this site included the patchily distributed forbs Canada thistle (*Cirsium arvense* L.) and bird's-foot trefoil (*Lotus corniculatus* L.). The mean annual temperature for the site is 7.5°C with a low monthly mean of –6.3°C (January) and a high monthly mean of 20.5°C (July), and mean annual precipitation of 818 mm, with a low monthly total of 61 mm (February) and a high monthly total of 97 mm (December) (1971-2000 Climate Normals, National Climate Data and Information Archive, Environment Canada).

In May 2010, three 1-m^2 N-fertilized plots were established at the site to generate forage material for the feeding experiments. Each forage plot received 2 g of N in the form of aqueous ammonium nitrate (2 L) in early June, July, and August for a total of 6 g N. This amount was chosen to match the addition rate used in the global change field experiment already established at this field site (see details below). An equivalent amount of de-ionized water (2 L per plot) was added to each of three non-fertilized forage plots, which were separated from nitrogen plots by a 1 m^2 buffer zone. Plot vegetation was kept short (approximately 3 cm) using a weed trimmer in order to maintain grass in a state of high quality re-growth similar to that of grass tillers fed upon by voles. Trimmed material was removed from all plot surfaces following each clipping event. Likewise, plot litter was removed before each clipping and subsequently replaced to maintain humidity at the soil surface. Approximately two weeks following each nitrogen application, both *P. pratensis* and *B. inermis* material was cut and collected by hand for use in cafeteria-style feeding experiments. A sub-set of the grass material was oven-dried for 72 hours at 65 °C in order to estimate initial dry weights of the material used for the feeding trials. Nitrogen content of the dried control material was quantified using a Kjeldahl digestion procedure modified from Shirai and Kawashima (1993). Briefly, grass material was digested in concentrated sulfuric acid with zinc powder, and NH_4-N in each digest was quantified colormetrically using a SmartChem 140 Discrete Autoanalyzer (Westco Scientific, CT, USA).

4.2.2 Patch use trials

Patch use trials were developed as part of a larger global change field experiment already established at the study site. The field experiment consisted of two temperature treatments (warmed and control), crossed with two levels of nitrogen addition (N fertilized and control) for a total of four treatment combinations. These treatments were applied to 1-m^2 circular plots distributed among ten blocks for a total of forty treatment plots; see Turner and Henry (2009) for full experiment details. Briefly, ceramic infrared heaters were used to warm the treatment plots by approximately 2-3°C at the soil surface, while nitrogen was added annually in two forms; a 2 g m^{-2} pulse of aqueous ammonium nitrate added at snowmelt in early-spring, while 4 g m^{-2} of slow-release ammonium nitrate pellets was added during early-summer. The application rate was based on estimates of increased atmospheric N deposition expected for this area by the year 2050

(Galloway et al. 2004). For the purpose of my experiment, each treatment plot was considered to be a resource patch. Although I did not anticipate that warming would have a direct effect on vole patch choice, I included these plots in my experiment because a previous study revealed that warming significantly increased plot biomass (Hutchison and Henry 2010), which in turn could influence the use of plots by voles based on the quantity of vegetative cover. In order to quantify meadow vole activity within resources patches tracking tunnels were constructed, with each consisting of a white tracking card and ink pad housed within a section of aluminum downspout measuring 35 × 8 × 6 cm. Once per month from June until September 2010, one tunnel was placed on the soil surface in each of the forty resource patches and retrieved following a 24 h period. Meadow vole prints were identified and track counts were used as an estimate of relative vole activity within resource patches.

4.2.3 Vole collection and feeding assays

A grid consisting of thirty Longworth traps was established across the entire field site (50 m × 75 m) to collect meadow voles for feeding trials, as well as to determine vole densities using the Schnabel method (Sutherland 1996). Each trap contained cotton bedding and was baited with sunflower seeds. Trapping for the purpose of density estimates occurred once per month, with each event consisting of three consecutive trapping days. Traps were set in the early evening and inspected the next morning at approximately 09:00 am. Prior to the initiation of monthly trapping events, trapping grids were set and locked open for three consecutive days in order to allow animals to become familiar with the presence of the traps. During feeding trial periods, captured voles were transported back to the university animal holding facility. A total of 15 male and 11 female meadow voles were used in the feeding trial assays, with average weights of 41.3 g and 33.9 g, respectively. At the holding facility the animals were fed a combination of dandelions and grass from the field site during a three day acclimation period, and were also provided access to water ad libitum. The light cycle was held at a constant 16L:8D for the duration of the experiment. For cafeteria feeding assays, voles were provided either a *P. pratensis* or *B. inermis* diet consisting of both N fertilized and non-fertilized material, with individual trials being terminated once voles had removed roughly 75% of either material as determined by visual estimation. Diet placement within each cage was randomly assigned to one of two locations in order to avoid positional bias. Following trial termination, all remaining material was oven dried at 65 °C for 72 h in order to determine consumption on a dry weight basis. A second set of trials was conducted 24 h following the termination of the first set. Values obtained over the two-day period were averaged within replicates (i.e. for each vole). Animals were returned to the field site once the experiment was concluded. Feeding experiments were repeated three times (once in June, July, and August) with no animal being used in more than one month. This research conformed to ASM guidelines as outlined by Sikes and Gannon (2011). All procedures were performed according to protocols specified within Animal Use Protocol 2010-043 granted to Dr. Hugh Henry by Animal Care and Veterinary Services at Western University. Meadow vole live capture was approved by the Ministry of Natural Resources, authorization number 1056963.

4.2.4 Statistical analyses

There was no significant difference between the consumption of *P. pratensis* and *B. inermis* material (P-values for June and July were 0.52 and 0.96, respectively; no analysis was performed for August because there was insufficient *B. inermis* material to perform species comparisons), so relative palatability values were pooled over grass species. For each of the three grass palatability trials, t-tests were performed to determine the effect of N addition on relative grass consumption. Because fertilized and non-fertilized consumption proportions sum to a value of 1 for each replicate, I compared fertilized grass consumption values to a hypothetical mean of 0.5, which represented zero feeding preference. I also performed t-tests to determine if nitrogen fertilization of forage plots significantly influenced grass N concentration. For each of the four resource patch use trials, a non-parametric Friedman test was conducted to determine if meadow vole track counts varied among treatment plots. T-tests were conducted using JMP 4.0 (SAS Institute), while Friedman tests were performed using SPSS 14.0 (Systat).

4.3 Results

4.3.1 Feeding trials

In the June feeding trial, the average consumption of the fertilized grasses was nearly double that of the control grasses (67% vs. 37%; p=0.02; Fig. 4.1), but there were no significant effects of N on diet choice in either July or August. Grass tissue N concentrations increased in response to N addition from 2.4% to 3.1% in June (p=0.006) and from 2.2% to 2.7% in July (p=0.01), but there was no significant difference among treatments in August (Fig. 4.2).

4.3.2 Density measurements and track counts

Based on Longworth trap data collected over summer 2010, the average meadow vole density across the entire field site was 239 voles/ha. In addition to meadow voles, other herbivorous rodents caught at this site included deer mice (*Peromyscus* spp.). However, trapping patterns suggest that deer mouse distributions were restricted to woodland habitats adjacent to the old field system. Tracking tunnels placed within experimental resource patches revealed that vole track counts were highly variable, with between 0 and 32 counts per card. However, there were no significant differences in counts among treatments during any month of the experiment (Table 4.1).

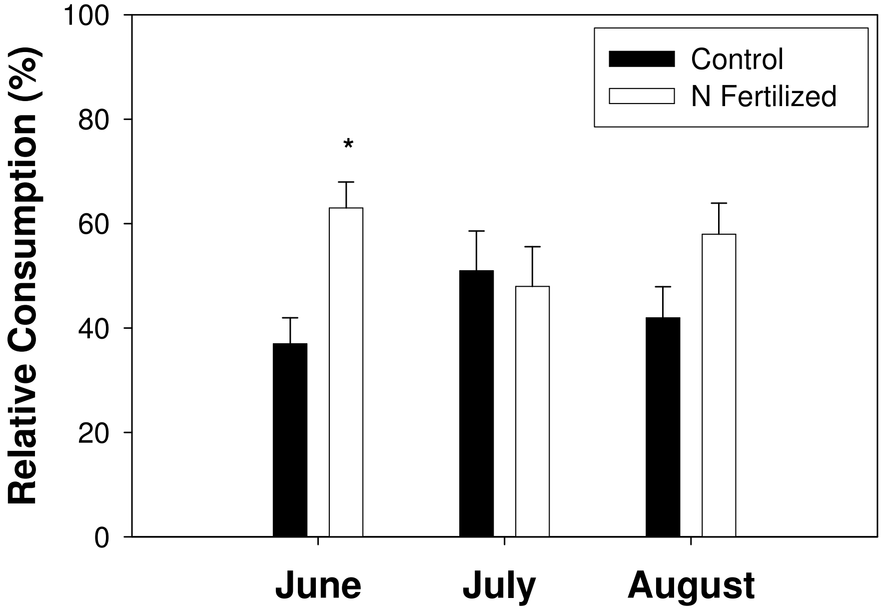

Figure 4.1. Effects of N addition on grass palatability to meadow voles. Bars represent mean relative consumption values (±SE, n=8, 10, 8 for June, July, and August, respectively). Asterisk denotes within-month significant difference between the relative consumption of fertilized grass and a hypothetical value of 0.5 representing zero preference.

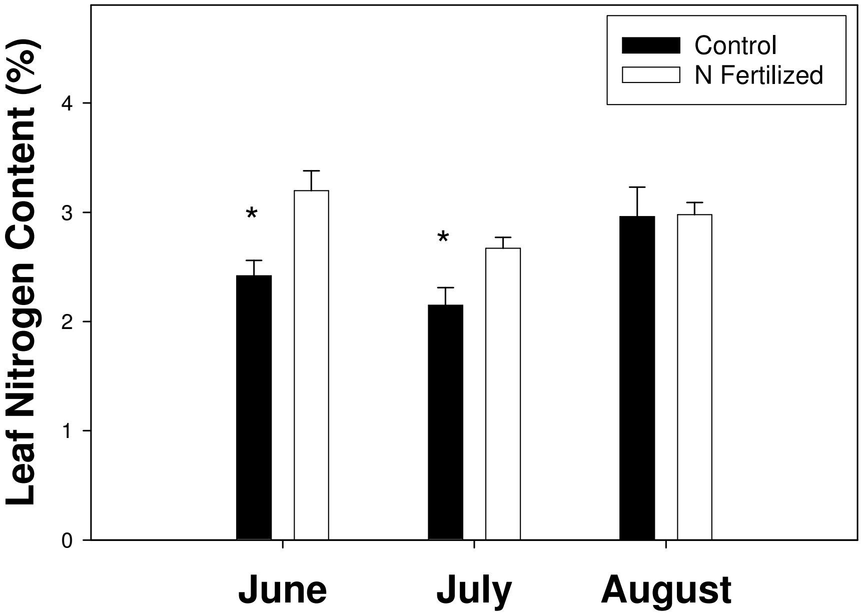

Figure 4.2. Effects of N addition on grass diet nitrogen content. Bars represent mean N concentration values (±SE, n=7, 7, and 6 for June, July, and August, respectively). Within-month significant differences are denoted by an asterisk.

Table 4.1. Summary of Friedman test P-values for warming (W) and nitrogen (N) effects on the number of meadow vole tracks (mean ± SE) in a plot-level field experiment set in a temperate old field. Data are arranged by month.

	Treatments					
	Control	W	N	W/N	X^2-value	P-value
June	4.89 (±1.37)	5.4 (±1.88)	5.44 (±1.18)	2.44 (±1.32)	0.5	0.92
July	0	4.5 (±1.97)	3.1 (±1.31)	4.56 (±1.54)	5.12	0.16
August	4.8 (±2.15)	2.1 (±1.45)	2.3 (±1.55)	0.8 (±0.8)	3.188	0.36
September	11.5 (±1.23)	3.4 (±2.56)	2.7 (±1.56)	4 (±2.27)	7.657	0.054

Degrees of freedom = 3 for each month

4.4 Discussion

4.4.1 Grass palatability trials

Despite the importance of energy acquisition, herbivore feeding dynamics can also be determined by the maximization of nutrient intake (Simpson et al. 2004), an idea supported by evidence that forage quality has a strong influence over herbivore diet choice (Mattson 1980, Stiling and Moon 2005). Available N in plant tissue is often low relative to herbivore N demand (White 1993), and positive herbivore responses to nitrogen fertilization are often associated with increased plant tissue N content (Ball et al. 2000, Throop and Lerdau 2004). My feeding experiments revealed that nitrogen addition significantly increased grass palatability to meadow voles in the June feeding trial (Fig. 4.1). This result is consistent with previous studies that have demonstrated positive responses of voles to the N fertilization of a variety of woody plants, including bilberry (Strengbom et al. 2003), as well as heather shrubs and Sitka spruce seedlings (Hartley et al. 1995). Also, as described earlier, interspecific variation in plant N content is significantly related to forage species preferences in meadow voles (Bergeron and Jodoin 1987, Marquis and Batzli 1989).

My observation that voles only preferred N fertilized grasses early in the summer was consistent with results from an exclosure experiment (Moise and Henry 2012) previously conducted at my field site. In the latter study, the exclosure effect increased in response to N addition, but only for the June sampling period, which could be explained by increased feeding by voles in the N addition plots at that time. The grass tissue N analyses I conducted in this chapter revealed that the effect of N addition on tissue N concentration was also highest early in the summer (Fig. 4.2), which coincides with the time when these cool-season (C3) grasses are most active, prior to seed set and the senescence of many leaves in July (Hutchison and Henry 2010). Although increased herbivore N demand may reflect greater nitrogen requirements during reproductive periods (Mattson 1980, Awmack and Leather 2002), meadow voles breed throughout both the spring and summer (Turner and Iverson 1973), and therefore breeding period would not explain why I observed meadow voles respond to increased grass N content in the June feeding experiments but not in July. It is possible that despite the statistically significant effect of N addition on tissue N content in July, the overall increase in tissue nitrogen content at that time was not biologically significant with respect to vole feeding preferences (grass nitrogen content in response to N addition was 2.7% in July, compared to 3.2% in June). This explanation is consistent with the finding of Hjältén et al. (2008) that differences in tissue N content between high N (2.8%) and low N (2.3%) poplar diets were too subtle to elicit a feeding response from *M. pennsylvanicus*.

Overall, my results suggest that increased forage palatability is likely an important mechanism governing meadow vole responses to changes in N availability, a finding that is particularly important given the current lack of knowledge regarding microtine foraging responses to variation in grass N content. Furthermore, differences in N effects on vole feeding preferences observed across the growing season suggest that both the community- and ecosystem-level consequences of changes in nitrogen availability on patterns of herbivory are likely subject to temporal variation in intraspecific plant nutrient availability.

4.4.2 Resource patch selection

Soil nutrient availability and the resulting changes in plant tissue quality can influence vegetation patch use by herbivores (Batzli and Lesieutre 1991, Treydte et al. 2011). In particular, herbivores may be attracted to patches in response to increased foliar N concentration (Drent and Van der Wal 1999, Bakker et al. 2005); however, I did not observe an effect of nitrogen fertilization or warming on meadow vole activity (Table 4.1). The scale of my patch manipulations (each block consisted of 1-m^2 plots, spaced approximately 1 m apart) was small relative to the extensive home range of meadow voles in grass dominated systems (~800-2000 m^2; Blair 1940). Therefore, it is possible that a larger patch size would be needed to influence vole activity. Although meadow voles possess some ability to select habitat at relatively small spatial scales, as population size increases space-use dynamics are predominantly determined at the landscape-scale by density-dependent processes such as conspecific interactions (Oatway and Morris 2007), and there was a relatively high population density over the experimental period. Ultimately, intense intraspecific competition may have necessitated the use of lower quality resources by subordinate individuals regardless of relative patch fitness value. Furthermore, regardless of density, herbivore interaction with less preferred patches may have occurred simply due to their proximity to high quality neighboring vegetation (Baraza et al. 2006).

The lack of a treatment effect on vole activity may have alternatively resulted from meadow voles exhibiting greater sensitivity to nutrients other than N in the field. In addition to N content, plant phosphorous availability is positively related to herbivore foraging preferences (Schütz et al. 2003) as well as herbivore abundance (Apple et al. 2009). Likewise, increased meadow vole activity has been observed following the fertilization of patches with sodium phosphate (Inoye et al. 1987), possibly owing to the positive relationship between diet sodium content and vole reproductive success (Batzli 1986).

Shifts in vegetation stature in response to fertilization can negate potential N effects on resource patch use. Grass biomass increased significantly in response to N addition in the field plots where I ran my experiment (Kim and Henry, in press), and the C:N ratio of grasses increases with increased tiller height (Novoa and Loomis 1981, Belanger and Gastal 2000). This growth dilution of nutrients can result in no increase in tissue N concentration, despite increases in total plant N. However, the grass collected from the field for the feeding trials was mowed, which meant that N fertilization could not result in large increases in tiller height. Although increased plot biomass and vegetation height may benefit small herbivores by visually obstructing predators (Mossman 1955, Koivunen et al. 1998), it does not always result in greater patch use (van de Koppel et al. 1996, Iason et al. 2002). Furthermore, the voles may have been relatively insensitive to increases in aboveground biomass in the N addition plots because a thick (approximately 5-10 cm deep) litter layer provided cover from predators across most of the site.

Overall, my tracking tunnel data suggest that N addition had no effect on relative meadow vole activity across resource patches at my scale of observation, although this may have resulted from the overriding effects of additional components of vegetation quality, or possibly density-dependent herbivore population dynamics. The arrangement of resource patches used for this study represents what is typical for many plot-level global change field experiments in grass-dominated systems. Therefore, my findings may be applicable to similar field manipulations subject to small mammal herbivory. Although tracking tunnel observations may highlight potential herbivore influences on overall plant responses to treatment effects within the context of plot-level global change experiments, they are unlikely to predict changes in herbivore population dynamics under future environmental scenarios *per se*, because animals may simply congregate within preferred resource patches in plot-level field manipulations (Moise and Henry 2010). Inferences from adaptive habitat selection strategies may provide a more useful approach for predicting shifts in herbivore population dynamics in response to drivers of global change (e.g. Morris et al. 2011).

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Chapter 5

5 Interactive responses of grass litter decomposition to warming, nitrogen addition, and detritivore access

5.1 Introduction

Responses of soil processes remain a major uncertainty in attempts to predict ecosystem responses to global change (Pendall et al. 2008). Changes to the dynamics of plant litter decomposition are an important component of these responses (Singh and Gupta 1977, Vitousek 1982), with the turnover of plant litter contributing to C flux between terrestrial and atmospheric pools (Chapin et al. 2002). Litter turnover also influences primary productivity because C and N mineralization are closely linked (McGill and Cole 1981), and litter can modify soil microclimate (Beatty and Sholes 1988, Deutsch et al. 2010), and interfere with the establishment of newly germinated plants (Bosy and Reader 1995).

Overall, litter mass loss is correlated with climate, tissue chemistry and soil biology (Swift et al. 1979, Aerts 1997), all of which can be influenced by drivers of global change, such as climate warming and nitrogen deposition (Knorr et al. 2005, Aerts 2006, Blankinship et al. 2011). Warming effects on decomposition can occur directly through changes to microbial activity and extracellular enzyme activity (Wan et al. 2007, Brzostek et al. 2012), and indirectly as a result of soil drying (Allison and Treseder 2008) or changes to plant tissue quality (An et al. 2005). Likewise, nitrogen addition can directly alter microbial activity (Carreiro et al. 2000), or may influence litter chemistry, altering its subsequent decomposition (Henry et al. 2005).

In addition to the influence of bacteria and fungi on organic matter decomposition (Pritchard 2011), soil detritivores also contribute substantially to litter breakdown (Swift et al. 1979, Wolters 2000, Wall et al. 2008). Soil fauna can respond to warming and N addition (Hättenschwiler and Bretscher 2008, Blankinship et al. 2011, Holmstrup et al. 2012), but the magnitude and direction of these responses can differ considerably from those of microbes. For example, in response to N addition, soil fauna can be sensitive to ammonium toxicity (Wei et al. 2012) or salt desiccation (Lohm et al. 1977). Furthermore, the combined effects of factors such as warming and fertilization on soil organisms can vary substantially from their individual effects (Sjursen et al. 2005). Evidence from microcosm experiments suggests that decomposition responses to global change treatments can be strongly influenced by detritivore effects (Coûteaux et al. 1991, Rouifed et al. 2010). Nevertheless, many studies examining global change effects on decomposition often exclude soil fauna, and macrofauna in particular, which could not only result in an underestimate of litter turnover, but the effects of the latter could interact with the global change factors (Wall et al. 2008).

The time scale of responses may further complicate the relationship between abiotic and biotic controls over decomposition. For example, positive responses of C turnover to warming can occur shortly after litter incubation (Xu et al. 2012b), yet changes in carbon pools, microbial biomass and carbon use efficiency can negate the effect of warming over longer time scales (Bradford et al. 2008, Allison et al. 2010). Likewise, despite short-term positive effects of N addition on the decomposition of newly senesced litter, the turnover of recalcitrant material that accumulates as decay progresses can be slowed by N addition (Fog 1988), possibly as a result of the inhibition of ligninolytic enzyme activity (Carreiro et al. 2000). The responses of detritivores to variation in substrate quality can differ substantially from those of microbes (Scheu and Schaefer 1998), suggesting that these two groups may be differentially affected by changes in litter chemistry during decomposition. In addition, because the composition of soil fauna communities changes seasonally (Levings and Windsor 1996, Zhu et al. 2010), temporal differences in treatment effects can result from interspecific variation in detritivore sensitivity to factors such as warming and drought (Kardol et al. 2011).

The objective of this study was to determine the interactive effects of warming, nitrogen addition and detritivore access on litter mass loss, both at six weeks post-incubation and after one year, in a global change experiment conducted in a temperate old-field. Based on the assumption that the responses of detritivores and microbes to warming and N addition would vary, I predicted there would be interactive effects of the global change treatments and detritivore manipulation on litter mass loss. To further explore the mechanisms (i.e. effects of litter origin and microenvironment) explaining mass loss responses in the global change experiment, I transplanted litter collected from the treatment plots into a common environment, and also transplanted untreated litter into the treatment plots. I predicted that the additive effects of the litter origin and microenvironment incubations would equal the integrated response of treated litter incubated in the treatment plots.

5.2 Methods

5.2.1 Study site

The experiment was conducted at an old field site in London, ON, Canada (43°1′46″ N, 81°12′52″ W) between October 2010 and October 2011. The site is a former agricultural field that has not been plowed or mowed in over 25 years. Dominant vegetation at the site includes two grass species, Kentucky bluegrass (*Poa pratensis* L.) and smooth brome (*Bromus inermis* Leyss.), with the forb Canada thistle (*Cirsium arvense* L.) and legume bird's-foot trefoil (*Lotus corniculatus* L.) present in patches. The mean annual temperature for the site is 7.5°C (8.1°C over the experimental period), with a low monthly mean of –6.3°C (January) and a high monthly mean of 20.5°C (July), and mean annual precipitation of 981 mm (1100 mm over the experimental period), with a low monthly total of 61 mm (February) and a high monthly total of 97 mm (December) (Canadian Climate Normals 1971–2000, Environment Canada, National Climate Data and Information Archive). The soil is classified as silt loam glacial till (Hagerty and Kingston 2011), with pH 7.6 and composed of approximately 50% sand, 41% silt, and 9% clay (Bell et al. 2010).

5.2.2 Warming and N addition experiment

Warming and N addition treatments were applied to 1 m^2 circular plots set up in a factorial block design, including two levels of warming (ambient and heated) crossed with two nitrogen treatments (control and N-fertilized). All four treatment combinations were replicated across ten different blocks for a total of 40 plots; see Turner and Henry (2009) for full details of the experiment. Increased temperature of approximately 2-3°C at the soil surface was achieved using 150 W ceramic infrared heaters (Zoo-Med Laboratories, San Luis Obispo, CA, USA) which mimic solar heating without the production of photosynthetically active radiation (Shen and Harte 2000). The plots were warmed continuously since late 2006. Soil temperature and moisture data were collected using 107-BAM-L temperature probes and CS-616 time domain reflectometers, respectively (Campbell Scientific Corp.). Nitrogen treatments began in early 2007 and were added annually in two forms: a 2 g m^{-2} pulse of aqueous ammonium nitrate added at snow melt in early-spring, and 4 g m^{-2} of slow-release ammonium nitrate pellets added in early-summer. Application rate was based on estimates of increased N deposition expected for this area by the year 2050 (Galloway et al. 2004).

5.2.3 Sample collection and litter bag design

In early September 2010, senesced tissue of both *Poa pratensis* and *Bromus inermis* was collected from the global change treatment plots, as well as from similar areas in the same field outside of the plots. Only standing dead material was collected in order to avoid including loose surface litter from previous years' growth. Material was pooled for each treatment and allowed to air dry for three days at room temperature. A sub-set of control samples was dried at 65 °C for three days in order to estimate initial sample dry weights prior to field incubation.

Litter subsamples were prepared for the decomposition experiment with a consistent 3:1:1 ratio of *B. inermis* stems to *B. inermis* leaves to *P. pratensis* tillers in order to standardize litter content among subsamples. Two different litter presentation techniques were employed in order to manipulate detritivore access to the material. A detritivore restriction treatment was achieved using standard nylon mesh litter bags (10 × 5 cm bag size, 100 µm mesh size, hereafter referred to as ‘mesh’ samples), which allowed for the exclusion of both meso and macro soil fauna. In order to allow free access to litter by detritivores, a second set of samples was constructed without the use of mesh by securing both ends of each litter sample using spring-loaded paper clamps (hereafter referred to as ‘open’ samples). Two mesh samples and two open samples were placed in each plot. The method of using bags with larger mesh sizes in order to allow detritivore access was not appropriate for my purposes because individual grass pieces were thin, and larger holes would have resulted in the loss of material. Litter pieces used for open samples were approximately 9 cm in length, similar to the length of pieces used in the mesh bags. Roughly 5% of the material was obstructed by the clamp contact points and hence restricted from detritivore access. To identify potential differences in the direct effects of the mesh bags vs. clamps on litter decomposition, control incubations were also conducted in the field to quantify the mass loss of mesh and open samples in the absence of soil fauna (through the use of plastic exclosures), both over six weeks (n=5) and over one year (n=5). The control incubations were based on an approach suggested by Bradford et al. (2002) for testing for the effects of different mesh sizes on mass loss in the absence of soil fauna.

5.2.4 Litter incubation

Litter samples (both mesh and open) removed from the experimental plots were subsequently incubated in their corresponding treatment plots (e.g. litter from N addition plots was returned to the N addition plots). In order to clarify the mechanisms (i.e. litter origin versus environmental conditions) underlying the treatment effects on litter mass loss, in addition to the aforementioned non-transplanted ‘integrated response’ samples, litter transplants were used to achieve two alternative incubation configurations: a ‘litter origin’ incubation, which involved the transplantation of litter from global change treatment plots into a common garden in the field, and a ‘microenvironment’ incubation, which involved the transplantation of untreated litter from the surrounding field into the global change plots. For all incubations, litter samples were placed in direct contact with the soil surface and then covered using the loose litter that had been displaced to insert them. Litter samples were recovered from the field at two different time periods; one following six weeks of incubation, and another after one year. Following the incubation period, litter samples were oven-dried at 65°C for three days and then weighed. Final dry weights were compared with the water mass-corrected initial air dry weights in order to determine % mass loss.

5.2.5 Data analyses

For each of the six combinations of the two sampling dates and the three incubation configurations, I used three-way fixed-effects ANOVAs to test for the response of mass loss to the effects of the between-subjects factors (N addition, warming, and detritivore access) as well as their interaction terms. For soil temperature and moisture I used two-way fixed-effects ANOVAs to test for the effects of warming and N addition, followed by Tukey's tests to determine significantly different treatment combinations. All data distributions were checked for normality by visual examination of histograms. My analyses were conducted using the Fit model platform in JMP 4.0 (SAS Institute). I used % mass loss as the dependent variable which was calculated as 1- (final dry mass/initial dry mass).

5.3 Results

5.3.1 Integrated response incubations

After six weeks of decomposition (Fig. 5.1a), mass loss varied on average from 13% to 23%. There was 4% greater mass loss in N fertilized plots than in control plots (p=0.0004, Table 5.1; all percent treatment effects are reported as absolute changes in % mass loss). Additionally, there was a significant interaction between warming and detritivore access (p=0.0007), with open samples experiencing 5% greater mass loss than mesh samples in ambient temperature plots, but not in heated plots, and open samples in ambient temperature plots experiencing 5% higher mass loss than open samples in heated plots. Heated plots experienced a significant decrease in soil moisture (Table 5.2). Control incubations confirmed that there were no direct effects of mesh bags vs. clamps on mass loss after six weeks (p=0.297; Open = 24.46% (±0.02%), Bag = 27.29% (±0.02%)).

After one year (Fig. 5.1b), mass loss varied on average from 47% to 75%. There were significant increases in mass loss of 4%, 16%, and 20% with warming ($p=0.017$), nitrogen addition ($p=0.004$) and detritivore access ($p<0.0001$), respectively, although these main effects were not completely additive (there was a significant three-way interaction; $p=0.032$). There was no significant effect of heating on mean soil temperature over one year (Table 5.2), because with increased melting of snow cover, the heated plots were exposed to cold air temperatures and were often colder than the ambient plots over winter. Control incubations confirmed that there were no direct effects of mesh bags vs. clamps on mass loss after one year ($p=0.638$; Open = 55.11% (±0.03%), Bag = 57.29% (±0.03%)).

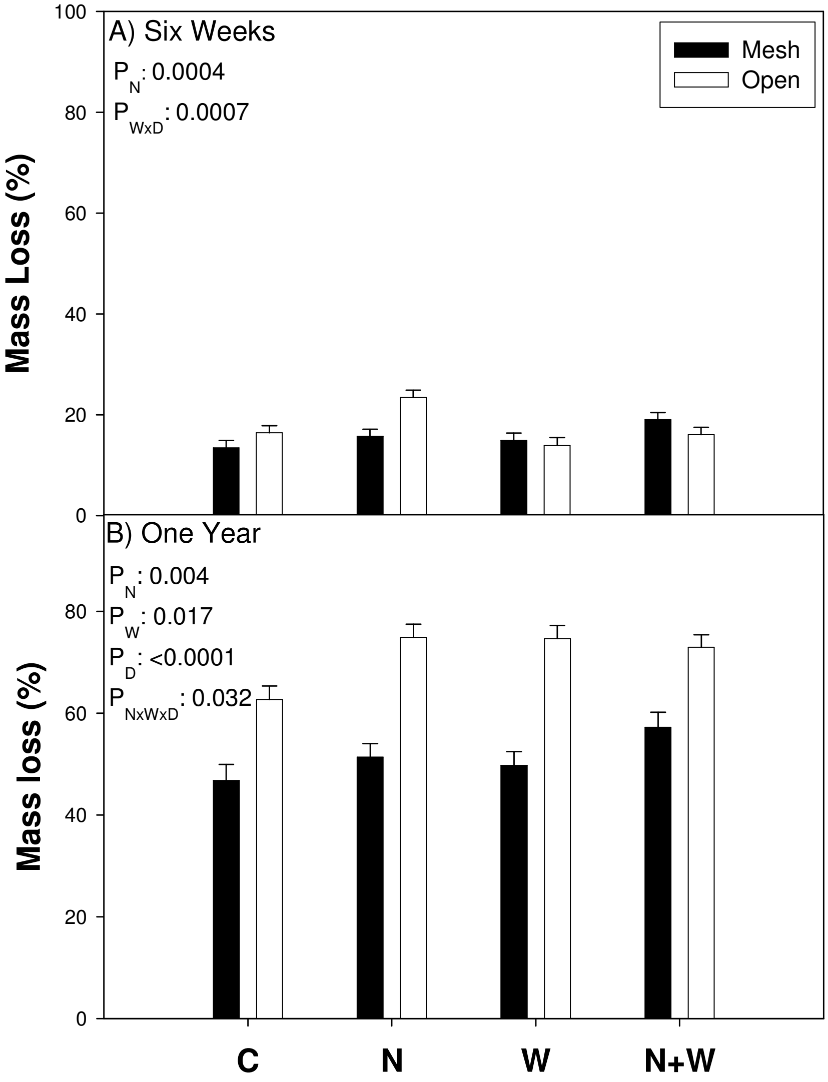

Figure 5.1. Interactive effects of N addition, warming, and detritivore access on litter mass loss (%) in the integrated response incubation after a) six weeks and b) one year. Bars represent means ± SE, with significant 3-way ANOVA results presented in the upper- left corner of the figure.

Table 5.1. Summary of ANOVA P-values for the effects of global change and detritivore access treatments on litter % mass loss in the field. Data are arranged by incubation regime nested within the six-week (Oct-Nov 2010) and one-year (October 2010-2011) sampling periods**.**

	Six-Week Incubation			One-Year Incubation		
	Integrated	Litter Origin	Microenvironment	Integrated	Litter Origin	Microenvironment
$W_{(1,67)}$	0.222	0.03*	0.437	0.017*	0.427	0.926
$N_{(1,67)}$	0.0004**	0.008**	0.793	0.004**	0.356	0.952
$D_{(1,67)}$	0.118	0.571	0.002**	<0.0001***	0.011*	<0.0001***
$WxN_{(1,67)}$	0.469	0.962	0.425	0.159	0.94	0.078
$WxD_{(1,67)}$	0.0007**	0.804	0.72	0.877	0.755	0.387
$NxD_{(1,67)}$	0.517	0.018*	0.024*	0.83	0.28	0.875
$WxNxD_{(1,67)}$	0.116	0.158	0.827	0.032*	0.421	0.937

Degrees of freedom are displayed in parentheses after the effects

W Warming, *N* Nitrogen, *D* Detritivore Access

Significance *0.05–0.01, **0.01–0.001, ***<0.001

Table 5.2. Summary of warming and N addition effects on mean (±SE) soil temperature (5 cm depth) and moisture (0-15 cm) measurements recorded in the global change field experiment after six weeks (Oct-Nov 2010) and one year (October 2010-2011) of litter incubation. Significant differences within a sampling period are denoted by different letter combinations for each soil variable.

	Soil Temperature (°C)		Soil Moisture (vol/vol)	
	Six Weeks	One Year	Six Weeks	One Year
Control	6.5 (±0.1)^{a}	10.0 (±0.1)	0.305 (±0.007)^{a}	0.316 (±0.006)^{a}
W	7.5 (±0.2)^{b}	10.3 (±0.2)	0.286 (±0.005)^{b}	0.301 (±0.005)^{b}
N	6.9 (±0.1)^{ab}	10.1 (±0.1)	0.304 (±0.004)^{a}	0.316 (±0.005)^{a}
WxN	7.1 (±0.2)^{b}	10.3 (±0.1)	0.284 (±0.007)^{b}	0.298 (±0.006)^{b}

W warming, N Nitrogen Addition

5.3.2 Litter origin incubations

For the litter origin incubations, after six weeks (Fig. 5.2a) there was a significant interaction between nitrogen addition and detritivore access (p=0.018), with material obtained from N fertilized plots experiencing 10% greater mass loss than material from non-fertilized plots when samples were open to detritivores, but not when detritivores were restricted. There was also increased mass loss of 4% in litter from warmed plots (p=0.03) and increased mass loss of 5% in litter from nitrogen addition plots (p=0.008). After one year (Fig 5.2b), detritivore access increased mass loss by 6% (p=0.011), but none of the other treatment effects were significant (Table 5.1).

5.3.3 Microenvironment incubations

For the microenvironment incubations, after 6 weeks (Fig. 5.3a) there was a significant interaction between nitrogen addition and detritivore access (p=0.024), with the mass loss of open samples double that of mesh samples in non-fertilized plots, but not in N-fertilized plots. Open samples also experienced 6% greater mass loss than mesh samples (p=0.002). After one year (Fig. 5.3b), detritivore access increased mass loss by 21% (p<0.0001), but there were no other significant treatment effects (Table 5.1).

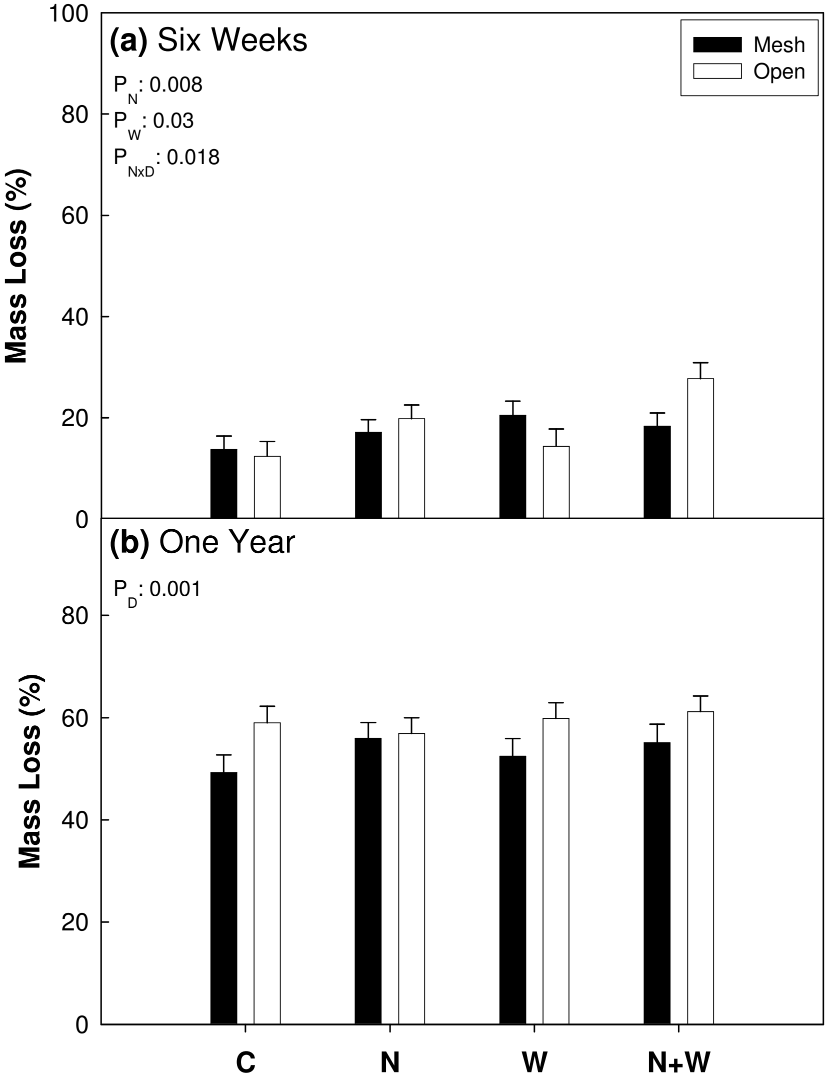

Figure 5.2. Interactive effects of N addition, warming, and detritivore access on litter mass loss (%) in the litter origin incubation after a) six weeks and b) one year. Bars represent means ± SE, with significant 3-way ANOVA results presented in the upper-left corner of the figure.

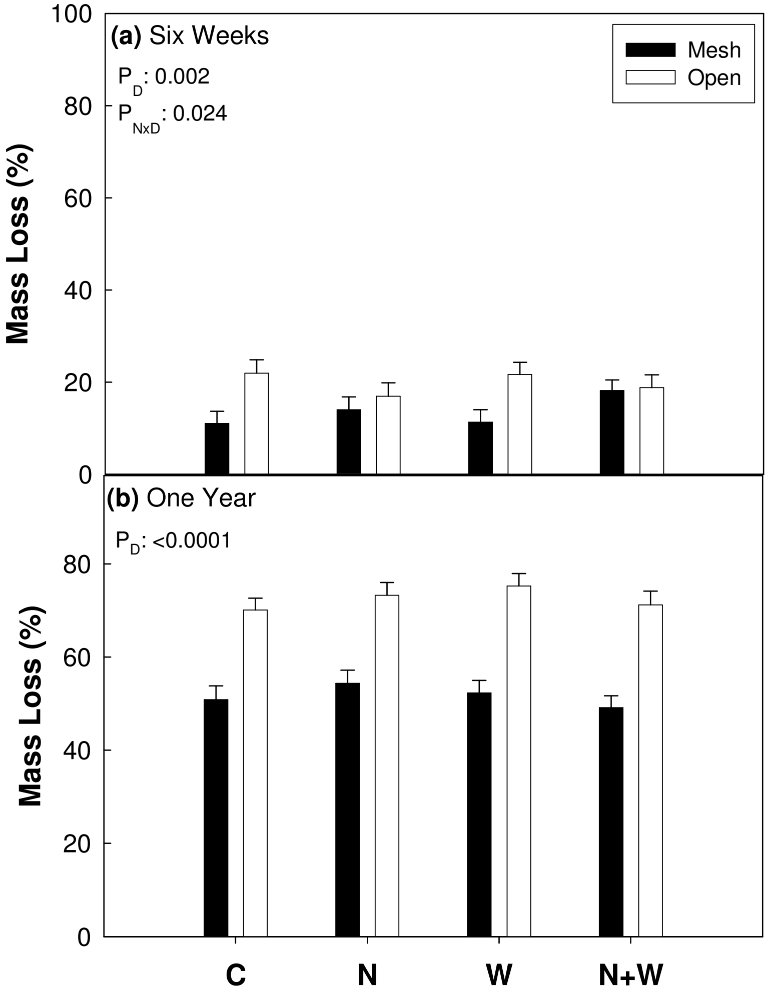

Figure 5.3 Interactive effects of N addition, warming, and detritivore access on litter mass loss (%) in the microenvironment incubation after a) six weeks and b) one year. Bars represent means ± SE, with significant 3-way ANOVA results presented in the upper-left corner of the figure.

5.4 Discussion

5.4.1 Integrated responses after six weeks

Soil fauna can contribute substantially to C cycling, both directly through fractionation and consumption of plant litter, and indirectly through interactions with the microbial community (Swift et al. 1979, Wolters 2000). It follows that alterations to soil faunal assemblages can modify litter turnover responses to drivers of global change (Coûteaux et al. 1991, Rouifed et al. 2010). After six weeks of incubation, open litter samples experienced greater mass loss than mesh samples in ambient temperature plots, but not in heated plots, suggesting that detritivore activities were reduced by warming. In grass-dominated systems, earthworms, mites, and other arthropods are the soil fauna that play the largest role in litter decomposition (Coleman et al. 1990, Bradford 2002, Uyl et al. 2002). Warming and associated decreases in soil moisture can impose climatic stress on the detritivore community (Pritchard 2011), decreasing soil fauna abundance, biomass, and diversity (Briones et al. 1997, Blankinship et al. 2011, Xu et al. 2012a). In my study, soil moisture from 0-15 cm depth was approximately 7% lower in warmed plots than in ambient temperature plots during the six week incubation period (Table 5.2). Given that soil buffers the effects of warming (Lavelle and Spain 2001), more extreme differences were likely present at the soil surface, enhancing desiccation stress for the detritivores.

My observation that samples in N addition plots experienced greater mass loss than samples in non-fertilized plots is consistent with other studies of the effects of N addition on grass turnover (Hunt et al. 1988). However, both the direction and magnitude of N effects on litter decomposition often depend on site-specific factors (Hobbie 2005), with the trend of positive responses in systems exposed to low levels of background N deposition (<5 kg N ha^{-1} y^{-1}; (Knorr et al. 2005). For comparison, the N deposition rate in the region of my study is approximately 6 kg N ha^{-1} y^{-1} (Total Inorganic N Deposition, National Atmospheric Deposition Program, 2006).

5.4.2 Integrated responses after one year

After one year of litter incubation, the negative effect of warming on mass loss for open samples observed after six weeks was no longer present, and there were no significant interactions between detritivore access and the warming and N addition treatments. Acclimation or recovery by the detritivore community may explain such transient effects of warming and drought on C turnover (Balser et al. 2006, Holmstrup et al. 2012). Alternatively, warming effects on C dynamics can vary seasonally (Templer and Reinmann 2011), and the lack of an interaction between warming and detritivore access may reflect an absence of water stress over the spring and summer during the incubation period (precipitation over this time was ~20% above normal). Regardless of the mechanism, mass loss in open samples after one year was 20% greater than in mesh samples, whereas the interaction between warming and detritivore access after six weeks only accounted for a difference of 2%, which likely explains why no residual effects of the six-week response were present after one year. In a previous study conducted in my field experiment, Hutchison and Henry (2010) reported estimates of aboveground net primary productivity ranging from 800 to 1500 g/m^2/y in response to the warming and N addition treatments. Given that the carbon content of aboveground grass tissue at this site is approximately 40% (M. Turner, unpublished results), a 20% increase in mass loss in response to detritivore access following one year of litter incubation is equal to a increased litter turnover of approximately 174 g C/m^2/y. Using these same correction factors, there was an overall positive effect of warming on mass loss after one year equal to increased litter turnover of approximately 28 g C/m^2/y, which is consistent with the general trend of increased decomposition with increased temperature in the absence of drought (Aerts 2006, Butenschoen et al. 2011). The temperature sensitivity of organic matter decomposition may also increase over time in response to declining C quality (Conant et al. 2008).

The positive effect of N addition on litter turnover observed after six weeks remained present after one year of incubation, at which point it was equal to increased litter turnover of approximately 30 g C/m^2/y (see the detritivore access discussion above for the calculation of this estimate). However, at later stages of decomposition, N fertilization can impede the decay of the lignin-dense, recalcitrant fraction of plant litter (Fog 1988) as a result of decreases in microbial ligninolytic enzyme activity (Carreiro et al. 2000). However, lignin content does not always predict long-term N fertilization effects on grass decomposition (Hobbie 2008), possibly because the lignin content of grasses such as *P. pratensis* is relatively low (Holman et al. 2007).

Although mesh litter bags are commonly used to study the dynamics of plant litter decomposition, the accuracy of results can be hindered by artifacts arising from spillage losses and changes to litter microclimate (Suffling and Smith 1974, Kampichler and Bruckner 2009). In my study, there was a risk that such artifacts could be confounded with differences in detritivore access between open and mesh-enclosed samples. As described above, I conducted additional trials using physical exclosures to test for possible differences in litter mass loss between open samples and mesh bags that might be unrelated to detritivore access (e.g. effects of the mesh bags on microbial decomposition or moisture). The lack of a difference between open and mesh sample controls for both time intervals suggests that the differences observed in the main experiment were caused primarily by detritivore access. However, in the case of the open samples, affixing the material at the ends likely obstructed the accessibility of some of this litter to some detritivores. Likewise, because microfauna have a body size <100 μm (Swift et al. 1979), this size class would not have been restricted from the mesh enclosed samples. Therefore, the detritivore access effects I observed are likely a conservative estimate of actual soil faunal effects.

5.4.3 Litter origin and microenvironment incubations

Although interactions between detritivore access and warming were observed for the integrated response incubations after six weeks, for the litter origin and microenvironment incubations there were instead interactions between detritivore access and N addition, but none for warming. With respect to litter origin, N-fertilized plants often exhibit high N content in their senesced tissues (Johnson 1992, Henry et al. 2005), and this N-rich material is often targeted by soil fauna (Hendriksen 1990, Hättenschwiler and Bretscher 2008), which is consistent with my results. N addition also increased mass loss to some extent in mesh-enclosed samples, which is consistent with the stimulation of microbial decomposition by N addition (Coûteaux et al. 1995, Aerts 1997). However, for the untreated litter transplanted into N addition plots detritivore access effects were reduced, suggesting that the added mineral N had a direct negative effect on detritivores. The opposing effects of litter origin and microenvironment on detritivore access effects may explain why no interactions between nitrogen addition and detritivore access occurred for the integrated response incubations. Nevertheless, it remains curious why the warming effects observed for the integrated response incubations were not also observed for the microenvironment incubations.

Following one year of incubation there were significant increases in mass loss with detritivore access, although the effect size for the litter origin incubations was less than a third of that of the other incubations, possibly as a result of low detritivore densities in the area of the field where the common garden plots were established. There were no interactions between N addition and detritivore access, which is similar to the results obtained for the integrated response incubations. Unlike the integrated response results, however, there were no effects of warming or N addition for either the tissue quality or microenvironment incubations. Much like the results obtained after six weeks, these results indicate unanticipated interactions between litter quality and microclimate in determining the integrated decomposition response.

5.4.4 Conclusions

Much of our current knowledge regarding the influence of environmental change on plant decomposition relies on evidence from mesh litter bag studies. As soil fauna may also exert significant influence over litter decay, further quantification of the detritivore response is critical to the understanding of the interactive effects between biotic and abiotic controls on litter turnover (Butenschoen et al. 2011, A'Bear et al. 2012). Interactions between global change and detritivore access treatments observed during the six week incubation highlight important differences between soil faunal and microbial responses that may ultimately influence the dynamics of litter turnover under future environmental conditions. Nevertheless, my data suggest that acclimation of the detritivore community may occur over time, although I cannot rule out the potential role of seasonal shifts in soil fauna composition (Levings and Windsor 1996, Zhu et al. 2010).

Furthermore, simple mechanistic explanations of litter responses in global change experiments (i.e. litter origin, plot microclimate) may not be adequate, as I observed substantial differences between treatment responses in my integrated response, litter origin and microenvironment incubations. Although many of the interactive effects observed in this study were transient, indicating that their long-term implications for ecosystem dynamics may be minimal (legacy effects notwithstanding), the additive effects of warming, N addition, and detritivore access following one year of litter incubation suggest that the omission of soil faunal effects in global change litter bag studies may underestimate the magnitude of overall mass loss substantially.

5.5 Acknowledgements

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Chapter 6

6 General Discussion

Processes such as plant carbon sequestration and litter turnover can be significantly altered by anthropogenic drivers of global change (Breymeyer and Melillo 1991, Gifford et al. 1995), yet the extent to which shifts in plant-herbivore and litter-detritvore relationships contribute to these changes has received little attention. In addition, faunal activities can substantially impede our ability to properly assess the direct responses of plant communities to experimental treatments within the context of plot-level global change field studies (Moise and Henry 2010). The main objective of my Ph.D. research was to quantify the interactive effects of biotic and abiotic controls on productivity and decomposition within the context of a global change field experiment. Furthermore, in order to investigate the potential mechanisms governing the observed responses, I conducted additional experiments to explore the effects of plant tissue quality and habitat microenvironment on both herbivore and detritivore foraging activity. Overall, these studies provided extensive insight into the consequences and underlying mechanisms of the altered plant-animal relationships that occurred in response to the manipulation of temperature and nitrogen availability. While detailed discussions of the experimental observations are included in the preceding chapters, here I synthesize the overall consequences of warming and N enrichment for ecosystem C dynamics, and highlight the potential mechanistic importance of factors such as plant quality and microenvironment, as observed from general trends across studies. In addition, I identify key research foci for future investigation that may further clarify important components of ecosystem responses to global change.

6.1 Global change and ecosystem C dynamics

The results presented in Chapters 3 and 5 indicate that faunal responses to drivers of global change can substantially alter plant productivity and litter turnover, respectively, suggesting that these relationships may have important implications for C-related processes such as carbon assimilation, sequestration, and subsequent mobilization.

Chapter 3 involved the quantification of herbivore exclusion effects on aboveground net primary productivity responses to warming and N addition. Similarly, Chapter 5 investigated the effects of global change on litter processing by soil fauna through the use of physical detritivore exclosures.

6.1.1 Plant-herbivore interactions and plot C dynamics

A key result from Chapter 3 was that the increase in grass biomass in response to the exclusion of rodents was significantly greater in nitrogen fertilized plots than non-fertilized plots. Likewise, the increase in grass biomass in response to the exclusion of molluscs from heated plots was significantly greater than the change in grass biomass when they were excluded from ambient temperature plots. Together, these findings revealed that not only can drivers of global change significantly influence plant-herbivore interactions, but also that these relationships can be further complicated by taxon-specific herbivore responses to experimental treatments. Understanding interspecific variation in community-level responses to drivers of global change is of critical importance for predicting shifts in ecosystem dynamics under future environmental scenarios (Gilman et al. 2010). Because plant biomass production is associated with carbon sequestration (Tilman et al. 2006), the increased quantity of phytomass removed by exaggerated herbivory has important implications for overall C storage dynamics. Despite the potential for both warming (Huttunen et al. 2007) and nitrogen enrichment (Staalduinen et al. 2009) to promote plant compensatory growth following defoliation, in my study significant herbivore effects remained present throughout the entire growing season.

In addition to the quantity of plant tissue removed, the timing of herbivory can have important implications for ecosystem C dynamics. In Chapter 3, grass biomass responses to herbivore exclusion were observed early in the growing season, which is consistent with the feeding habits exhibited by the dominant herbivores present at the field site (e.g. slugs preferentially consume new vegetation such as seedlings; Strauss et al. 2009). The defoliation of immature plants may have disproportionally strong effects on plant fitness as a result of the pre-emption of future phytomass production (Marquis 1992, Peters 2007). Therefore, herbivore effects established early in the growing season would negatively impact plot C storage potential by limiting the biosynthesis of new photosynthetic tissue.

Although both the herbivore access and global change treatments had strong effects on grass productivity, previous studies within the same field experiment revealed that plot C dynamics may be equally sensitive to background climate variability. While both warming and N addition significantly increased plant productivity in 2008, reduced precipitation in 2007 was suggested to inhibit grass responses to the treatment effects (Hutchison and Henry 2010). This explanation is consistent with the positive relationship between terrestrial plant N responses and the quantity of annual precipitation (Xia and Wan 2008). Likewise, grass productivity can be insensitive to temperature manipulation when also subjected to drought conditions (Peñuelas et al. 2004). Therefore, during relatively dry years, when changes to plot vegetation structure or plant N status are absent or otherwise nonsignificant, it is unlikely that herbivore pressure would vary among treatment plots.

Belowground plant-herbivore interactions were not explored as part of this dissertation, but a previous study at the same field site revealed that root biomass decreased in response to warming, and it remained insensitive to N enrichment (Hutchison and Henry 2010). These results suggest that the global change manipulations would, at most, have the effect of minimizing the role of belowground NPP as a carbon sink. Moreover, despite general patterns of high C allocation to root structures in grassland systems (Sims et al. 1978), long-term plant carbon sequestration is likely primarily associated with aboveground storage, because the dominant graminoid species in this experiment exhibit rapid root turnover (Stewart and Frank 2008). Even when carbon is stored in root systems for any relevant period of time, aboveground herbivory has substantial effects on soil processes (Bardgett et al. 1998), and it can indirectly deplete belowground plant C stores as a result from the upregulation of root carbon exudation in response to defoliation (Holland et al. 1996). This relationship suggests that shifts in the C dynamics of belowground tissues can both reflect as well as amplify carbon losses resulting from aboveground tissue removal.

6.1.2 Litter-detritivore interactions and plot C dynamics

The relationship between fauna and litter turnover is important to understand in the context of global change, because the manipulation of detritivore communities can have significant implications for decomposition responses to factors such as increased CO_2 concentration and warming (Coûteaux et al. 1991, Rouifed et al. 2010). Much like for the plant-herbivore interactions, the effects of global change on the litter-detritivore interactions in Chapter 5 occurred shortly following initiation of the experiment. My results indicated that following six weeks of litter incubation, detritivore contributions to litter turnover were significantly greater in ambient temperature plots than in heated plots. These results are consistent with previous observations of the negative effects of warming on soil fauna activity in other systems (Lavelle et al. 1997). From an ecosystem perspective, this result may have important implications for carbon cycling dynamics, because detritivore litter processing facilitates plant decomposition (Swift et al. 1979). However, these interactive effects were no longer present following one year of litter incubation. Instead, litter mass loss increased substantially in the presence of detritivores, regardless of the presence of other treatments. Furthermore, additional evidence suggests that, much like the observations for grass productivity, the influence of climate variability on overall patterns of carbon turnover can override global change treatment effects. For example, Bell et al. (2010) reported significant seasonal variation in microbial extracellular enzyme activity (EEA), despite observing minimal responses to warming or N addition.

Implications of the one-year detritivore effects from Chapter 5 are two-fold. With regard to ecosystem dynamics, increased mass loss can have important consequences for soil resource availability, because carbon turnover is positively related to the mobilization of nutrients such as nitrogen (McGill and Cole 1981). Likewise, increased mass loss can promote plant productivity by negating the suppressive effects of litter on the establishment and growth of newly germinated plants (Bosy and Reader 1995). Secondly, the ecosystem consequences of increased carbon turnover may enhance net C efflux. Although grasslands often exhibit extensive soil C sequestration (Scurlock and Hall 1998), a previous study of soil C flux at my field site revealed that the experimental plots acted predominantly as a carbon source over two consecutive years (Kim and Henry, in press). Moreover, measurements of carbon gas exchange were insensitive to the global change treatments, much like the observed detritivore effects following one year of litter incubation in my experiment, suggesting that soil fauna may strongly contribute to overall C losses. Also, because heating lamps were temporarily shut off in the Kim and Henry study in order to allow for the physical placement of the CO_2 flux chambers over treatment plots, the carbon flux data would have only represented possible indirect respiration responses to warming effects such as soil drying. Therefore, this result supports the suggestion that temperature effects on soil fauna desiccation stress were insufficient to influence C turnover over the long-term.

6.2 Mechanisms governing shifts in plant-animals interactions in response to global change

In order to investigate the potential mechanisms governing shifts in plant-animal interactions observed in Chapters 3 and 5, I conducted additional experiments to explore the effects of plant tissue quality and plot microenvironment on herbivore and detritivore activity. Results from Chapters 3, 4 and 5 indicated that shifts in diet and habitat patch quality in response to nitrogen manipulation can have important implications for both herbivore and detritivore foraging dynamics, while the warming effects observed in Chapters 3 and 5 suggest that temperature may also play a role.

6.2.1 The role of plant quality

Nitrogen enrichment had a substantial effect on plant-animal interactions, which reflects the central role of nitrogen in heterotroph feeding dynamics (Mattson 1980, White 1993). As mentioned above, the exclosure effects observed in Chapter 3 suggest that rodent herbivores responded positively to the nitrogen fertilization of resource patches, which is consistent with previous reports of increased herbivore foraging pressure in response to soil nutrient amendment (Ball et al. 2000, Throop and Lerdau 2004). While it was surprising that similarly disproportionate mollusc foraging effects were not observed in N-fertilized plots, molluscan herbivores common to the field site included the grey field slug, *Deroceras reticulatum*, and the foraging activities of this species have been previously shown to be unaffected by plot N fertilization (Cleland et al. 2006).

Evidence obtained from Longworth trapping revealed that the meadow vole*, Microtus pennsylvanicus*, was the most common rodent herbivore found at my study site, suggesting that its sensitivity to the manipulation of plant N availability strongly contributed to the exclosure effects. In general, meadow vole foraging dynamics have important implications for the structure of grassland communities (Howe et al. 2006), and meadow voles exhibit diet preferences that relate positively to natural variation in interspecific plant N content (Marquis and Batzli 1989). Likewise, some vole species have been shown to preferentially consume shrubs (Pedersen et al. 2011) and tree seedlings (Hartley et al. 1995) that have been grown under high N conditions. Although the perennial graminoid *Poa pratensis* (Kentucky bluegrass) is a dominant component of the meadow vole diet in many regions, surprisingly, meadow vole responses to the manipulation of grass nitrogen availability had previously received very little attention.

In order to explore the potential influence of meadow vole feeding preferences on the plot biomasss responses to nitrogen enrichment observed in Chapter 3, part of Chapter 4 focused on herbivore responses to grass N manipulation in a cafeteria-style feeding experiment. When provided the choice between grasses grown in either high-N or ambient-N conditions, grasses obtained from fertilized plots comprised 66% of meadow vole diet intake. This preference was consistent with increased grass tissue N content in response to nitrogen addition, providing support for the influence of plant quality as a mechanism driving shifts in plant-herbivore interactions under global change. This observation likely reflects herbivore sensitivity to the increased nutritive value of plants grown under high N availability including greater protein content and digestible energy (Mårell et al. 2002). Herbivores fed high protein diets often incur fitness benefits such as earlier sexual maturation (Cameron and Eshelman 1996), as well as improved reproductive success (White 1978).

Although the fertilization effect on grass nitrogen content was significant for both the June and July (but not August) feeding assays, only the June trial resulted in a significant meadow vole feeding response. Provided that changes in plant tissue chemistry were an influential component of meadow vole feeding patterns in the field experiment, this observation has important implications for variation in biomass production among plots. Because the N effect on meadow vole feeding preferences was only observed in the first of three feeding trials conducted over the field season, it coincided with the early-season establishment of rodent exclusion effects in N fertilized plots reported in Chapter 3, further suggesting a link between resource quality, plant-herbivore interactions and C dynamics in the field experiment. With regards to the July feeding experiment, it is possible that the differences in diet N were too subtle to result in a significant feeding effect. This explanation is consistent with a previous study where there were significant differences in N content between experimental diets, but the effect of N addition on plant N (2.8% versus 2.3% for control diets) was likely too small to allow for significant discrimination by meadow voles (Hjältén et al. 2008). Moreover, because grass N uptake can vary across the growing season (Wherley et al. 2009, Larsen et al. 2012), then it is possible that disproportionate meadow vole feeding preferences across treatment plots might only occur following the establishment of a detectable, threshold difference in vegetation N content between nitrogen-fertilized and non-fertilized plots.

In addition to N effects on plant-herbivore interactions, evidence from Chapter 5 suggests that nitrogen addition influenced litter processing by detritivores. In order to explore the individual effects of litter quality on detritivory, grass litter from N fertilized and non-fertilized plots was removed from the field experiment and placed into a common garden environment. Although litter quality was not explicitly quantified in my study, a previous experiment conducted at my field site revealed that grass litter N content increased from 0.79% to 0.97% in response to nitrogen addition (M. R. Vankoughnett, unpublished data). Following six weeks of litter incubation, detritivore effects on litter mass loss were significantly greater for fertilized litter than non-fertilized litter. This result is consistent with previous observations of detritivore preference for plant litter containing high tissue N content (Hendriksen 1990). Much like for plant-herbivore interactions, this relationship highlights a possible mechanistic role of diet quality on shifts in litter-detritivore interactions in response to N deposition. While this result could have important implications for short-term C cycling dynamics, the effect was no longer present following one year of decomposition, possibly in response to the general decline in litter lability and tissue quality over time. Likewise, similar N effects were absent from the main global change field experiment. Overall, results from my resource quality assays suggest that herbivores were more sensitive to shifts in plant quality in response to N addition than were detritivores, which is consistent with differential N effects observed across trophic levels in previous studies (Murphy et al. 2012). Grass ontogenic dynamics likely contribute to this relationship; the effect of nitrogen enrichment on the N content of live tissue at my field site can reach as high as double the N effect on plant litter (M. R. Vankoughnett, unpublished data), which may be partially attributed to nitrogen resorption during the onset of senescence.

6.2.2 The role of plot microenvironment

Results from Chapters 3 and 5 suggest that in addition to the importance of plant quality, shifts in plant-animal interactions may be related to the effects of nitrogen addition and warming on the microenvironment of experimental plots. The positive responses of slug herbivory to warming in Chapter 3 likely reflect increases in invertebrate feeding activity in response to increased temperature (Lactin and Johnson 1995, Kozłowski et al. 2011). Although water-sensitive fauna such as molluscs are subject to desiccation in response to increased temperature (Carne-Cavagnaro et al. 2006), the grey field slug, *Deroceras reticulatum*, is primarily nocturnal (Hommay et al. 1998), therefore its feeding activities are restricted to cool periods of the day. The average minimum nightly temperature over the course of the experiment was 10.2°C (the feeding rate of *D. reticulatum* increases with temperature until an optimum of 14°C; Wareing and Bailey 1985), thus it is possible that the increased soil surface warming of 2-3°C provided by the infrared heaters increased slug metabolic activity and resource demand.

In contrast to the mollusc responses, evidence from the tracking tunnel observations suggests that microenvironment had a minimal effect on grass responses to rodent exclusion in N addition plots. Although increased sward height can benefit voles, because they rely on overhead vegetative cover to hide from predators (Mossman 1955), results from Chapter 4 indicated that *M. pennsylvanicus* activity was insensitive to increased plot biomass in N fertilized plots. This result was surprising given previous reports of the association between mammalian herbivore habitat preference and resource patch vegetation structure (Van de Koppel et al. 1996, Koivunen et al. 1998). One possible explanation is that meadow voles were more responsive to components of vegetation other than plot structure, such as plant tissue phosphorous (Schütz et al. 2003) or sodium (Inoye et al. 1987) content. However, this explanation would not account for the substantial effects of rodent exclusion in N plots observed during the previous growing season. Although meadow voles possess the capacity to select habitat at a relatively small spatial scale, this process is replaced by foraging decisions at the landscape level when population densities are high (Oatway and Morris 2007). The latter scenario is supported by the relatively high vole population (239 voles/ha) observed at my field site during the microenvironment assay period, which is slightly higher than maximum meadow vole densities reported elsewhere (Christian 1971, Madison 1980). More importantly, this relationship suggests that interannual fluctuations in meadow vole population size may substantially modify herbivore impacts on plot biomass responses to the global change treatments.

Similar to the mollusc responses observed in Chapter 3, the results in Chapter 5 also suggest that invertebrate foraging activity was influenced by physiological effects of the global change treatments. Soil moisture was significantly lower in warmed plots than in ambient plots following six weeks of litter incubation, and reduced detritivore effects can therefore possibly be attributed to desiccation stress. Previous studies have reported negative effects of warming on detritivore activity, particularly when precipitation is limited (Blankinship et al. 2011). This relationship likely reflects the overall importance of water relations on soil faunal dynamics (Lindberg et al. 2002). However, following one year of decomposition, detritivore effects were no longer suppressed in warmed plots, which may reflect potential faunal recovery or acclimation. This explanation is consistent with previous reports of the recovery by soil organisms following drought stress (Maraldo and Holmstrup 2009, Holmstrup et al. 2012). It is also supported by the observation that mesic environments are capable of supporting a larger abundance of detritivores than xeric environments (Wardle 2002). In addition to the influence of warming, the effects of detritivory were reduced in N addition plots when litter material was added from untreated areas of field. This relationship suggests that the detritivores may have experienced direct negative effects of fertilization such as ammonium toxicity (Wei et al. 2012) or salt desiccation (Lohm et al. 1977). However, similar to the influence of warming, this effect was also transient.

In general, evidence from the microenvironment assays suggests that ectotherms such as slugs and insect detritivores were more sensitive to the physiological effects of global change treatments than were meadow voles. Likewise, although the absence of mollusc exclusion effects from N addition plots suggests that shifts in forage quality do not influence their feeding preferences, without conducting explicit palatability studies this conclusion would be premature. Moreover, previous feeding studies have reported significant effects of N enrichment on the diet preferences of several invertebrate herbivore taxa (Gebauer 2002, Throop and Lerdau 2004, Asplund et al. 2010). Together, these relationships suggest that, owing to their sensitivity to both direct and indirect treatment effects, ectotherms are likely to be more sensitive to the impacts of global change than are endotherms. This idea is supported by the prediction that climate change will cause significantly greater community disruption to plant interactions with ectotherms than with endotherms, owing primarily to the physiological regulation of ecothermic metabolism by environmental temperature (Sheldon et al. 2011). Ultimately, organismal physiology represents such a pertinent component to ecosystem dynamics that its principles serve as the foundation for the Metabolic Theory of Ecology which posits that the metabolic rate of biota regulate patterns and processes at all levels of ecological organization (Brown et al. 2004). A central component of this theory is the role of temperature on physiology, suggesting that the ecosystem consequences of warming will be substantial. With respect to herbivory, physiological responses of ecothermic herbivores to warming manipulations strongly contribute to shifts in consumer feeding rates (Dangles et al. 2013).

6.3 Future research directions

This dissertation has provided much support for the importance of shifting plant-animal relationships as drivers of ecosystem responses to global change. However, there remain several research questions that would provide further insight into the consequences of anthropogenic disturbance on biotic/abiotic interactions and their effects on ecosystem cycles and processes. While herbivores such as voles and slugs can exhibit significant control over plant community composition, other fauna such as herbivorous insects also contribute substantially to ecosystem structure and function. Likewise, although warming and N deposition are important components of global change, other factors such as increased CO_2 and altered precipitation are equally important to explore, and may interact with warming and N addition. Finally, in addition to nutritive quality, shifts in plant defense secondary chemistry in response to drivers of global change may have a prominent role in governing changes in plant-animal interactions under future environmental scenarios.

6.3.1 The ecological importance of insects

Insects are a potentially influential group of herbivores that were not addressed by my thesis, primarily because their effects are diverse and can be episodic and spatially patchy in nature. Nevertheless, the patterns and magnitude of insect herbivory can influence plant fitness (Maron 1998), population (Crawley 1989) and community (Clay et al. 2005) dynamics, as well as ecosystem nutrient cycling (Kaukonen et al. 2013). Consequences of global change such as shifts in plant quality can strongly influence plant-herbivore interactions through changes in insect density, herbivory pressure, and growth rate (Throop and Lerdau 2004). Much like the distinct feeding patterns observed in voles and slugs, insects often exhibit strong feeding preferences for specific host plants (Mayhew 1997), the hierarchal ordering of which can be switched in response to abiotic disturbance (Agrell et al. 2005). Insects are also sensitive to the direct effects of climate warming (Bale et al. 2002), and one of the greatest concerns with respect to insect responses to global change is the potential for pest outbreaks (Ayres and Lombardero 2000). The effects of increased temperature are of particular importance because warmer winters may result in increased insect survival (Cannon 1998). Moreoever, climate warming may also facilitate the invasion of pests into regions that have historically been too cold to allow for their establishment (Battisti et al. 2005). Conversely, warming may negatively impact insects living near or at their thermal optima (Deutsch et al. 2008). Shifts in plant-insect interactions may be especially important in northern ecosystems, where warming is expected to be considerably more intense than at lower latitudes (Hodkinson et al. 1998).

In addition to herbivores, insect detritivores are an important component of ecosystem dynamics (Swift et al. 1979, Wolters 2000). Although Chapter 5 explored broadscale functional shifts in detritivore responses to global change, it did not include an investigation of the potential mechanistic role of changes in the composition of detritivore communities resulting from treatment manipulations. Previous studies have reported changes in the structure of soil fauna communities in response to factors such as increased CO_2, warming, and N deposition (Hagvar and Klanderud 2009, Kardol et al. 2011). Moreover, shifts in the relative abundance of important grassland detritivores including mites, nematodes, and earthworms can have substantial consequences for processes such as decomposition (Bradford 2002). Although it is possible that reduced litter turnover by soil fauna in warmed plots reported in Chapter 5 may simply reflect suppressive temperature effects at the whole-community level, species-specific drought effects on mites are consistent with interspecific variation in their temperature tolerances (Siepel 1996).

6.3.2 Other drivers of global change

In addition to warming and N deposition, other drivers of global change, such as elevated atmospheric CO_2 concentrations and altered precipitation regimes, will have important implications for ecosystem dynamics (Cramer et al. 2001, Weltzin et al. 2003). Moreover, the co-occurrence of these factors with the factors examined in this dissertation is likely to reveal important treatment interactions not readily apparent from the simple additive effects of individual treatments in isolation (Rustad 2008, Leuzinger et al. 2011). Owing to processes such as deforestation and fossil fuel combustion, the global atmospheric concentration of CO_2 is predicted to double by 2100 relative to pre-industrial revolution values (Houghton et al. 1995). Similar to N deposition, increased CO_2 can increase plant productivity (Wand et al. 1999), with associated influences on herbivore feeding dynamics (Heagle 2003). However, in contrast to increases in plant tissue quality in response to N deposition, CO_2 fertilization dilutes plant nutrient content (Cotrufo et al. 1998), which may negate positive herbivore responses to nitrogen fertilization, such as the increase in plant palatability observed in Chapter 4.

Alternatively, herbivores may increase consumption of plant material grown under high CO_2 conditions in order to compensate for the reduction in tissue quality (Lincoln et al. 1993).

Another important component of climate change is the predicted increase in the variability of precipitation patterns (IPCC 2007). Both increases and decreases in precipitation quantity can influence processes such as ecosystem productivity and carbon exchange (Wu et al. 2011), while shifts in the timing of rain events may also play a crucial role (Chou et al. 2008). Altered precipitation patterns can modify plant-animal interactions through changes in plant productivity (Zhou et al. 2002) and chemistry (Torp et al. 2009). Likewise, insect herbivores respond positively to increased plant water content (Scriber 1979), although their responses to plants subjected to drought show mixed results (Larsson 1989). Changes in precipitation will also have important implications for decomposition, because both detritivore (Pritchard 2011) and microbial (Orchard and Cook 1983, Liu et al. 2009) communities are sensitive to soil moisture conditions. Moreover, because the effect of increased temperature on soil dynamics is amplified in dry environments (Blankinship et al. 2011), it is likely that changes in climate such as warming and drought will exhibit synergistic effects on litter turnover and soil respiration.

6.3.3 Plant defenses and global change

In addition to nutrient content, plant secondary metabolites play a critical role in mediating interactions with herbivores (Berenbaum 1995, Kliebenstein 2004). Compounds such as phenolics, tannins, and alkaloids both deter plant consumption and impede post-ingestion dry matter digestion and nitrogen assimilation (Rhoades and Cates 1976). Similarly, the attraction of parasitoids to plants undergoing herbivore attack is related to shifts in the profile of green leaf volatiles (De Moraes et al. 1998). Drivers of global change will substantially alter the dynamics of plant chemical defense systems (Bidart-Bouzat and Imeh-Nathaniel 2008). For instance, increases in CO_2 and N availability can increase plant tissue concentration of phenolics and alkaloids, respectively (Gerson and Kelsey 1999, Zvereva and Kozlov 2006). Likewise, both increased CO_2 and O_3 can affect the overall inducibility of plant defense pathways

(Bidart-Bouzat et al. 2005, Himanen et al. 2007), as well as the efficacy of plant volatile cues (Yuan et al. 2009). Changes in plant secondary chemistry may further influence decomposition dynamics owing to shifts in tissue lignin content (Sandermann Jr 1996, Matros et al. 2006). Ultimately, the modification of plant defense chemistry in response to global change is important for plant-animal interactions, including variation in feeding rate and herbivore performance (Lindroth et al. 1993). Exploring the extent to which these relationships indirectly contribute to plant community responses in global change field experiments may provide considerable mechanistic insight to future research.

Despite established relationships between global change factors and plant chemical defenses, comparatively little is known regarding interactions of the former with physical defenses. For instance, in response to herbivory, graminoids can accumulate disproportionately large quantities of silicon in their leaves (Hodson 2005), forming opaline phytoliths, which may be an important defense against insect herbivores (Reynolds et al. 2009). Furthermore, grass silicon accumulation in response to defoliation is hypothesized to function as a deterrent to grazing herbivores (McNaughton and Tarrants 1983, Massey et al. 2007). This suggestion is supported by observed vole responses to silicon-fertilized diets, including reduced grass dry matter digestibility and palatability, decreased body weight, as well as greater fecal N content (Massey and Hartley 2006, E. R. D. Moise, unpublished). Drivers of global change such as N deposition may counteract silicon effects, because nitrogen addition is associated with increases in plant digestibility (Johnson et al. 2001). In a recent experiment, I observed that the survival rate of armyworms was significantly greater when fed leaves from corn plants grown under high-silicon, high-nitrogen conditions relative to the high-silicon treatment alone (E. R. D. Moise, unpublished). With respect to shifts in precipitation patterns, plant silicification may function to mitigate drought stress (Liang et al. 2007). Because plot-scale global change field experiments are often established in grassland systems, is it likely that Si-accumulating plant species contribute substantially to observed trends in plant community responses. Moreover, because the quantification of silicon effects is absent from these studies, the importance of physical defenses remains unknown in the context of environmental change.

6.4 Concluding remarks

Shifts in species interactions in response to global change are well established in the literature (Tylianakis et al. 2008), yet the implications of these consequences remain largely unexplored in plot-level field experiments designed to assess direct plant responses to future environmental conditions. Much like moths being attracted to a light source, fauna may preferentially restrict their activities to the plots of specific experimental treatments (Moise and Henry 2010). The results presented in this dissertation reveal significant interactions between biotic and abiotic drivers of carbon-related processes such as plant productivity and litter turnover. However, at the ecosystem scale there was strong evidence in this experiment for the insensitivity of carbon flux to indirect warming effects (Kim and Henry, in press), possibly related to detritivore dynamics, suggesting that overall terrestrial C dynamics may not be regulated at the community level. This disassociation between processes at differing levels of ecological organization supports the importance for a holistic approach to environmental research (Odum 1971). Regardless, the destabilization of community interactions in response to global change will have important implications for other facets of ecosystem dynamics, particularly with respect to environmental change, species relationships, and the consequences of changes in biodiversity on ecosystem structure and function (Vitousek 1997, Chapin et al. 2000).

The effects of climate variability represent another important component to C dynamics in my field experiment. Evidence from this thesis coupled with observed trends from work by others at this site suggest that temporal variation in climate parameters may override plant responses to warming and N addition treatments. However, this relationship may have implications beyond the mitigation of treatment effects, because increases in the variation of temperature and precipitation are predicted as a consequence of global change (IPCC 2007). Because changes in climate variability, rather than averages, are likely to have a greater effect on ecosystem processes (Katz and Brown 1992), there is a growing emphasis on the importance of ecosystem responses to extreme events such as heat waves (De Boeck et al. 2010) severe drought (Bréda et al. 2006), and soil freezing effects from reduced snow cover (Groffman et al. 2001). Likewise, there is a critical need to better incorporate such variability into community-level global change field manipulations (Thompson et al. 2013). Ultimately, the application of these factors to the study of plant-animal relationships will contribute to a more comprehensive understanding of potential shifts in biotic/abiotic interactions under future environmental scenarios, and highlight how these changes may be reflected in modifications to ecosystem structure and function.

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Appendices

Appendix A: Permission to reproduce published material

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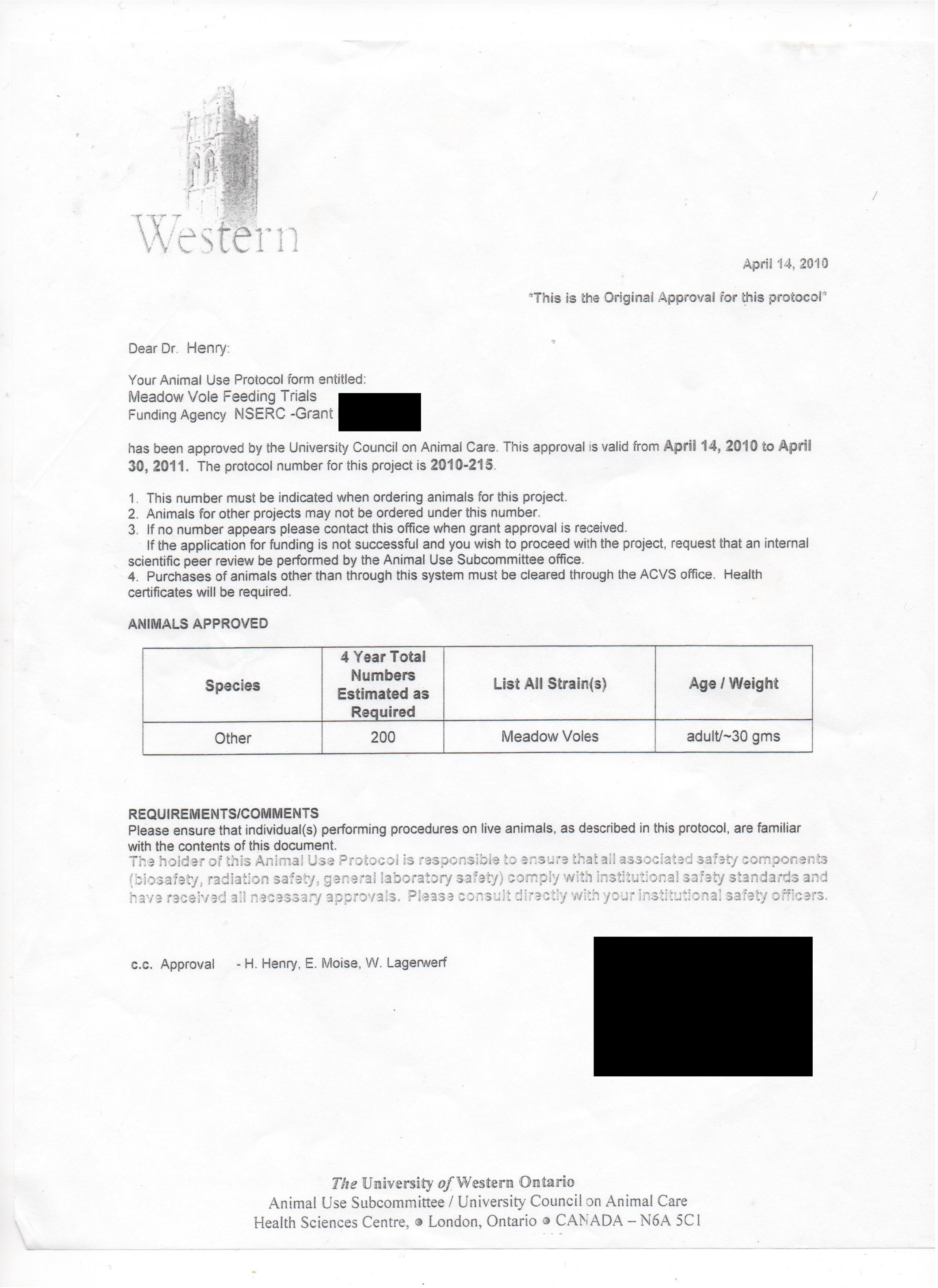
A version of Chapter 3 was published in *Oecologia*. As per Springer-Verlag copyright policy, manuscript reproduction for the purpose of thesis publication was obtained by acquiring an electronic republishing permit, license ID 3158310788161

A version of Chapter 4 has been submitted to the *Journal of Mammalogy* for publication. As per American Society of Mammalogists copyright assignment policy, “ASM grants back to the Author the right to use or republish, with a citation to the source of the published article, all or part of the material from the published manuscript in oral presentations and future published works written or edited by the author.” (American Society of Mammalogists copyright assignment and author disclosure form)

A version of Chapter 5 has been submitted to *Functonal Ecology* for publication. In its current state of review, no special permissions are required to reproduce the manuscript.

Appendix B: Animal use protocol approval



April 14, 2010

This is the Original Approval for this protocol

Dear Dr. Henry:

Your Animal Use Protocol form entitled:
Meadow Vole Feeding Trials
Funding Agency NSERC -Grant [redacted]

has been approved by the University Council on Animal Care. This approval is valid from **April 14, 2010 to April 30, 2011.** The protocol number for this project is **2010-215**.

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

ANIMALS APPROVED

Species	4 Year Total Numbers Estimated as Required	List All Strain(s)	Age / Weight
Other	200	Meadow Voles	adult/~30 gms

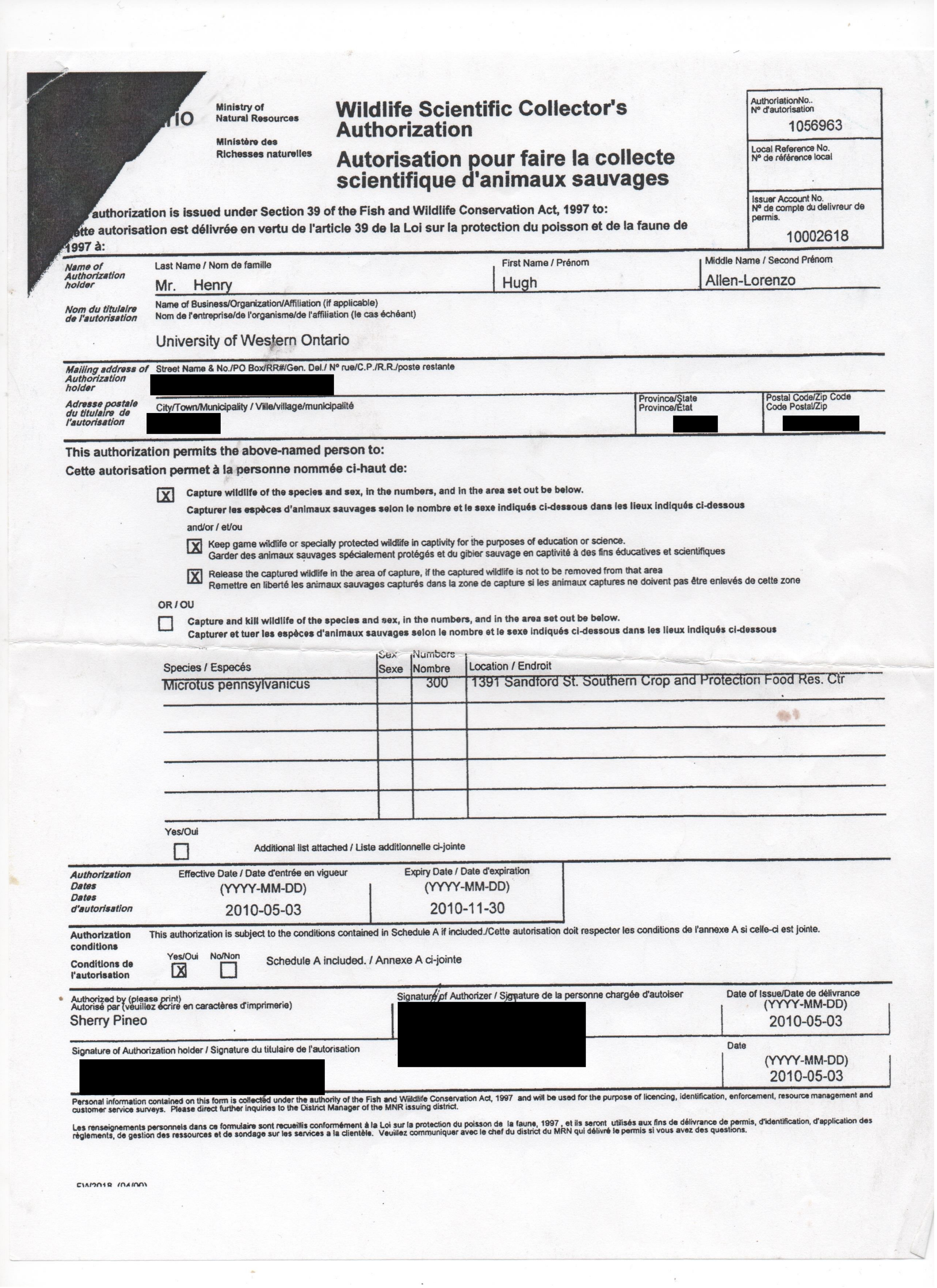
REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.
The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

c.c. Approval - H. Henry, E. Moise, W. Lagerwerf

[redacted]

The University *of* Western Ontario
Animal Use Subcommittee / University Council on Animal Care
Health Sciences Centre, ● London, Ontario ● CANADA – N6A 5C1

Appendix C: Scientific collector's permit



rio

Ministry of Natural Resources

Ministère des Richesses naturelles

Wildlife Scientific Collector's Authorization

Autorisation pour faire la collecte scientifique d'animaux sauvages

AuthoriationNo.. Nº d'autorisation	1056963
Local Reference No. Nº de référence local	
Issuer Account No. Nº de compte du delivreur de permis.	10002618

authorization is issued under Section 39 of the Fish and Wildlife Conservation Act, 1997 to:
tte autorisation est délivrée en vertu de l'article 39 de la Loi sur la protection du poisson et de la faune de 1997 à:

Name of Authorization holder / Nom du titulaire de l'autorisation

Last Name / Nom de famille: Mr. Henry
First Name / Prénom: Hugh
Middle Name / Second Prénom: Allen-Lorenzo
Name of Business/Organization/Affiliation (if applicable) / Nom de l'entreprise/de l'organisme/de l'affiliation (le cas échéant): University of Western Ontario

Mailing address of Authorization holder / Adresse postale du titulaire de l'autorisation

Street Name & No./PO Box/RR#/Gen. Del./ Nº rue/C.P./R.R./poste restante: [redacted]
City/Town/Municipality / Ville/village/municipalité: [redacted]
Province/State / Province/État: [redacted]
Postal Code/Zip Code / Code Postal/Zip: [redacted]

This authorization permits the above-named person to:
Cette autorisation permet à la personne nommée ci-haut de:

☒ **Capture wildlife of the species and sex, in the numbers, and in the area set out below.**
Capturer les espèces d'animaux sauvages selon le nombre et le sexe indiqués ci-dessous dans les lieux indiqués ci-dessous

and/or / et/ou

- ☒ Keep game wildlife or specially protected wildlife in captivity for the purposes of education or science.
  Garder des animaux sauvages spécialement protégés et du gibier sauvage en captivité à des fins éducatives et scientifiques
- ☒ Release the captured wildlife in the area of capture, if the captured wildlife is not to be removed from that area
  Remettre en liberté les animaux sauvages capturés dans la zone de capture si les animaux captures ne doivent pas être enlevés de cette zone

OR / OU

☐ **Capture and kill wildlife of the species and sex, in the numbers, and in the area set out below.**
Capturer et tuer les espèces d'animaux sauvages selon le nombre et le sexe indiqués ci-dessous dans les lieux indiqués ci-dessous

Species / Especés	Sex Sexe	Numbers Nombre	Location / Endroit
Microtus pennsylvanicus		300	1391 Sandford St. Southern Crop and Protection Food Res. Ctr

Yes/Oui ☐ Additional list attached / Liste additionnelle ci-jointe

Authorization Dates / Dates d'autorisation	Effective Date / Date d'entrée en vigueur (YYYY-MM-DD)	Expiry Date / Date d'expiration (YYYY-MM-DD)
	2010-05-03	2010-11-30

Authorization conditions / Conditions de l'autorisation This authorization is subject to the conditions contained in Schedule A if included./Cette autorisation doit respecter les conditions de l'annexe A si celle-ci est jointe.

Yes/Oui ☒ No/Non ☐ Schedule A included. / Annexe A ci-jointe

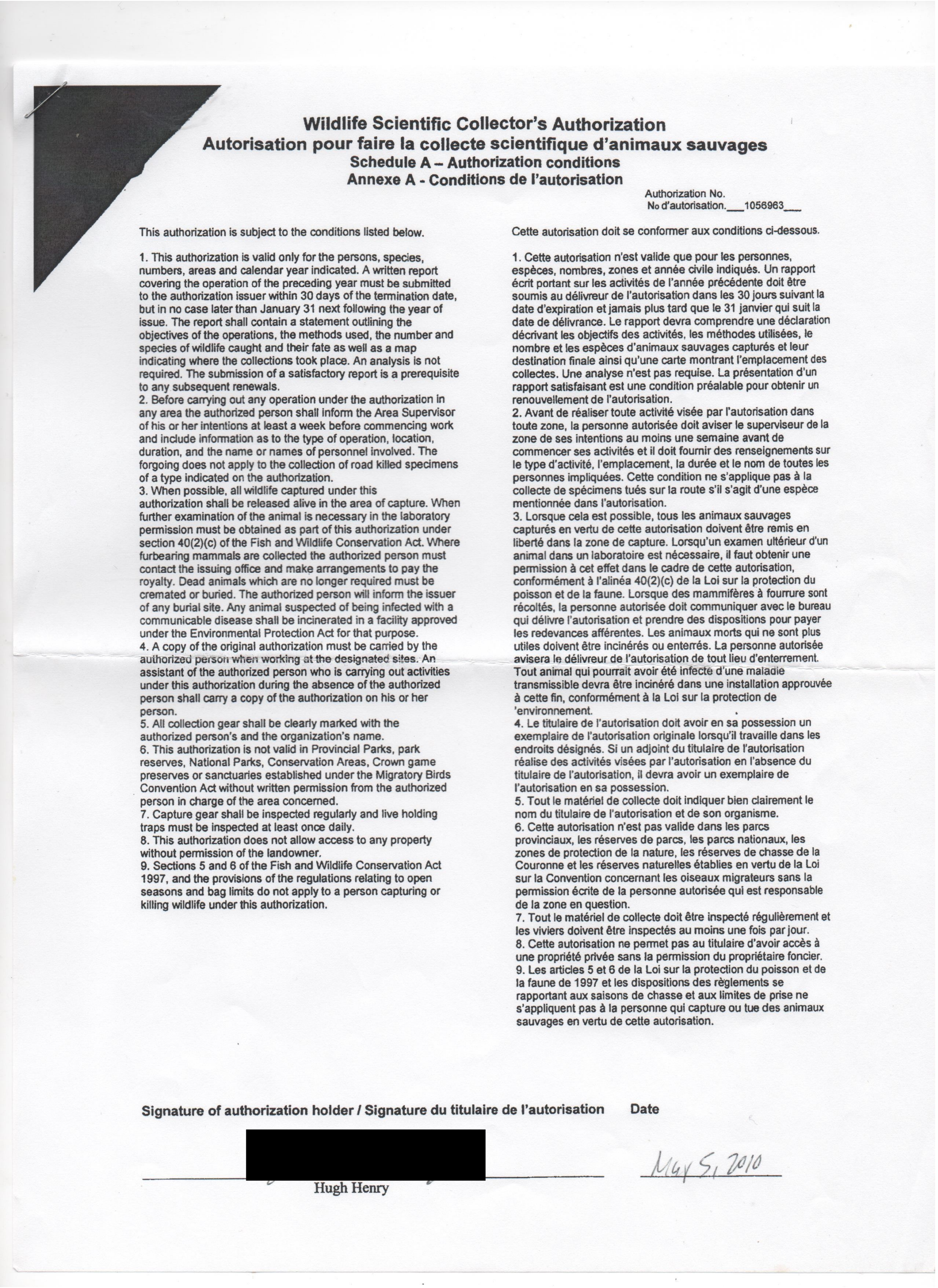
Authorized by (please print) / Autorisé par (veuillez écrire en caractères d'imprimerie): Sherry Pineo
Signature of Authorizer / Signature de la personne chargée d'autoiser: [redacted]
Date of Issue/Date de délivrance (YYYY-MM-DD): 2010-05-03

Signature of Authorization holder / Signature du titulaire de l'autorisation: [redacted]
Date (YYYY-MM-DD): 2010-05-03

Personal information contained on this form is collected under the authority of the Fish and Wildlife Conservation Act, 1997 and will be used for the purpose of licencing, identification, enforcement, resource management and customer service surveys. Please direct further inquiries to the District Manager of the MNR issuing district.

Les renseignements personnels dans ce formulaire sont recueillis conformément à la Loi sur la protection du poisson de la faune, 1997 , et ils seront utilisés aux fins de délivrance de permis, d'identification, d'application des règlements, de gestion des ressources et de sondage sur les services a la clientèle. Veuillez communiquer avec le chef du district du MRN qui délivré le permis si vous avez des questions.

FW2018 (04/00)



Wildlife Scientific Collector's Authorization
Autorisation pour faire la collecte scientifique d'animaux sauvages
Schedule A – Authorization conditions
Annexe A - Conditions de l'autorisation

Authorization No.
No d'autorisation.___1056963___

This authorization is subject to the conditions listed below.

1. This authorization is valid only for the persons, species, numbers, areas and calendar year indicated. A written report covering the operation of the preceding year must be submitted to the authorization issuer within 30 days of the termination date, but in no case later than January 31 next following the year of issue. The report shall contain a statement outlining the objectives of the operations, the methods used, the number and species of wildlife caught and their fate as well as a map indicating where the collections took place. An analysis is not required. The submission of a satisfactory report is a prerequisite to any subsequent renewals.
2. Before carrying out any operation under the authorization in any area the authorized person shall inform the Area Supervisor of his or her intentions at least a week before commencing work and include information as to the type of operation, location, duration, and the name or names of personnel involved. The forgoing does not apply to the collection of road killed specimens of a type indicated on the authorization.
3. When possible, all wildlife captured under this authorization shall be released alive in the area of capture. When further examination of the animal is necessary in the laboratory permission must be obtained as part of this authorization under section 40(2)(c) of the Fish and Wildlife Conservation Act. Where furbearing mammals are collected the authorized person must contact the issuing office and make arrangements to pay the royalty. Dead animals which are no longer required must be cremated or buried. The authorized person will inform the issuer of any burial site. Any animal suspected of being infected with a communicable disease shall be incinerated in a facility approved under the Environmental Protection Act for that purpose.
4. A copy of the original authorization must be carried by the authorized person when working at the designated sites. An assistant of the authorized person who is carrying out activities under this authorization during the absence of the authorized person shall carry a copy of the authorization on his or her person.
5. All collection gear shall be clearly marked with the authorized person's and the organization's name.
6. This authorization is not valid in Provincial Parks, park reserves, National Parks, Conservation Areas, Crown game preserves or sanctuaries established under the Migratory Birds Convention Act without written permission from the authorized person in charge of the area concerned.
7. Capture gear shall be inspected regularly and live holding traps must be inspected at least once daily.
8. This authorization does not allow access to any property without permission of the landowner.
9. Sections 5 and 6 of the Fish and Wildlife Conservation Act 1997, and the provisions of the regulations relating to open seasons and bag limits do not apply to a person capturing or killing wildlife under this authorization.

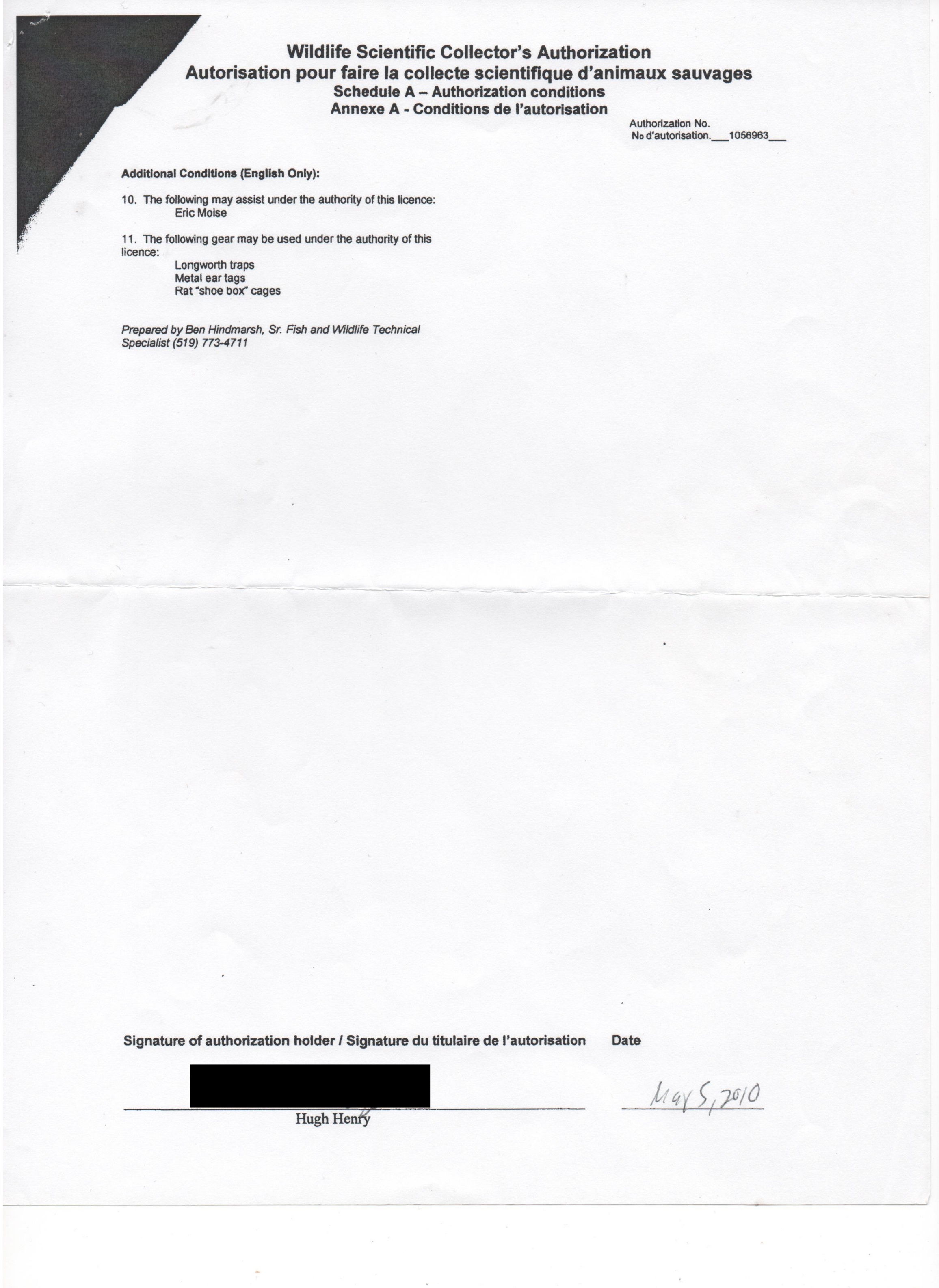
Cette autorisation doit se conformer aux conditions ci-dessous.

1. Cette autorisation n'est valide que pour les personnes, espèces, nombres, zones et année civile indiqués. Un rapport écrit portant sur les activités de l'année précédente doit être soumis au délivreur de l'autorisation dans les 30 jours suivant la date d'expiration et jamais plus tard que le 31 janvier qui suit la date de délivrance. Le rapport devra comprendre une déclaration décrivant les objectifs des activités, les méthodes utilisées, le nombre et les espèces d'animaux sauvages capturés et leur destination finale ainsi qu'une carte montrant l'emplacement des collectes. Une analyse n'est pas requise. La présentation d'un rapport satisfaisant est une condition préalable pour obtenir un renouvellement de l'autorisation.
2. Avant de réaliser toute activité visée par l'autorisation dans toute zone, la personne autorisée doit aviser le superviseur de la zone de ses intentions au moins une semaine avant de commencer ses activités et il doit fournir des renseignements sur le type d'activité, l'emplacement, la durée et le nom de toutes les personnes impliquées. Cette condition ne s'applique pas à la collecte de spécimens tués sur la route s'il s'agit d'une espèce mentionnée dans l'autorisation.
3. Lorsque cela est possible, tous les animaux sauvages capturés en vertu de cette autorisation doivent être remis en liberté dans la zone de capture. Lorsqu'un examen ultérieur d'un animal dans un laboratoire est nécessaire, il faut obtenir une permission à cet effet dans le cadre de cette autorisation, conformément à l'alinéa 40(2)(c) de la Loi sur la protection du poisson et de la faune. Lorsque des mammifères à fourrure sont récoltés, la personne autorisée doit communiquer avec le bureau qui délivre l'autorisation et prendre des dispositions pour payer les redevances afférentes. Les animaux morts qui ne sont plus utiles doivent être incinérés ou enterrés. La personne autorisée avisera le délivreur de l'autorisation de tout lieu d'enterrement. Tout animal qui pourrait avoir été infecté d'une maladie transmissible devra être incinéré dans une installation approuvée à cette fin, conformément à la Loi sur la protection de 'environnement.
4. Le titulaire de l'autorisation doit avoir en sa possession un exemplaire de l'autorisation originale lorsqu'il travaille dans les endroits désignés. Si un adjoint du titulaire de l'autorisation réalise des activités visées par l'autorisation en l'absence du titulaire de l'autorisation, il devra avoir un exemplaire de l'autorisation en sa possession.
5. Tout le matériel de collecte doit indiquer bien clairement le nom du titulaire de l'autorisation et de son organisme.
6. Cette autorisation n'est pas valide dans les parcs provinciaux, les réserves de parcs, les parcs nationaux, les zones de protection de la nature, les réserves de chasse de la Couronne et les réserves naturelles établies en vertu de la Loi sur la Convention concernant les oiseaux migrateurs sans la permission écrite de la personne autorisée qui est responsable de la zone en question.
7. Tout le matériel de collecte doit être inspecté régulièrement et les viviers doivent être inspectés au moins une fois par jour.
8. Cette autorisation ne permet pas au titulaire d'avoir accès à une propriété privée sans la permission du propriétaire foncier.
9. Les articles 5 et 6 de la Loi sur la protection du poisson et de la faune de 1997 et les dispositions des règlements se rapportant aux saisons de chasse et aux limites de prise ne s'appliquent pas à la personne qui capture ou tue des animaux sauvages en vertu de cette autorisation.

Signature of authorization holder / Signature du titulaire de l'autorisation **Date**

[redacted] May 5, 2010

Hugh Henry



Wildlife Scientific Collector's Authorization
Autorisation pour faire la collecte scientifique d'animaux sauvages
Schedule A – Authorization conditions
Annexe A - Conditions de l'autorisation

Authorization No.
No d'autorisation.___1056963___

Additional Conditions (English Only):

10. The following may assist under the authority of this licence:
    Eric Moise

11. The following gear may be used under the authority of this licence:
    Longworth traps
    Metal ear tags
    Rat "shoe box" cages

Prepared by Ben Hindmarsh, Sr. Fish and Wildlife Technical Specialist (519) 773-4711

Signature of authorization holder / Signature du titulaire de l'autorisation **Date**

[redacted]
Hugh Henry

May 5, 2010

Curriculum Vitae

Name:	Eric Moise
Post-secondary Education and Degrees:	The University of Western Ontario London, Ontario, Canada 2003-2008 B.Sc. Zoology
	The University of Western Ontario London, Ontario, Canada 2008-2013 Ph.D. Biology
Honours and Awards	2011 - NSERC Post-Graduate Scholarship ($42,000) - Ontario Graduate Scholarship ($15,000) – Declined 2010 - Ontario Graduate Scholarship ($15,000) 2009 - Ontario Graduate Scholarship in Science and Technology ($15,000) 2008 - Ontario Graduate Scholarship ($15,000) - Helen I. Battle Scholarship in Zoology ($500) - Helen I. Battle Gold Medal in Zoology
Related Work Experience	Teaching Assistant The University of Western Ontario 2008-2013

Publications

Submitted:

Moise, E.R.D. and Henry H.A.L. Nitrogen effects on diet choice and habitat use by the meadow vole, *Microtus pennsylvanicus*. Journal of Mammalogy.

Moise E.R.D. and Henry H.A.L. Interactive responses of grass litter decomposition to warming, nitrogen addition and detritivore access in a temperate old field. Functional Ecology.

Published:

Moise E.R.D. and Henry H.A.L. 2012. Interactions of herbivore exclusion with warming and N addition in a grass-dominated temperate old field. Oecologia 169: 1127-1136.

Moise E.R.D. and Henry H.A.L. 2010. Like moths to a streetlamp: exaggerated animal densities in plot-level global change field experiments. Oikos 119:791-795