Long-term vs. Short-term Plant Responses to Warming and Nitrogen Addition in a Temperate Old Field

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Biology

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Abstract

Climate warming and atmospheric nitrogen (N) deposition are expected to have strong effects on plant productivity in temperate ecosystems over the next century. However, short-term experiments may not adequately address their potential effects because of lags in changes in species composition. I added new plots to a pre-existing field experiment to compare the short-term (1-2 year; new plots) vs. long-term (14-15 year; old plots) effects of warming and N addition on plant productivity, relative species abundances, plant tissue N content, and litter decomposition. In 2020, N addition increased aboveground plant productivity most in old plots and only increased belowground biomass in the old N plots. In 2021, N addition effects did not differ among old and new plots. There were no significant treatment effects on forb species composition. Overall, non-native C3 grasses appear to impede additional long-term responses of plant productivity to global change by suppressing changes in species composition.
Keywords

Atmospheric nitrogen deposition, warming addition, plant productivity, temperate old-field, long-term study, species composition, *Poa pratensis, Bromus inermis*, NDVI, decomposition.
Across the globe, industrial and agricultural activities have intensified over the last century, which have led to an increase in greenhouse gas emissions. These gases are causing an acceleration in climate warming, and as a result, average annual air temperature is predicted to increase by 1.5 °C to 2 °C in the coming decades. Climate warming increases will also intensify most towards the poles. Moreover, advancements in agricultural during the twentieth century has led to the overuse of nitrogen (N) fertilizers in many northern temperate regions, causing increased rates of N pollution in rain and snow. This N pollution is predicted to continue rising over the next century, and to negatively impact ecosystems by promoting undesirable changes in plant species composition and by increasing N runoff from land to water. I conducted a field experiment to examine the effects of warming and N addition on plant species composition, plant growth and the breakdown of dead plant material. In particular, I wanted to compare whether there were differences between short-term (1-2 year) vs. long-term (14-15 year) effects on plants. Overall, fertilized plots exhibited increases in aboveground grass biomass (primarily Kentucky Bluegrass) and increased breakdown of grass litter. Warming effects were minor and only influenced the breakdown of Canada Thistle litter. Lastly, over the past 15 years, there were no changes in the abundances of non-grass species, and the plots remained largely dominated by the grasses (Kentucky Bluegrass and Smooth Brome grass) present at the beginning of the experiment. These grasses appear to impede additional long-term aboveground responses of plant biomass and species diversity to global change (warming and N addition).
Co-Authorship Statement

Dr. Hugh Henry, who supervised my project, will be included as a co-author on any manuscripts arising from this thesis.
Dedication

I would like to dedicate this thesis to my loving parents, Rick and Vicki. They support me in everything I do, encourage me to go above and beyond, and have instilled in me that I am braver than I believe, stronger than I seem, and smarter than I think.
Acknowledgments

I would like to thank my supervisor, Dr. Hugh Henry, for his continuous encouragement; invaluable insights and guidance throughout my master’s degree; and his sense of humor which helped eased the frustration of completing my project throughout a global pandemic. I am truly grateful for the all the time and effort he spent mentoring me, as he went above and beyond his duty, and as a result has made me a better scientist.

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I am thankful to have such wonderful lab mates Dr. Ricky Kong, Spencer Heuchan, Samuel Rycroft, and Erica Stroud, who supported me throughout my degree, shared adorable photos of their pets, and funny memes.

I’d like to thank the friends I made during my teaching assistantship, Andrew Pitek and Colleen Wardlaw, who helped me with course work, but more importantly made marking term papers and exams a fun and a memorable experience. Lastly, I’d like to thank the close group of friends I made during my degree, Michelle O’Donnell, Becky Poisson, Renée Resendes, and Jessica Sinka. These brilliant women were continuous sources of support, encouragement, and laughter. I am truly grateful for their friendships as they inspired me to be my best self, and who I know will continue to encourage and support me during my future endeavors.
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<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>$C_3$</td>
<td>type of carbon fixation that creates a 3-carbon organic molecule during photosynthesis</td>
</tr>
<tr>
<td>$C_4$</td>
<td>type of carbon fixation that creates a 4-carbon organic molecule during photosynthesis</td>
</tr>
<tr>
<td>CAM</td>
<td>Crassulacean acid metabolism</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>ANPP</td>
<td>Aboveground Annual Net Primary Productivity</td>
</tr>
<tr>
<td>$\text{NH}_4^+$</td>
<td>Ammonium</td>
</tr>
<tr>
<td>$\text{NO}_3^-$</td>
<td>Nitrate</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>Carbon Dioxide</td>
</tr>
<tr>
<td>Tg</td>
<td>Teragram</td>
</tr>
<tr>
<td>NDVI</td>
<td>Normalized Difference Vegetation Index</td>
</tr>
<tr>
<td>NuR</td>
<td>Nutrient Resorption</td>
</tr>
<tr>
<td>NRE</td>
<td>Nitrogen Resorption Efficiency</td>
</tr>
<tr>
<td>PRE</td>
<td>Phosphorus Resorption Efficiency</td>
</tr>
<tr>
<td>NRP</td>
<td>Nitrogen Resorption Proficiency</td>
</tr>
<tr>
<td>CHNS Analyzer</td>
<td>Carbon, Hydrogen, Nitrogen, Sulfur Analyzer</td>
</tr>
<tr>
<td>MLCF</td>
<td>Mass Loss Correction Factor</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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1 Chapter 1: Introduction

1.1 Global Climate Change

The intensification of industrial and agricultural activities has led to substantial increases in greenhouse gas emissions over the last century. Consequently, at the global scale, climate warming is intensifying, and it is predicted that the mean annual air temperature of 1.5 °C and 2°C (relative to 1850-1900) will be exceeded by the end of the 21st century, with the most severe warming predicted to occur at high latitudes (IPCC, 2021). In addition, warming has been greater over land than in the oceans (IPCC, 2021). Warming in the Arctic has already led to substantial melt from ice sheets and glaciers, reductions in snow cover, and sea ice extent for all months has decreased since 1979 (IPCC, 2021). Unless there are drastic reductions in carbon dioxide along with other greenhouse gas emissions within the coming decades, warming will continue to intensify and impact various ecosystems and ecosystem processes.

Temperature plays a key role in regulating various terrestrial ecosystem processes, such as soil respiration (Rustad et al., 2001), litter decomposition (Aerts, 2006; Petraglia et al., 2019), N mineralization and nitrification (Sierra, 1997; Zak et al., 1999), and plant productivity (Grace, 1988; Rustad et al., 2001). A recent study predicted the effect on plants that could occur by limiting global warming to 1.5 °C, rather than the 2 °C previously agreed upon in the Paris Agreement on climate change: the integrated range loss at 2 °C for plants would be about 30%, while at 1.5 °C the loss would be about 24% (Warren et al., 2018). The effects of warming on ecosystems may also influence other global change factors, such as increases in atmospheric CO₂, which could create a positive feedback, further amplifying climate change (Davidson & Janssens, 2006; Melillo et al., 2017).

1.2 Warming treatments in field experiments

To understand and predict biological responses to climate change, field experiments designed to manipulate temperature are frequently used. In contrast with observational studies, which pose limitations such as the inability to fully differentiate effects of warming from other factors that have changed over time (e.g., plant succession or land use change) (Ettinger et al., 2019), controlled field experiments can establish causality and provide important mechanistic information. Moreover, unlike experiments conducted in growth chambers or glasshouses,
field experiments provide contextual relevance with respect to species interactions and environmental conditions. Infrastructure commonly used to study warming in the field includes open-top chambers (Marion et al., 1997; Norby et al., 1997), polyethylene tents (Havström et al., 1993; Wookey, 1993), heated fluid-filled tubes on the soil surface (Hillier et al., 1994), buried cables (Peterjohn et al., 1994), passive night-time warming (Hollister & Webber, 2000), and overhead infrared heaters (Nijs et al., 1996). The latter method, while costly to run and limited by access to electricity, is considered to be the least affected by experimental artefacts (Harte et al., 1995).

1.3 Effects of warming on herbaceous plants

1.3.1 Changes in phenology

Plants are synchronized with seasonal changes in their environment, and there is evidence for shifts in the timing of developmental stages (e.g., phenology) of plants in response to recent climate change, and specifically increased temperature (Cleland et al., 2007; Huang et al., 2020; Piao et al., 2019). Changes in phenophases, such as leaf unfolding, flowering, plant growth, fruiting, and senescence in response to changes in climate have been documented in the Northern Hemisphere (Gill et al., 2015; Myneni et al., 1997; Parmesan & Yohe, 2003; Root et al., 2003; Schwartz et al., 2006; Walther et al., 2002). As well, recent increases in vegetation activity and carbon uptake have primarily resulted from changes in phenology in response to warming (Piao et al., 2017).

As the Northern Hemisphere experiences increasing temperatures (IPCC, 2014), shifts in the growing season have also occurred (Fu et al., 2014; Jeong et al., 2011; Myneni et al., 1997; Schwartz et al., 2006), with a steady increase in temperature lengthening the growing season through the earlier arrival of spring and the delay of autumn events (Cleland et al., 2006; Ma & Zhou, 2012). Jeong et al. (2011) reported that at a hemispheric scale, the start of the growing season advanced by 5.2 days during the period of 1982-1999, but advancement was slower during the period of 2000-2008, at only 0.2 days. As well, the end of the growing season in North America was delayed by 8.1 days during 1982-1999, and then by another 1.3 days between 2000-2008 (Jeong et al., 2011).
While much of the literature on phenological shifts in response to climate change has focused on woody plants (Cleland et al., 2007; Fu et al., 2015; Ma & Zhou, 2012; Menzel et al., 2006; Orlandi et al., 2014), a recent meta-analysis on phenological changes in herbaceous plants found that, in most cases, spring phenophases (e.g., first leaf date and first flowering date) had advanced over the previous 30 years (Huang et al., 2020). A 25-year-long study of herbaceous species found that long-term shifts with climate warming varied among phenological events (e.g., emergence, the end of expansion, beginning of flowering, end of flowering, beginnings of senescence and dormancy) and were species specific (Augspurger & Zaya, 2020). Furthermore, for a given species, most phenological events have been advancing and most durations of these events are shortening, by 68% and 74%, respectively (Augspurger & Zaya, 2020). Shifts in the phenological cycles of plants caused by climate change, specifically the lengthening of the growing season, strongly positively correlates with annual gross primary productivity and net primary productivity (Piao et al., 2007).

1.3.2 Effects on aboveground plant productivity

Plant growth and development are temperature dependant, and each species has a set range over which growth can occur, represented by a maximum, minimum, and optimum temperature (Hatfield & Prueger, 2015). Therefore, changes in temperature can result in both positive or negative plant responses (Piao et al., 2007). For example, in a meta-analysis of experimental warming at the ecosystem level, while warming increased aboveground plant productivity by 19%, this response was greatest in cold ecosystems, such as tundra sites (Rustad et al., 2001). An additional meta-analysis demonstrated that while warming significantly increased biomass (+ 12.3%) across all terrestrial plants, these responses depended on plant species and/or functional type (Lin et al., 2010a).

While longer growing seasons can increase plant productivity, the effects of warming on photosynthesis (which, from a mechanistic standpoint, can drive productivity) are inconsistent, with some studies reporting no changes in photosynthetic rate (Bronson & Gower, 2010; Li et al., 2019), while others found decreases (Jochum et al., 2007; Rachmilevitch et al., 2006) or increases (Danby & Hik, 2007; Wan et al., 2009; Yamaguchi et al., 2016; Yuan et al., 2018). This variability in results suggests that photosynthetic responses to warming could be species dependant (Liang et al., 2013; Niu et al., 2008). Moreover, the ability for plants to adapt their
photosynthetic characteristics to their current growth temperature (temperature acclimation) has been found to be varied among C\textsubscript{3}, C\textsubscript{4}, and CAM species, as well as plant functional types within C\textsubscript{3} plants (Yamori et al., 2014).

Interannual variation can also influence the plant productivity response to warming. For example, Hutchison and Henry (2010) found that while warming did not significantly affect aboveground biomass in every year, it increased biomass in a year when the treatment advanced spring snow melt, and then decreased biomass in years with a severe late spring frost. Furthermore, warming-induced increases in plant productivity could be driven indirectly by enhanced nutrient availability, given that warming can increase microbial activity (section 1.8.1), which increases rates of nutrient mineralization (Lu et al., 2011; Rustad et al., 2001).

1.3.3 Effects on belowground plant productivity

Similar to aboveground biomass, belowground biomass responses to experimental warming vary, with some studies finding that warming increases (Flanagan et al., 2013; Hutchison & Henry, 2010; Liu et al., 2017; Ma et al., 2017; Salazar et al., 2020), decreases (Bronson et al., 2008; Parts et al., 2019), or has no significant effect on root biomass (Hoeppner & Dukes, 2012; Lin et al., 2010; Wu et al., 2011). This variation may be attributed to multiple factors, such as root diameter and depth (Hoeppner & Dukes, 2012), ecosystem type (Salazar et al., 2020), and the amount of precipitation (Flanagan et al., 2013; Liu et al., 2017). Furthermore, if a study only reports root samples collected once a year, this can underestimate root biomass, because it does not account for root turnover throughout the growing season.

Experimental duration may also play a role in root biomass responses to warming treatments. Wu et al. (2011) found that warming significantly stimulated total biomass (above and below ground) when accounting for experiment length, with positive responses becoming pronounced over the longer term (as elaborated on in section 1.7). Perennial species such as some grasses develop large root systems over time. Root depth defines plant-available water capacity (Macleod et al., 2007), thus having a larger root system aids in drought tolerance (Ludlow & Muchow, 1990; Wasaya et al., 2018).
1.3.4 Shifts in species range and diversity

Physiological levels of tolerance, such as the capacity to survive frost and drought, primarily limit the spatial distribution of plant species (with the exception of equatorial regions, where range limits are influenced more by competition, not abiotic tolerance) (O’Brien, 1998; Currie et al., 2004; Clifford & Booth, 2015; Bucher & Rosbakh, 2021). Globally, latitudinal, longitudinal, and altitudinal shifts in species range as a result of climate warming are increasing rapidly and are expected to continue over the next decades (Lenoir & Svenning, 2015). Species richness and abundance are likewise affected by climate, with water availability being a crucial limiting factor (Cook et al., 2015; Harrison & Grace, 2007; Huang et al., 2016; Sommer et al., 2010). Therefore, dry regions, where plant growth is mainly water-limited, will likely experience decreases in species richness under a warming climate; however, in cold temperature-limited regions, it is possible that species richness will increase, but this would likely occur at a smaller scale (Harrison, 2020).

1.4 Nitrogen as a limiting nutrient in ecosystems

Nitrogen (N) is a major component of proteins and amino acids, which are required in large quantities for various physiological processes and for overall plant development, and it is also a component of chlorophyll, the primary pigment involved in light capture for photosynthesis (Leghari et al., 2016). N is one of the most limiting nutrients for plant growth and productivity in northern terrestrial systems due to it being relatively scarce in forms that are available for plant uptake (Morgan & Connolly, 2013; Vitousek et al., 1997). While the atmosphere is made up of approximately 78% N, the main constituent, dinitrogen gas (N₂), features a strong triple covalent bond, and only a select group of microorganisms can convert it into more broadly available forms (e.g., ammonium) via biological nitrogen fixation (Davison, 1995). However, advancements in technology in the twentieth century led to the development of the Haber-Bosch process, which is used for large-scale industrial N fixation (Smill & Streatfeild, 2002).

The main anthropogenic activities that account for 60% of global atmospheric deposition are food production (agricultural use of fertilizers) and energy production (use of fossil fuels for industry and transportation) (Kanakidou et al., 2016). Both of these activities have expanded in many northern temperate regions over the last century, which has increased rates of
atmospheric N deposition (Galloway et al., 2004), which is defined as the input of reactive, biologically available N from the atmosphere to the biosphere, either in the form of wet deposition (precipitation) or dry deposition (dust particles). It is estimated that in 1860 the global anthropogenic inputs of available N were about 15 Teragrams (Tg) N yr\(^{-1}\) and that increased to 156 Tg N yr\(^{-1}\) in 1995 and then to 187 Tg N yr\(^{-1}\) in 2005 (Galloway et al., 2004), thereby increasing ~13-fold. It was projected that in the next 30 years rates of N deposition would reach 2 – 5 g N m\(^{-2}\) yr\(^{-1}\) near industrial regions around the globe, and these figures approximate an increase in global N deposition to 270 Tg N yr\(^{-1}\) by 2050 (Galloway et al., 2004).

In recent years, air pollution legislation from North America (Gilliam et al., 2019; Houle et al., 2015; Li et al., 2016c) and Europe (Waldner et al., 2014) has resulted in regionally decreased rates of atmospheric N deposition; however, in other regions such as Asia, rates have increased (Vet et al., 2014), which influences global atmospheric levels of N deposition.

1.5 Ecosystems effects of increased atmospheric nitrogen addition

1.5.1 Effects on aboveground plant productivity

With the combination of a warmer, longer growing season and increased rates of N mineralization, net primary productivity is projected to increase for many ecosystems (Rustad et al., 2001). Given that N is the key limiting nutrient for productivity in most terrestrial ecosystems (Vitousek et al., 1997), increased rates of N deposition could further drive changes in plant productivity. Plant biomass typically increases in response to N addition in the field; a recent global meta-analysis found that N addition increased aboveground biomass by 31% (Yue et al., 2020). However, such increases tend to depend on plant functional type, and are greater in herbaceous species than in woody species (Xia & Wan, 2008). N addition also can increase above- and below-ground competition in plants (Wilson & Tilman, 1991). However, the relationship between aboveground competition and productivity is strongly correlated with neighbour size, because shading can negatively impact growth (Tilman, 1988; Keddy et al., 2002; Lamb et al., 2007). Increased rates of N deposition can also have negative impacts on plant productivity, especially when plants are exposed to long-term levels of increased N, which may result in N saturation (section 1.5.4).
1.5.2 Effects on belowground plant productivity

Plant root systems are significantly affected by atmospheric N deposition, which influences morphology, biomass, and various functions related to carbon cycling (Nadelhoffer, 2000). For example, a meta-analysis found that simulated N deposition increased total root biomass by 20%; however, this was a combined total of fine root biomass decreasing by 12% and coarse roots increasing by 56% (Li et al., 2015a). Moreover, there are contradictory results regarding how belowground productivity responds to increased available N, with root biomass increases (Li et al., 2015a; Ren et al., 2019; Zhang et al., 2015), decreases (Ren et al., 2019; Xu et al., 2017), and no significant changes (Chen et al., 2018; Henry et al., 2015; Hutchison & Henry, 2010; Vankoughnett & Henry, 2014). This variation may be attributed to many factors, such as ecosystem type, water availability, seasonal variation, species, and/or duration of experiment (Chen et al., 2018; Hutchison & Henry, 2010; Li et al., 2015a; Ren et al., 2019).

1.5.3 Species diversity

Increases in plant productivity as a result of terrestrial eutrophication can have detrimental effects on plant diversity (Yue et al., 2020). For example, a regional meta-analysis from Chinese sites found that N addition reduced species richness in temperate ecosystems and negatively affected plant diversity over the long-term (3-10 years) vs. the short-term (≤ 1 year) (Han et al., 2019), and a recent meta-analysis at a global scale found that N addition reduced plant diversity across all terrestrial ecosystem types (Yue et al., 2020). N addition can favour the dominance of nitrophilic species by disfavouring competitors and/or other species that are sensitive to changes in soil chemistry caused by the addition of N (Zhou et al., 2018). Moreover, in the absence of other confounding factors (e.g., drought), N addition can allow fast-growing species with inefficient resource-use strategies to outcompete species that have conservative resource-use strategies (Liu et al., 2011). For example, in a mature grassland community, N addition resulted in the reduction of species richness, driven by a loss of perennial grasses and forbs at all N input rates, but an increased dominance of early successional annuals (Bai et al., 2010). These findings also demonstrated that plant responses, instead of soil responses, could provide more sensitive measures of the impacts of anthropogenic N deposition, as plant responses occurred at lower N addition rates compared to soil responses (Bai et al., 2010).
1.5.4 Nitrogen saturation

Temperate ecosystems are typically N-limited, but long-term increases in N deposition (chronic N deposition) can result in N saturation. N saturation occurs when N inputs equal or exceed an ecosystem’s ability to absorb the added N (Tipping et al., 2017), causing changes to processes such as N mineralization, nitrification and carbon sequestration (Aber et al., 1998), increasing nitrate motility in soils (Payne et al., 2017), and altering soil fertility (Komatsu et al., 2019). Aber et al. (1998) described how aboveground net primary productivity (ANPP) changes as a function of N deposition over time, as ecosystems progress towards and exceed N saturation. Under N limiting conditions, ANPP increases linearly with additional N inputs. When continuous N availability exceeds plant N demand, the increase in ANPP levels off, resulting in a saturation threshold of N input (Aber et al., 1998). A recent global meta-analysis estimated an average N saturation threshold of 5–6 g m$^{-2}$ y$^{-1}$ for terrestrial ecosystems (Tian et al., 2016), but the response patterns varied among ecosystem types, rates of N addition and other environmental factors (i.e., temperature and precipitation). Nevertheless, being able to quantify plant N saturation along N gradients in terrestrial ecosystems enhances the ability to predict possible responses at both the community and ecosystem levels.

A chronic increase of N, even at low levels, can cause eutrophication and soil acidification, with wide-ranging negative consequences for ecosystem services and biodiversity (Bobbink et al., 2010; Clark et al., 2017; Lu et al., 2018; Stevens et al., 2018; Vitousek et al., 1997). While ANPP initially increases with N addition, chronic N addition can lead to the reduction of overall productivity through the loss of dominant species (Isbell et al., 2013). For example, in a tallgrass prairie, productivity returned to ambient levels after 4 years of N addition as a result of a decline in species abundance and a shift in the community to non-N-fixing and annuals forbs (Avolio et al., 2014). Such changes have the potential to alter the stability of ecosystems function and processes over time. For example, Emmett (2007) summarized findings from various conceptual frameworks that described changes associated with increased N availability, and these included early loss of low N tolerant plants, enhanced NH$_4^+$ concentrations associated with suppressed microbial immobilization of deposited NO$_3^-$, increased NO$_3^-$ leaching, and decreased decomposition of soil organic matter.
1.5.5 Combined effects of warming and nitrogen on plant productivity

As previously described, both climate warming and atmospheric N deposition can increase plant productivity. When combined, added N and increased warming have been found to have positive additive effects on aboveground plant productivity (Hutchison & Henry, 2010) and positive interactive effects on belowground productivity (Zhang et al., 2015). Furthermore, while a global meta-analysis found that the combined effects of added N and increased warming have resulted in losses of species richness (Midolo et al., 2019), one study found that the combined effects of warming and added N caused a species composition shift towards more thermophilic and eutrophic plant communities, culminating in increased species richness (Boutin et al., 2017). These results are the first to suggest that shifts towards enhanced species diversity may occur under N deposition and climate warming.

1.6 Long-term global change experiments

1.6.1 Temporal context

In recent decades, there has been a strong focus in ecosystem ecology to quantify the impacts of various global change factors (including climate warming and increased atmospheric N deposition) on plant species composition and productivity (Komatsu et al., 2019). As described above, field studies can provide valuable contextual relevance with respect to species interactions and environmental variability. However, even field experiments are typically limited by temporal scale, and the question arises as to whether their short-term findings can be extrapolated to the longer term. For example, short-term field experiments are not able to address the cumulative effects that can occur over the longer term, particularly for communities comprised of perennial, long-lived plants (Dentener et al., 2006).

Short-term field experiments also can be disproportionately influenced by year-to-year variation in weather. For example, in a study of warming in a temperate old field, productivity responses to warming in each given year depended on spring snow cover; productivity increased when plot warming promoted early snow melt in the absence of severe frost, whereas productivity decreased in response to warming in a year when warming-induced snow melt...
was followed by a severe frost event (Henry et al., 2015). Furthermore, interannual variation in precipitation can play a strong role in influencing warming responses, with water loss worsening in drought years when warming is applied (Hutchison & Henry, 2010; Zhang et al., 2015). It is also possible that biomass production in short-term field experiments may be driven disproportionately by feedbacks with litter deposition (section 1.6.4).

Moreover, plant responses to combined N addition and warming have also been recorded in long-term experiments, with one study reporting that long-term N addition and warming had a negative additive effect on community stability, with the similar effects of warming and N originating from different mechanisms (Wu et al., 2020). While warming decreased community stability by reducing the stability of dominant species, N addition reduced community stability by decreasing species asynchrony and the stability of both dominant and common species (Wu et al., 2020). Similarly, a global meta-analysis found that greater losses of species richness from combined N addition and increased warming occurred at sites with longer experimental durations (Midolo et al., 2019).

Of important note is the high variation in the duration of experiment in the literature considered to be ‘long-term’. For example, Komatsu et al. (2019) refer to long-term as ≥ 10 years, while An et al. (2004) consider 4 years to be long-term. However, the term is often relative to what is being studied. Using the previous examples, Komatsu et al. (2019) were looking at the plant community responses to various global change drivers, something that can take years to emerge. In contrast, An et al. (2004) looked at the effects that long-term experimental warming had on green leaf N concentrations, where long-term patterns can be exhibited under a shorter period of time. It has been found that continuous directional responses are frequent in longer-term experiments (>11 years), and in some cases are accompanied by large shifts in community composition (Smith et al., 2015). Furthermore, the results of the latter meta-analysis suggest that while responses of ANPP to chronic resource manipulations are variable, once a response arises it persists (Smith et al., 2015).

1.6.2 Lag effects

Time lags are an important component of vegetation responses to climate change. A global meta-analysis by Wu et al. (2015) revealed that time-lags in vegetation responses to climate
change differed among vegetation types and climates, and that climatic factors even caused variation in time lags within the same vegetation type. Similarly, Ding et al. (2020) observed that time lags varied with vegetation types/biomes, climatic factors and regions. Vegetation lag responses may be particularly important in the context of species such as ecosystem engineers (Jones et al., 1994), and therefore have consequences for ecosystem structure and functioning, by impacting productivity, biodiversity and nutrient cycling.

In the context of global change experiments, a recent meta-analysis found that while in the short-term (<10 years), plant communities appeared resistant to experimental manipulation of global change drivers, over the long-term (≥ 10 years), there was increasing community divergence of treatments in comparison to the control (Komatsu et al., 2019). Similarly, another global meta-analysis found that increases in plant productivity with long-term N addition resulted in decreased plant diversity (Yue et al., 2020). Moreover, as described above, lagged responses of plant species composition to environmental change can be particularly evident in communities comprised of perennial plants. For example, Cotto et al. (2017) showed that perennial species can endure inadequate habitats longer than predicted by their climatic tolerance, thus delaying range loss.

1.6.3 Litter accumulation

Plant litter can negatively affect plant growth by blocking photosynthetically active radiation, decreasing germination, impeding seed penetration into the soil or having allelopathic effects, and increasing the risk of fungal infection (Kelemen et al., 2013). Changes in light availability caused by variation in litter input can consequently drive shifts in species and functional group composition (Amatangelo et al., 2008). For example, it has been reported that increasing litter mass reduces species richness and can either increase (Letts et al., 2015) or decrease species evenness (Lamb, 2008). Litter accumulation also can favour rhizomatous grasses over bunch grasses in grasslands, as a result of increased soil moisture (Hou et al., 2019), and it can create a positive feedback loop with invasive species, negatively affecting native species establishment and diversity (Mariotte et al., 2017). A substantial increase in biomass production in response to warming and/or N addition in the first year of a field experiment could result in a thick litter layer the subsequent spring, thereby suppressing biomass production in the second year of the experiment; the resulting low litter production in the
second year could then allow for high biomass production the following year, pending adequate decomposition of the plant litter from the first year of the experiment. However, these observations remain anecdotal, and it is unclear to what extent such oscillations may occur and how long they may take to dampen.

1.6.4 NDVI: measuring plant productivity in long-term global change experiments

In long-term field experiments it is important to limit the amount of disturbance to plots. Therefore, non-destructive methods of biomass estimation can be useful, particularly for exploring seasonal trends. There are multiple non-destructive methods that can be used for biomass estimation, such as the point intercept method (Jonasson, 1988) and the use of allometric equations to convert measurements of shoot height into shoot mass (Money & Niklas, 1995; Niklas, 1994). While these methods are effective and do not require biomass removal from the sampling area, they are also very time consuming. A quicker alternative to estimate aboveground biomass is the use of spectral measures. Normalized difference vegetation index (NDVI) is a type of spectral vegetation index that uses the contrast between the red and near-infrared wavelengths reflected from a green vegetation canopy to distinguish living plants from plant litter, soil or standing water. Although it can be used to estimate aboveground plant biomass (Gamon et al., 1995), a previous study conducted at my site found that NDVI was not a good indicator of aboveground biomass at peak grass biomass because the response saturated after the canopy filled in, and thereafter further vertical growth by the grasses was undetected (Hutchison & Henry, 2010). However, NDVI remains useful in grass-dominated communities for estimating aboveground biomass during spring green-up, or to quantify plant senescence in the fall (Henry et al., 2015).

1.7 Litter decomposition

1.7.1 The role of litter decomposition

Litter decomposition responses to climate change drivers have been studied widely due to their important implications for carbon cycling and productivity; decomposition roughly balances ANPP by emitting approximately 5 – 6 times the amount CO₂ than current rates of fossil fuel
combustion (Gregorich et al., 2017). When plant litter undergoes decomposition, nutrients that were not resorbed during senescence (section 1.9) are re-mineralized (Wang et al., 2017). Climate (temperature and moisture) and litter quality are the key drivers of litter decomposition at regional to global scales (Aerts, 1997; Austin & Vitousek, 2000; Coûteaux et al., 1995; Cornwell et al., 2008; Gregorich et al., 2017; Petraglia et al., 2019; Zhang et al., 2008). Nevertheless, decomposition of plant litter is facilitated by soil biota (Petraglia et al., 2019), and decomposer (microbial) biomass can strongly regulate decomposition at regional scales (Bradford et al., 2017).

In some cases, plants can interact with soil microorganisms to drive positive nutrient cycling feedbacks (Hobbie, 1992). For example, in nutrient-poor ecosystems, plants use nutrients efficiently, thus producing low quality litter which decomposes slowly, limiting rates of nutrient remineralization, because it is less desirable to soil biota; in contrast, plants in nutrient rich ecosystems produce rich litter that is readily utilized by soil biota, thus increasing subsequent nutrient availability for plants (Gessner et al., 2010; Hobbie, 1992).

### 1.7.2 Warming effects on decomposition

Litter decomposition rates typically increase with increasing temperature (Sihi et al., 2018). Due to decomposition having a positive relationship with temperature, there is concern of a positive feedback, whereby increased decomposition releases more CO₂ (via increased microbial activity), thus further intensifying climate change. However, increased rates of decomposition as a result of global warming may only occur if there is sufficient soil moisture (Aerts, 2006). For example, within a given site, the positive effect of temperature on decomposition can be diminished or even reversed when warming increases water stress (Butenschoen et al., 2011). Nevertheless, soil moisture effects on decomposition rates may be strongest in the short-term, with temperature overwhelming these effects over the longer term (Gregorich et al., 2017).

Although temperature effects on decomposition are largely attributed to changes in microbial activity, there are contradictory reports regarding the effects of soil warming on microbial biomass, ranging from positive effects (Belay-Tedla et al., 2009) to negative effects (Frey et al., 2008) or no effects (Biasi et al., 2008; Wang et al., 2014b). Variation in the spatial and
temporal scale of the study may be important in explaining variation in warming effects on microbial activity (Carey et al., 2016; Crowther et al., 2016; Hartley et al., 2008; Luo et al., 2001; Melillo et al., 2017; Melillo et al., 2002). Overall, this uncertainty has diminished agreement regarding the potential for feedbacks between decomposition and climate change (Walker et al., 2018).

Climate may also cause shifts in soil decomposer communities. For example, warm and moist conditions favour fast growing, competitive decomposers, and the latter can drive greater rates of various ecosystem processes than organisms that are more stress-adapted (Crowther & Bradford, 2013; de Vries et al., 2012). While still little is known about how climate can influence the functional abilities of soil decomposer communities, the direct positive effects of a more favourable climate for decomposition rates appear to be correlated with greater functional potential of the decomposer communities (Keiser & Bradford, 2019).

### 1.7.3 N addition effects on decomposition

Plants are able to meet much of their N requirement through N mineralized from decomposing litter (Suseela, 2019). However, increasing rates of N deposition can drastically change soil N availability as well as N cycling (Liu et al., 2013), altering the quality and quantity of litter and the soil physiochemical environment (Liu & Greaver, 2010). Thus, atmospheric N deposition can affect litter decomposition both directly, by modifying microbial activity (Knorr et al., 2005) or indirectly, through its effect on litter N content (Averill & Waring, 2018). Within plant communities, decomposition responses to N addition can be species specific and time-scale dependent, and changes in the relative abundances of species can have an important effect on overall decomposition as a result of intraspecific variation in litter quality (Henry & Moise, 2015).

Microbial activity and its impact on decomposition is strongly influenced by N availability. Thus, if microbes are N limited, then added N should increase their biomass. However, soil N addition experiments often show a decline in microbial biomass and organic matter decomposition (Treseder, 2008; Zhou et al., 2017). Furthermore, microbial N limitation has been found to increase decomposition through a process referred to as ‘microbial mining’, which is continuously suppressed by a high soil N supply (Craine et al., 2007).
mining occurs when low N availability forces microbes to use labile substrates (i.e., compounds that are easily transformed by biological activity) to gain N from recalcitrant organic matter (i.e., compounds resistant to microbial decomposition) (Craine et al., 2007). Therefore, this process would be consistently suppressed under increased N. Nitrogen addition has also been found to reduce microbial diversity (Wang et al., 2018b) and decrease microbial biomass by 15% (Treseder, 2008). A recent global meta-analysis also found N addition reduced microbial biomass, and that negative effects of N addition on microbial composition and abundance increased with experimental duration and amount of N deposition added (Zhang et al., 2018). Averill & Waring (2018) argued that the lack of a positive response between added N and microbial biomass was likely an indirect effect of increased nitrification as a result of N fertilization induced soil acidification. Thus, microbial growth and decomposition would continue to slow to the degree in which N stimulates acidification (Averill & Waring, 2018).

1.8 Measure of nutrient resorption

1.8.1 Resorption efficiency

In response to limited soil nutrient availability, plants have evolved mechanisms to recover nutrients from senescing and/or old tissues; these nutrients are then reallocated to produce new tissue or reserved in storage structures (Aerts & Chapin, 1999; Brant & Chen, 2015; Killingbeck, 1996; Prieto & Querejeta, 2020). This process is referred to as nutrient resorption (NuR), and it increases nutrient use efficiency (Yuan et al., 2006) by conserving nutrients that would otherwise be lost to the soil via litterfall (Killingbeck, 1996). While NuR occurs year-round, it is most prominent during autumn, as this is when plants begin to senesce and enter dormancy before winter (Freschet et al., 2010). Nitrogen resorption efficiency (NRE), an index of internal nutrient cycling (Wang et al., 2018b) is the measure of nutrients resorbed during leaf senescence expressed as a proportion of total nutrients (i.e. [(nutrient in green leaves – nutrient in senesced leaves)/ nutrient in green leaves] ×100) (Killingbeck, 1996). Global estimates of the average N resorbed back into live plant tissues is 62% (Vergutz et al., 2012), however resorption rates vary by plant functional type, and a recent global meta-analysis found that while the mean NRE for all herbaceous species was 59%, graminoids specifically had a NRE of 61% (Wang et al., 2018b).
Long-term, cumulative responses to global change may be revealed by changes in plant nutrient content, which provide integrated information regarding plant nutrient availability. N addition tends to increase plant tissue N content, with a negative effect on plant NuR (Yuan & Chen, 2015). This is attributed to increased availability of N to a plant requiring less energy to be spent on resorbing N from its structures. However, documented responses of plant NRE to changes in soil fertility via N addition have been inconsistent, ranging from no effect (Li et al., 2015b; Wang et al., 2014a) to reduced NRE (Huang et al., 2008) to increased NRE (Wang et al., 2020).

Moreover, the effects of warming on nutrient content can be variable, with reports of both increases in plant N concentration (Ren et al., 2018; Volder et al., 2015) and decreases in plant N concentration (An et al., 2005; Wu et al., 2019). Aerts et al. (2007) found that a warming treatment had little impact on NuR in a high-latitude peatland, but this response may be attributed to the small temperature increase (0.9°C) that was applied. Ren et al. (2018) found that in a desert grassland, NRE decreased during wet years, which they attributed to variation in the nutrient concentrations of plants and soils or to increased stimulation of microbial activity. Warming alone was also found to increase the rate of NRE by 12% at the community level, but increased by 16% when combined with a N addition treatment of 40 kg N ha\(^{-1}\) year\(^{-1}\) (Zong et al., 2018).

Another factor that can affect NuR is climate, but little research has been done on its effects, with most studies focused at a local or regional scale (Aerts et al., 2007; Luo et al., 2018; Norby et al., 2000; Prieto & Querejeta, 2020; Ren et al., 2018). Globally, it has been suggested that climate can greatly influence NuR, based on the finding that NRE is positively correlated with latitude, but negatively correlated with mean annual temperature and mean annual precipitation (Vergutz et al., 2012; Yan et al., 2018; Yuan & Chen, 2009). There have been few long-term studies (>3 years) on the effects N addition on NRE. While N addition can decrease NRE (Liu et al., 2021; Su et al., 2021), the effects on NRE can be both species and dose specific (Huang et al., 2008; Zong et al., 2018).
1.8.1.1 Nutrient resorption efficiency an indicator for N saturation

Methods for inferring ecosystem N saturation include examining foliar N:P (phosphorus) ratios (Tessier & Raynal, 2003) and the difference between NRE and PRE (phosphorus resorption efficiency) (Han et al., 2013). NRE decreases with increasing N availability (Li et al., 2016a; Li et al., 2010; Lü et al., 2013, 2016; Lü & Han, 2010), thus demonstrating a reciprocity between plant nutrient status and nutrient uptake from its environment. Thus, low NRE may suggest N saturation at a site.

1.8.2 Resorption proficiency

Nutrient resorption can also be assessed using resorption proficiency, defined as the level to which nutrients are reduced in senescing leaves. In general, the lower the nutrient concentration in senesced leaves, the lower the N loss through leaf fall, and the higher the N resporion proficiency (NPR); thus, plants living in fertile conditions are less proficient (Killingbeck, 1996). While resorption efficiency relates to plant physiology and metabolic processes, proficiency is directly associated with decomposition processes and therefore nutrient cycling (Wang et al., 2014a). Because proficiency determines the degree to which plants can reduce nutrients in their senesced leaves, this influences leaf chemistry, a key factor of decomposition rate (Aerts, 1999; Killingbeck, 1996). It follows that plants with high proficiency produce leaf litter with low nutrient concentrations and slow decomposition rates (Aerts & Chapin, 1999; Killingbeck, 1996; Xu et al., 2020), which affects nutrient cycling.

Different plant functional types have varying rates of N proficiency which results in differences in nutrient cycling. For example, evergreen species have higher proficiency rates than woody deciduous species, which in turn have higher rates than herbaceous species. Consequently, the nutrient content of leaf litter returned to the soil in herbaceous and deciduous species is higher than that of evergreen species (van Heerwaarden et al., 2003). However, N proficiency also can be impacted by nutrient inputs, such as atmospheric N deposition, especially in regions with limited nutrient availability.

Compared to efficiency, proficiency may be more responsive to nutrient availability. For example, in a global meta-analysis, N fertilization increased senesced leaf N concentration
(i.e., resorption proficiency) by 36% on average, a response that depended strongly on the rate of N addition, and was stronger than the resorption efficiency response (Yuan & Chen, 2015). This difference could be attributed to efficiency being a calculated proportion; as argued by Killingbeck (1996), resorption proficiency may be more conclusive and objective for estimating the extent to which selection has acted to reduce nutrient loss, because selection acts upon traits and not proportions.

1.9 Temperate old fields as a study system

Old fields are areas of previously human-managed land (often farmland) that have been abandoned and left to naturally recover through ecological succession. Years of cultivation, fertilization, alteration of biomass, and changes in hydrology impact ecosystem processes and cause shifts in species composition (Cramer et al., 2008; Dambrine et al., 2007; Foster et al., 2003; McLauchlan, 2006). Some old fields assemble along broadly repeatable patterns of succession that resemble the structure, composition and function of the historical vegetation state that existed before human intervention (Cramer et al., 2008). However, not all old fields follow this pattern, as some newly abandoned fields may be colonized by non-native invasive species due to increased soil nutrient availability from previous fertilization (Standish et al., 2008). Long-lived exotic invasive species can persist for decades (Cramer & Hobbs, 2007) by disturbing natural succession pathways and modifying patterns of vegetation recovery (Meiners et al., 2002), therefore inhibiting late-successional species from establishing and prolonging early succession stages (Cramer et al., 2008). Eventually old fields enter into later stages of succession whereby shrubs and then trees take over and form a stable community.

Old fields have been identified as useful test sites, both for observational studies and for experiments designed to study existing ecological theory (Cramer & Hobbs, 2007). In particular, old fields comprised mostly of herbaceous plants are convenient for inferring the community and ecosystems level effects of global change drivers such as warming and atmospheric N deposition, given that even when the treatments are applied at a relatively small spatial scale (e.g., 1 m² plots), many individuals and even species can be present within each plot. In the temperate regions of North America, herbaceous old field communities typically transition rapidly from weedy annual species to perennial community comprised of grasses, forbs, and legumes. The grasses are dominated by C₃ rhizomatous species, which are
categorized as cool-season grasses because they grow most vigorously from the spring through early summer, with some additional growth in the fall. These species are typically found in areas that have hot, dry summers and cold winters that experience freezing (Casler & Undersander, 2018).

For my thesis I conducted experiments in a temperate old field dominated by the C₃ grasses *Poa pratensis* and *Bromus inermis* L. Neither species is native to North America, and over time they have invaded and out-competed native species in a range of communities, with prairies and native grasslands experiencing the greatest impact on their diversity (DeKeyser et al., 2015; Otfinowski et al., 2007).

1.10 Objectives and Predictions

The objective of my study was to compare the short-term (1-2 year) vs. long-term (14-15 year) effects of warming and N addition on plant relative species abundances, productivity, litter decomposition, and shoot nutrient resorption efficiency in the context of a field experiment conducted in a temperate old field. Warming treatments were applied all year round using overhead infrared heaters, which increased soil surface temperature by 2-3°C, and these were crossed with a N addition treatment (6 g m⁻² y⁻¹) in a factorial design. To control for factors such as interannual variation in weather for the comparison of short-term vs. long-term responses, I added new N addition, warming, and control plots to a pre-existing 14-year-old field experiment.

Given the lags inherent in the turnover of perennial species in response to environmental change, I predicted treatment effects on relative species abundances would be the most pronounced in the old plots, and that these changes in the relative abundances of plant species would drive differences in plant litter decomposition at the plot level. I also predicted that the new plot treatment effects on productivity would be suppressed in the second year as a result of disproportionately high litter accumulation and the resulting delay in green-up. Lastly, based on the increased likelihood of N saturation in the old plots, I predicted the plants in the latter would exhibit lower NRE and be less proficient than those in the new plots.
Chapter 2: Materials and Methods

2.1 Study site description

My study was conducted at an old field site in London, Ontario, Canada (43° 01′ 46″ N, 81°12′ 52″). The site used to be an agricultural field, but was not plowed, fertilized, or mowed in over 30 years. Site vegetation was largely comprised of the dominant perennial C₃ grasses Poa pratensis L. (Kentucky bluegrass) and Bromus inermis Leyss (Smooth brome), while the forb Cirsium arvense L. (Canada thistle) was ubiquitous, but less dominant. Also present in some plots but at a lower density were the forbs Asclepias syriaca L. (common milkweed), Aster ericoides L. var. ericoides, (heath aster), Solidago canadensis L. var. scabra Torr. & A. Gray (tall goldenrod), and the legume Lotus corniculatus L. (bird’s foot trefoil). The soil of this region is classified as a well to imperfectly drained loam glacial till (Hagerty & Kingston, 1992), and those at the site were comprised of approximately 9% clay, 41% silt, and 50% sand, with an average pH of 7.6 (Bell et al., 2010). The mean annual air temperature was 7.9°C, with a mean monthly low of -5.6°C (January), a mean monthly high of 20.8°C (July), and a mean annual precipitation of 84.3 mm (Table 2.1) (Canadian Climate Normals 1981–2010, Environment Canada, National Climate Data and Information Archive).

2.1: Mean Temperature and Total Precipitation over the 2020 Plant Growing Season (1 April to 31 October) for the Experimental Site Relative to 1981-2010 Climate Normals.

<table>
<thead>
<tr>
<th></th>
<th>1981-2010</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean growing season temperature (°C)</td>
<td>14.7 (4.9)</td>
<td>15.0</td>
</tr>
<tr>
<td>Mean growing season precipitation (mm)</td>
<td>86.1 (8.8)</td>
<td>80.0</td>
</tr>
<tr>
<td>Total growing season precipitation (mm)</td>
<td>602.5</td>
<td>560.3</td>
</tr>
</tbody>
</table>

Data from Environment Canada, National Climate Data and Information Archive.

Standard deviation indicated in parentheses.
2.2 Experimental design

The pre-existing experimental treatments, which have run since late 2006, consisted of two warming treatments (year-round warming and ambient) combined with two nitrogen (N) addition treatments (N addition and no N addition), which were organized in a randomized block factorial design (n=10). There were 10 replicates blocks each containing 9 plots distributed within a 0.5 ha area. Within each block warming and N addition treatments were applied at the plot level, resulting in a total of four treatment combinations per block and 40 plots (Fig. 2.1) (20 plots were used for a winter-warming treatment, which has since been discontinued, and the remaining 30 plots were reserved for future use. Each plot was 113 cm in diameter and extended outwards by a buffer zone of 10 cm, which also received the experimental treatments. Warming was administered using 150 W ceramic infrared heaters (Zoo-Med Laboratories, San Luis Obispo, CA, USA), which were suspended 50 cm above the plot centers, but from mid-June to late-September were raised by 25 cm to avoid scorching the grass inflorescences. These heaters provided warming without giving off photosynthetically active radiation (Harte et al., 1995). Microclimatic effects on the soil temperature and moisture were recorded hourly, year-round. Soil temperature was monitored using CR1000 dataloggers connected to 107-BAM-L temperature probes at both 1 and 5 cm soil depth, and soil moisture was monitored using CS616-L time domain reflectometry probes located at 0–15 and 0–30 cm soil depth (all equipment from Campbell Scientific Canada Corp., Edmonton, AB, Canada).

Nitrogen was added annually, through a pulse of aqueous ammonium nitrate that was added at a rate of 2 g m$^{-2}$ y$^{-1}$ of N in late March, and slow-release ammonium nitrate pellets (Florikan ESA, Sarasota, FL) added at a rate of 4 g m$^{-2}$ y$^{-1}$ of N in late May. The total addition rate of 6 g m$^{-2}$ y$^{-1}$ of N was consistent with the high estimate of the predicted increase in N addition expected in this region by 2050 (Galloway et al., 2004). In addition to the 40 pre-existing old treatment plots (which had been receiving their treatments since 2006), I established from the reserved plots new sets of 10 warmed plots, 10 N addition plots, and 10 new control plots (the latter were included as untreated plots that could be compared to the long-term control plots, to examine whether there were residual effects of sampling disturbance in the latter). These new plots received the same treatments as the old plots, but no new combined warming and N addition plots were established.
2.3 Biomass sampling

During both years of the experiment, coinciding with peak plant biomass (early July), I destructively harvested the dominant grass species *Poa pratensis* and *Bromus inermis* from each plot within a 10 × 10 cm quadrat. Samples were oven dried at 65°C for 72 hr and then weighed to determine the biomass. For forb species, I estimated percent cover within each plot, both at peak biomass and then again later in the growing season (August). To estimate root biomass, I used soil cores collected from each plot using a 2 cm × 15 cm deep corer. I soaked the soil cores in water and separated the roots from the soil particles using a 35-mesh sieve (0.5 mm openings), dried the roots at 65°C for 72 hr, and then weighed the roots.
2.4 NDVI sampling

I used a portable spectrophotometer (FieldSpec Handheld, Analytical Spectral Devices, Boulder, CO, U.S.A.) to measure the normalized difference vegetation index (NDVI) in mid-to late spring (May 2020 and April to May 2021) to assess plant green-up. An additional measurement was taken during senescence in late September (2020). The spectral measures were taken on cloudless days when wind speed was not sufficient enough to sway the plant shoots, and within 1 hr of noon. Each recorded spectrum was the average of 10 spectra collected with a fiber optic suspended 1 m above the soil, using a 20° field of view foreoptic, to give a 30 cm diameter circle of view. NDVI was calculated as (NIR - RED)/(NIR + RED), where NIR (near infrared) = the average reflectance from 720 to 740 nm and RED (visible red) = the average reflectance from 660 to 680 nm (Hutchison & Henry, 2010).

2.5 Tissue sample analyses and nutrient resorption measures

Nitrogen content and nutrient resorption measures were only collected in the first year of observation (2020). During the destructive harvest, leaf subsamples (green tissue) of *Poa pratensis*, as it was the only grass between the two dominant grasses present in every plot, was collected and additional samples were collected in the fall (senesced tissue) from each plot for tissue N analysis and nutrient resorption efficiency estimations. Both green and senesced samples were dried at 65°C for 72 hr, then ground to a fine powder using a Wiley mill followed by a ball mill. Powder samples were then weighed into tin boats (4 mm × 6 mm), compressed, and analyzed for %N in a CHNS Analyzer (make: Elementar, model: Vario Isotope Cube) in the University of Western Ontario Biotron analytical facility.

Leaves lose mass as a result of soluble carbon compounds that are resorbed during senescence (van Heerwaarden et al., 2003; Yan et al., 2018), which can cause bias of estimates of resorption efficiency (van Heerwaarden et al., 2003). Therefore, a mass loss correction factor (MLCF) was used to account for the percentage of leaf mass lost during senescence, and was applied as follows (Yuan & Chen, 2015):

\[
NRE = \left(1 - \frac{[N_s]}{[N_g]} \times MLCF \right) \times 100
\]

(1)
where [N_s] and [N_g] are the N concentrations for senesced and green leaves, and the MLCF is 0.713 for graminoids (Vergutz et al., 2012). Nutrient resportion proficiency (NPR) is the final nutrient level left in senesced leaves, thus NPR= N_s (Killingbeck, 1996).

2.6 Litter decomposition

In the fall of 2020, I collected litter samples (recently senesced tissue) of the two dominant grass species to measure treatment effects on decomposition rates. The samples were air dried for 72 hr at room temperature and then combined, at a 3:1:1 ratio of Bromus inermis stems to B. inermis leaves to Poa pratensis tillers, for a total of 500 mg of litter (Henry & Moise, 2015). These subsamples were then placed in nylon mesh bags (10 cm × 5 cm dimension, with 1.0 mm openings). Additional litter bags containing litter from Cirsium arvense L., the only forb species that was found in the majority of plots, were assembled for each plot. These samples were also dried for 72 hr at room temperature and then combined, at a 3:1: ratio of stem to leaves, for a total of 400 mg, and placed in nylon mesh bag as described above. For plots that did not contain C. arvense, material from another plot of the same treatment was used. Additional litter samples of B. inermis, P. pratensis, and C. arvense were collected and dried at 65°C for 72 hr following air drying to estimate the initial water content for the litter placed in the bags, thus allowing expression of the latter a dry weight basis. All bags were placed on the soil surface, and were collected 32 and 30 weeks after placement (grass and thistle, respectively). Upon collection, litter samples were oven-dried at 65°C for 72 hr, separated by species, and weighed, with total mass loss used to estimate decomposition. I calculated the weighted average of decomposition for each plot by combining the species-level estimates of initial litter mass in each plot with the mass loss data.

2.7 Data analyses

I analyzed the dependant response variables for my biomass samples using two separate 2-way block ANOVAs: the first with plot age and warming as the fixed factors and the second with plot age and N addition as the fixed factors. For the long-term plots I also ran a 2-way block ANOVA with warming and N addition as the fixed factors to test whether a warming by N addition interaction had emerged in the later years of the experiment. Analyses were run using R version 4.0.2. The 2020 biomass data was square-root transformed prior to analysis to satisfy
the assumptions of normality and homogeneity of variances. The same analysis was run for
NDVI, tissue N content, NRE, and decomposition data. Forb percent cover estimates did not
meet the assumptions of normality and therefore were analyzed non-parametrically via
Kruskal-Wallis H tests.
Chapter 3: Results

3.1 Volumetric water content and soil temperature

The weather varied between the two years of observation (2020 and 2021) following the establishment of the new plots experiment, with the primary difference being that it was warmer and drier during the second year during the period of most rapid plant growth (March to the end of June) (Figs. 3.1 and 3.2). In the first year, during this period, volumetric water content in the warmed plots was on average 0.38 (0.39 in the ambient plots), while soil temperature at 5 cm soil depth in the warmed plots was on average 10.5 °C (9.6 °C in the ambient plots). In the second season, the corresponding volumetric water content value for the warmed plots was 0.33 (0.34 for the ambient plots) and soil temperature in the warmed plots was on average 13.6°C (12 °C in the ambient plots).
Figure 3.1: Average volumetric water content at 5 cm soil depth for warmed (n=30) and ambient (n=40) plots.

Figure 3.2: Average soil temperature at 5 cm (°C) soil depth for warmed (n=30) and ambient (n=40) plots.
3.2 Productivity and cover estimates

In the first year, there was evidence for stronger nitrogen addition effects on productivity in the old plots than in the new plots. Specifically, for total aboveground productivity, nitrogen addition significantly increased total productivity in the old plots ($P_N=0.002$; Fig. 3.3c), and there was a marginally significant interaction between plot age and nitrogen addition ($P_{N\times age}=0.056$; Fig. 3.3a). This marginally significant effect became significant ($P<0.05$) when block 10 was excluded from the analysis (block 10 was an outlier compared to the other blocks because it had been largely overrun by an adjacent patch of *Solidago canadensis* since the old plots were established 15 years ago). These responses of total productivity were explained in part by the response of *Poa pratensis*, which increased significantly in the nitrogen addition plots ($P_N=0.002$; Fig. 3.4a). There also was a marginally significant interaction between plot age and nitrogen addition for *Bromus inermis* ($P_{N\times age}=0.069$) and a marginally significant increase for *B. inermis* in response to nitrogen addition in the old plots ($P_N=0.060$; Table 3.1). As with total productivity, these marginally significant effects became significant ($P<0.05$) when block 10 was excluded from the analysis. There were no significant effects of warming on aboveground productivity, nor significant interactions between nitrogen addition and warming in the old plots (Table 3.1) (as described above, the experimental design precluded the testing of a nitrogen by warming interaction in the new plots). Likewise, there were no significant treatment effects on cover for any of the forb species (Table 3.2a). However, root biomass increased significantly in response to nitrogen addition in the old plots ($P_N=0.027$; Fig. 3.5c).

In the second year, nitrogen addition was again the only factor that elicited a significant aboveground productivity response; namely, the aboveground productivity of *P. pratensis* increased significantly in the nitrogen addition plots ($P_N=0.018$; Fig. 3.6 a). However, unlike the first year, there were no interactions between plot age and nitrogen addition. There were no significant treatment effects on cover for any of the forb species (Table 3.2b). Likewise, there were no significant treatment effects on root biomass in either the old or new plots (Table 3.1).
Figure 3.3: Total aboveground productivity at peak biomass during 2020 in response to a) nitrogen addition (new and old plots), b) warming (new and old plots) and c) nitrogen and warming (old plots only) (n=10). Boxes contain the interquartile range (the distance between the 25th and 75th percentiles), whiskers represent the minimum and maximum values in the data set, excluding statistical outliers (open circles; statistical outliers were retained in the analyses, with data transformations used when applicable to meet the assumption of normality). Significant and marginally significant 2-way ANOVA P-values are displayed on the panels, with all ANOVA P-values shown in Table 3.1. The data were square-root transformed prior to analysis.
Table 3.1: Summary of 2-way ANOVA P-values for effects of treatment and plot age on plant productivity

<table>
<thead>
<tr>
<th></th>
<th>2020 Aboveground Productivity</th>
<th>2020 Root mass (all species)</th>
<th>2021 Aboveground Productivity</th>
<th>2021 Root mass (all species)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td><em>P. pratensis</em></td>
<td><em>B. inermis</em></td>
<td>Total</td>
</tr>
<tr>
<td>N and plot age</td>
<td>N</td>
<td>0.056*</td>
<td>0.002**</td>
<td>0.621</td>
</tr>
<tr>
<td>age</td>
<td></td>
<td>0.069*</td>
<td>0.437</td>
<td>0.931</td>
</tr>
<tr>
<td>N x age</td>
<td></td>
<td>0.055*</td>
<td>0.635</td>
<td>0.069*</td>
</tr>
<tr>
<td>Warming/plot age</td>
<td>W</td>
<td>0.472</td>
<td>0.642</td>
<td>0.277</td>
</tr>
<tr>
<td>age</td>
<td></td>
<td>0.572</td>
<td>0.986</td>
<td>0.577</td>
</tr>
<tr>
<td>W x age</td>
<td></td>
<td>0.825</td>
<td>0.151</td>
<td>0.661</td>
</tr>
<tr>
<td>N and Warming (old plots only)</td>
<td>W</td>
<td>0.002**</td>
<td>0.012*</td>
<td>0.060*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.381</td>
<td>0.444</td>
<td>0.578</td>
</tr>
<tr>
<td>N x W</td>
<td></td>
<td>0.747</td>
<td>0.853</td>
<td>0.994</td>
</tr>
</tbody>
</table>

W- warming; N – Nitrogen; Age – Plot age. Asterisks denote significant results (*0.05 – 0.01, **0.01-0.001, ***<0.001), and marginally significant P values (P<0.1) are denoted by *. 
Figure 3.4: Aboveground productivity of *Poa pratensis* at peak biomass during 2020 in response to a) nitrogen addition (new and old plots), b) warming (new and old plots) and c) nitrogen and warming (old plots only) (n=10). Boxes contain the interquartile range (the distance between the 25th and 75th percentiles), whiskers represent the minimum and maximum values in the data set, excluding statistical outliers (open circles; statistical outliers were retained in the analyses, with data transformations used when applicable to meet the assumption of normality). Significant and marginally significant 2-way ANOVA P-values are displayed on the panels, with all ANOVA P-values shown in Table 3.1. The data were square-root transformed prior to analysis.
Figure 3.5: Total belowground productivity of all species at peak biomass during 2020 in response to a) nitrogen addition (new and old plots), b) warming (new and old plots) and c) nitrogen and warming (old plots only) (n=10). Boxes contain the interquartile range (the distance between the 25th and 75th percentiles), whiskers represent the minimum and maximum values in the data set, excluding statistical outliers (open circles; statistical outliers were retained in the analyses, with data transformations used when applicable to meet the assumption of normality). Significant and marginally significant 2-way ANOVA P-values are displayed on the panels, with all ANOVA P-values shown in Table 3.1. The data were square-root transformed prior to analysis.
Table 3.2: Summary of Kruskal-Wallis P-values for effects of treatment and plot age on forb percent cover estimates for (a) July and August 2020 and (b) July 2021.

a)

<table>
<thead>
<tr>
<th></th>
<th>July</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td>New plots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>0.054</td>
<td>0.156</td>
</tr>
<tr>
<td>age</td>
<td>0.778</td>
<td>0.445</td>
</tr>
<tr>
<td>W</td>
<td>0.759</td>
<td>0.544</td>
</tr>
<tr>
<td>Old Plots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>0.232</td>
<td>0.789</td>
</tr>
<tr>
<td>W</td>
<td>0.550</td>
<td>0.154</td>
</tr>
</tbody>
</table>

b)

<table>
<thead>
<tr>
<th></th>
<th>Forb Percent Cover Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. arvense</td>
</tr>
<tr>
<td>New Plots</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>0.880</td>
</tr>
<tr>
<td>age</td>
<td>0.505</td>
</tr>
<tr>
<td>W</td>
<td>0.535</td>
</tr>
<tr>
<td>Old Plots</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>0.732</td>
</tr>
<tr>
<td>W</td>
<td>0.773</td>
</tr>
</tbody>
</table>

W- warming; N – Nitrogen; Age – Plot age. Asterisks denote significance (*0.05 – 0.01, ** 0.01-0.001, *** <0.001). Note: data did not meet the assumptions of normality, thus a non-parametric test (Kruskal-Wallis) was performed.
Figure 3.6: Aboveground productivity of *Poa pratensis* at peak biomass during 2021 in response to a) nitrogen addition (new and old plots), b) warming (new and old plots) and c) nitrogen and warming (old plots only) (n=10). Boxes contain the interquartile range (the distance between the 25th and 75th percentiles), whiskers represent the minimum and maximum values in the data set, excluding statistical outliers (open circles; statistical outliers were retained in the analyses, with data transformations used when applicable to meet the assumption of normality). Significant and marginally significant 2-way ANOVA P-values are displayed on the panels, with all ANOVA P-values shown in Table 3.1.
3.3 NDVI (normalized difference vegetation index)

In the first year, there was a significant interaction between plot age and nitrogen addition for late spring NDVI ($P_{N\times\text{age}}=0.028$), whereby the plots greened up fastest in the old plots with nitrogen addition (Fig. 3.7a). There also was a significant interaction between nitrogen addition and warming in the old plots ($P_{N\times W}=0.025$), explained by accelerated green up in both the nitrogen addition and warmed plots, but no additive effects of the treatments (Fig. 3.7c). However, there were no significant treatment effects on fall senescence, nor were there significant treatment effects on NDVI the following spring (Table 3.3).
Figure 3.7: May 2020 (late-spring) NDVI measurements in response to a) nitrogen addition (new and old plots), b) warming (new and old plots) and c) nitrogen and warming (old plots only) (n=10). Boxes contain the interquartile range (the distance between the 25th and 75th percentiles), whiskers represent the minimum and maximum values in the data set, excluding statistical outliers (open circles; statistical outliers were retained in the analyses, with data transformations used when applicable to meet the assumption of normality). Significant 2-way ANOVA P-values are displayed on the panels, with all ANOVA P-values shown in Table 3.3.
Table 3.3: Summary of ANOVA P-values for effects of treatment and date on NDVI.

<table>
<thead>
<tr>
<th></th>
<th>2020 May 13</th>
<th>2020 October 6</th>
<th>2020 April 23</th>
<th>2021 May 6</th>
<th>2021 June 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N and plot age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>0.005**</td>
<td>0.529</td>
<td>0.782</td>
<td>0.744</td>
<td>0.152</td>
</tr>
<tr>
<td>Age</td>
<td>0.792</td>
<td>0.401</td>
<td>0.955</td>
<td>0.523</td>
<td>0.310</td>
</tr>
<tr>
<td>N × Age</td>
<td>0.028*</td>
<td>0.774</td>
<td>0.538</td>
<td>0.201</td>
<td>0.320</td>
</tr>
<tr>
<td><strong>Warming/plot age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.116</td>
<td>0.939</td>
<td>0.986</td>
<td>0.986</td>
<td>0.450</td>
</tr>
<tr>
<td>Age</td>
<td>0.810</td>
<td>0.402</td>
<td>0.954</td>
<td>0.954</td>
<td>0.317</td>
</tr>
<tr>
<td>W × Age</td>
<td>0.332</td>
<td>0.618</td>
<td>0.215</td>
<td>0.215</td>
<td>0.329</td>
</tr>
<tr>
<td><strong>N and Warming</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Old plots only)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>&lt;0.001***</td>
<td>0.402</td>
<td>0.920</td>
<td>0.251</td>
<td>0.352</td>
</tr>
<tr>
<td>W</td>
<td>0.014*</td>
<td>0.992</td>
<td>0.253</td>
<td>0.194</td>
<td>0.278</td>
</tr>
<tr>
<td>N × W</td>
<td>0.025*</td>
<td>0.888</td>
<td>0.831</td>
<td>0.302</td>
<td>0.651</td>
</tr>
</tbody>
</table>

W- warming; N – Nitrogen; Age – Plot age. Asterisks denote significance (*0.05 – 0.01, ** 0.01-0.001, *** <0.001).
3.4 N content and nutrient resorption

Green tissue N increased significantly with N addition ($P_N=0.0002$), and it also was significantly higher in old plots than in new plots ($P_{age}=0.029$; Fig. 3.8a). Green tissue N did not respond to warming in the new plots, but it decreased in response to warming in the old plots ($P_{W\times age}=0.063$). Senesced N tissue concentration increased significantly with N addition ($P_N=0.002$; Figure 3.9a and c). On balance, there were no significant treatment effects on NRE (Table 3.4).
Figure 3.8: Green tissue nitrogen concentration (as a percent) in response to a) nitrogen addition (new and old plots), b) warming (new and old plots) and c) nitrogen and warming (old plots only) (n=10). Boxes contain the interquartile range (the distance between the 25th and 75th percentiles), whiskers represent the minimum and maximum values in the data set, excluding statistical outliers (open circles; statistical outliers were retained in the analyses, with data transformations used when applicable to meet the assumption of normality). Significant 2-way ANOVA P-values are displayed on the panels, with all ANOVA P-values shown in Table 3.4.
Table 3.4: Summary of ANOVA P-values for effects of treatments on N concentration and NRE.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Green Tissue</th>
<th>Senesced Tissue</th>
<th>NRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>N and plot age</td>
<td>N</td>
<td>&lt;0.001***</td>
<td>0.002**</td>
</tr>
<tr>
<td>Age</td>
<td>0.029*</td>
<td>0.134</td>
<td>0.579</td>
</tr>
<tr>
<td>N × Age</td>
<td>0.374</td>
<td>0.357</td>
<td>0.784</td>
</tr>
<tr>
<td>Warming/plot age</td>
<td>W</td>
<td>0.019*</td>
<td>0.201</td>
</tr>
<tr>
<td>age</td>
<td>0.039*</td>
<td>0.167</td>
<td>0.578</td>
</tr>
<tr>
<td>W × Age</td>
<td>0.063*</td>
<td>0.775</td>
<td>0.876</td>
</tr>
<tr>
<td>N and Warming (Old plots only)</td>
<td>N</td>
<td>0.014*</td>
<td>0.001**</td>
</tr>
<tr>
<td>W</td>
<td>0.215</td>
<td>0.598</td>
<td>0.698</td>
</tr>
<tr>
<td>N × W</td>
<td>0.615</td>
<td>0.980</td>
<td>0.798</td>
</tr>
</tbody>
</table>

W- warming; N – Nitrogen; Age – Plot age; [N] – Nitrogen concentration. Asterisks denote significance (*0.05 – 0.01, ** 0.01-0.001, *** <0.001) and marginally significant P values (P<0.1) are denoted by +.
Figure 3.9: Senesced tissue nitrogen concentration (as a percent) in response to a) nitrogen addition (new and old plots), b) warming (new and old plots) and c) nitrogen and warming (old plots only) (n=10). Boxes contain the interquartile range (the distance between the 25th and 75th percentiles), whiskers represent the minimum and maximum values in the data set, excluding statistical outliers (open circles; statistical outliers were retained in the analyses, with data transformations used when applicable to meet the assumption of normality). Significant 2-way ANOVA P-values are displayed on the panels, with all ANOVA P-values shown in Table 3.4.
3.5 Litter decomposition

For total mass loss from grass litter (Table 3.5), there was a marginally significant increase in response to nitrogen addition (P_N=0.0505), which was driven by a significant increase in *B. inermis* leaf mass loss (P_N=0.045; Figure 3.10a). For *Cirsium arvense* total mass loss (Table 3.6) there was a significant interaction between plot age and warming (P_{age\times W}=0.012), whereby mass loss increased in the new plots, but decreased in the old plots (Fig. 3.11b). There also was a significant interaction between nitrogen addition and warming in the old plots (P_{N\times W}=0.007), with mass loss being low in the warmed plots in the absence of nitrogen addition (Figure 3.11c). The latter interaction was driven by the response of *C. arvense* leaves (P_{N\times W}=0.001, Fig. 3.12c). For *C. arvense* stems, nitrogen addition significantly increased mass loss (P_N=0.034, Fig. 3.13a).

| Table 3.5: Summary of ANOVA P-values for effects of treatments on decomposition of grass litter bags. |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Effect                                         | Total           | *B. inermis* Stem | *B. inermis* Leaf | *P. pratensis* Tiller |
| N and plot age                                 | N               | 0.485            | 0.045*           | 0.939            |
|                                                | Age             | 0.387            | 0.575            | 0.445            |
|                                                | N \times Age    | 0.384            | 0.650            | 0.960            |
| Warming/plot age                               | W               | 0.960            | 0.257            | 0.661            |
|                                                | Age             | 0.381            | 0.585            | 0.443            |
|                                                | W \times Age    | 0.122            | 0.705            | 0.594            |
| N and Warming (old plots only)                 | N               | 0.667            | 0.190            | 0.439            |
|                                                | W               | 0.262            | 0.859            | 0.526            |
|                                                | N \times W      | 0.561            | 0.813            | 0.709            |

W- warming; N – Nitrogen; Age – Plot age. Asterisks denote significance (*0.05 – 0.01, **0.01-0.001, ***<0.001) and marginally significant P values (P<0.1) are denoted by +.
Figure 3.10: Percent mass loss during decomposition of *Bromus inermis* leaves in response to a) nitrogen addition (new and old plots), b) warming (new and old plots) and c) nitrogen and warming (old plots only) (n=10). Boxes contain the interquartile range (the distance between the 25th and 75th percentiles), whiskers represent the minimum and maximum values in the data set, excluding statistical outliers (open circles; statistical outliers were retained in the analyses, with data transformations used when applicable to meet the assumption of normality). Significant 2-way ANOVA P-values are displayed on the panels, with all ANOVA P-values shown in Table 3.5.
Figure 3.11: Percent mass loss during decomposition of *Cirsium arvense* (leaves + stems) in response to a) nitrogen addition (new and old plots), b) warming (new and old plots) and c) nitrogen and warming (old plots only) (n=10). Boxes contain the interquartile range (the distance between the 25th and 75th percentiles), whiskers represent the minimum and maximum values in the data set, excluding statistical outliers (open circles; statistical outliers were retained in the analyses, with data transformations used when applicable to meet the assumption of normality). Significant 2-way ANOVA P-values are displayed on the panels, with all ANOVA P-values shown in Table 3.6.
Figure 3.12: Percent mass loss during decomposition of *Cirsium arvense* leaves in response to a) nitrogen addition (new and old plots), b) warming (new and old plots) and c) nitrogen and warming (old plots only) (n=10). Boxes contain the interquartile range (the distance between the 25th and 75th percentiles), whiskers represent the minimum and maximum values in the data set, excluding statistical outliers (open circles; statistical outliers were retained in the analyses, with data transformations used when applicable to meet the assumption of normality). Significant 2-way ANOVA P-values are displayed on the panels, with all ANOVA P-values shown in Table 3.6.
Figure 3.13: Percent mass loss during decomposition of *Cirsium arvense* stems in response to a) nitrogen addition (new and old plots), b) warming (new and old plots) and c) nitrogen and warming (old plots only) (n=10). Boxes contain the interquartile range (the distance between the 25th and 75th percentiles), whiskers represent the minimum and maximum values in the data set, excluding statistical outliers (open circles; statistical outliers were retained in the analyses, with data transformations used when applicable to meet the assumption of normality). Significant 2-way ANOVA P-values are displayed on the panels, with all ANOVA P-values shown in Table 3.6.
Table 3.6: Summary of ANOVA P-values for effects of treatments on decomposition of C. arvense litter bags.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>C. arvense Stem</th>
<th>C. arvense Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>N and plot age</td>
<td>N</td>
<td>0.065*</td>
<td>0.034*</td>
</tr>
<tr>
<td></td>
<td>age</td>
<td>0.750</td>
<td>0.861</td>
</tr>
<tr>
<td></td>
<td>N x age</td>
<td>0.422</td>
<td>0.929</td>
</tr>
<tr>
<td>Warming/plot age</td>
<td>W</td>
<td>0.054*</td>
<td>0.173</td>
</tr>
<tr>
<td></td>
<td>age</td>
<td>0.736</td>
<td>0.859</td>
</tr>
<tr>
<td></td>
<td>W x age</td>
<td>0.029*</td>
<td>0.062*</td>
</tr>
<tr>
<td>N and Warming (old plots only)</td>
<td>N</td>
<td>0.110</td>
<td>0.066*</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>0.216</td>
<td>0.582</td>
</tr>
<tr>
<td></td>
<td>N x W</td>
<td>0.007**</td>
<td>0.159</td>
</tr>
</tbody>
</table>

W- warming; N – Nitrogen; Age – Plot age. Asterisks denote significance (*0.05 – 0.01, ** 0.01-0.001, *** <0.001) and marginally significant P values (P<0.1) are denoted by +.
Chapter 4: Discussion and Conclusions

4.1 Above and belowground biomass responses to nitrogen addition and warming

In the first year of the experiment, total aboveground biomass was greater in the old nitrogen (N) addition plots than in the new N addition plots, which was explained by significant increases of *Poa pratensis* aboveground biomass and marginally significant increases in *Bromus inermis*. Even though both new and old plots received the same amount of N that year, it is likely that the greater N response in the old plots could have resulted from residual N from previous years of fertilization, which can be retained either in the soil or as stored N in plant tissue (White, 1957). For example, a study that examined switchgrass biomass responses to N fertilization found that residual N effects occurred up to one year after N fertilization ceased (Springer, 2017). In the current study, greater N retention in the old N addition plots also may have been explained by their increased belowground biomass. The latter would be consistent with the results of a previous $^{15}$N retention study, where high root biomass significantly increased $^{15}$N retention directly by increasing $^{15}$N uptake, and indirectly by increasing microbial $^{15}$N uptake, thereby reducing the amount of $^{15}$N leached from the system (de Vries & Bardgett, 2016).

Given there was not a significant N addition effect on total aboveground productivity in the first year of treatment for the new plots, contrary to my prediction, there would not have been a strong feedback resulting from increased litter accumulation suppressing growth in the second year. However, there was nevertheless a significant increase in the aboveground productivity of *P. pratensis* in the new N addition plots in the first year. A different result was observed 15 years previously in the first year of treatment for the old plots, when neither warming nor N addition had significant effects on plant growth (Hutchison & Henry, 2010). In the latter study, it was speculated that this result could have been explained by interannual variation in precipitation over the summer (i.e., it was unusually dry in the first summer of the experiment for the old plots). In contrast, the new plots experienced wetter conditions than the old plots had in their first year of treatment, which could explain why they exhibited a significant N response. However, in the second year of treatment for the new plots, there were
dry conditions in the spring and early summer (Figs. 3.1 and 3.2), when the growth of the cool season grasses is highest. These conditions were similar to those experienced in the first year of the experiment 15 years earlier (as described in Hutchison and Henry, 2010). Both the new and old plots exhibited significant increases of *P. pratensis* biomass in the second year of treatment for the new plots, but the N addition effect was weaker than in the previous, wetter year. These results suggest that the year in which an experiment is started may play an important role in the outcome, as described previously in a review by Vaughn and Young (2010), who observed that in ecological field experiments where identical replicates were initiated in multiple years (<5% of all experiments), 76% exhibited significant differences in results among initiation years.

As described earlier, a global meta-analysis by Komatsu et al. (2019) found that plant community responses to global change drivers emerged over longer periods of time compared to the short-term (< 10 y), and that responses were more frequent when three or more global change drivers were being manipulated. Based on their findings, I predicted that the old plots in the current experiment would exhibit substantial shifts in relative species abundances relative to the new plots, with the latter being greatest in the combined N and warming treatment plots. In contrast, however, my results showed that even over the long-term (≥ 15 years) and with two global change drivers being manipulated, not only did aboveground grass productivity not differ among the old and new plots after two years of the latter being established, but there also were no significant treatment effects on forb species cover in the new and old plots. This result is likely due to the particular species (the non-native C₃ grasses *Poa pratensis* and *Bromus inermis*) that were seeded at the site and already dominant when the experiment was initiated. These species have remained dominant at the study site over the last 15 years, and my results were consistent with the findings of Deák et al. (2011), who found graminoid biomass and the subsequent litter accumulation reduce species richness and suppress the growth of forbs. Additionally, increases in aboveground biomass increase litter accumulation, which often further favours rhizomatous grasses at the expense of forbs (Hou et al., 2019; Zavaleta et al., 2003). While I did not observe a decrease in percent forb cover in response to N addition, the concomitant increase in grass productivity meant that the total biomass of forbs would have decreased relative to the grasses. Therefore, overall, it appears
that the non-native C₃ grasses are impeding potential long-term aboveground responses of plant productivity and species composition to the global change treatments in the experiment.

Further insight into potential future changes in species composition at the study site can be gained by considering the changes that occurred over the last 15 years in block 10 of the experiment. This block as a whole had become dominated by *Solidago canadensis* since the establishment of the old plots 15 years previously. *Solidago canadensis* grows and reproduces clonally via ramets (Trenbath & Harper, 1978), and when able to grow in pure stands it can prevent the invasion of neighbours, buffer against neighbour interference, and continue to expand uniformly outwards regardless of the identity of neighbours (Hartnett & Bazzaz, 1985). While this stand of *S. canadensis* did not respond to the N and warming treatments at the scale of the experimental plots (i.e., it moved as a front across the entire block, independent of the treatments), its establishment and potential expansion could likely be affected by such treatments over a larger spatial scale (e.g., Peng et al., 2019; Ren et al., 2019). Likewise, parts of block 7 and 8 had progressively been encroached upon by a stand of dogwood (*Cornus* spp.). These shrubs grow in thickets, and once established they spread primarily through vegetative reproduction via horizontal roots (runners) (Boeken & Canham, 1995). Like *Solidago canadensis*, the dogwood was present prior to the initiation of the long-term experiment, and its expansion through the plots appeared to be independent of the treatments. Therefore, replacement of the grasses at the site has been following the stages expected with old field succession (e.g., Cramer & Hobbs, 2007), yet it remains unclear how the speed and direction of such successional processes might be altered by warming and N addition. This question would likely need to be addressed at a much larger spatial scale than 1 m² plots.

### 4.2 Effects of nitrogen addition and warming on NDVI

In the first year of treatment for the new plots, there was a significant interaction between N addition and plot age, which was driven by rapid green up in the old N addition plots. In contrast, a recent meta-analysis found that N addition often delays the onset of phenological events; however, this effect was smallest for forbs and grasses (Wang & Tang, 2019). In the current study, it is likely that the N effects on early green up occurred as a result of the old N plots having residual N from previous years of fertilization, as described above (section 4.1). Surprisingly, there was no warming effect on plant green up in the new plots. However, there
was a significant interaction between N addition and warming in the old plots, which was explained by accelerated green up in response to both N addition and warming alone, but no additive effect. Cleland et al. (2006) reported in a long-term grassland study that warming and N addition had a negative interactive effect on plant green up, but the latter study was conducted in an annual grassland in a region characterized by a Mediterranean-type climate, which was quite different than my study system and its climate type. Advances in plant phenology in response to warming, which were observed in both studies, have been documented widely elsewhere (e.g., Aerts, 2006; Price & Waser, 1998; Walker et al., 2006).

Despite the trend in the old warmed plots of accelerated green up, by the time of peak plant biomass (early July) there was no significant effect of warming on aboveground productivity, which was consistent with the findings of a similar warming study conducted in an old field (Hoeppner & Dukes, 2012). This may have resulted from the indirect effects of warming on plant growth, in that in addition to the possible direct benefits of warming (Lin et al., 2010; Rustad et al., 2001), warming can also impede aboveground productivity by decreasing soil moisture (Fu et al., 2013; Wu et al., 2021). Therefore, a lack of positive warming effect in my plots could be due to warming effects depending on adequate precipitation (Hossain & Beierkuhnlein, 2018; Wu et al., 2011). As for NDVI in the fall, although warming (Fu et al., 2018; Huang et al., 2020; Jeong, 2020) and N addition (Wang & Tang, 2019) often delay plant senescence, in fall 2020, there were no significant effects of N addition or warming on NDVI.

In the second year following the initiation of the new plots, there were no significant treatment effects on plot green up in either the old or new plots. A previous analysis of warming effects in this experiment (i.e., for the old plots; Henry et al., 2015) revealed that when snowmelt occurs synchronously across all of the plots (i.e., there is a sudden, intense spring melt that affects all plots, independent of the warming treatment), there are no significant effects of warming on plant growth. These synchronous snow melt dynamics were observed in the second year of the treatment for the new plots.
4.3 Effects of nitrogen addition and warming on grass N content and NRE

Green tissue N was highest in the N addition plots, with the highest values present in the old plots. This result supports the findings from previous studies, where increased nutrient availability resulted in increased plant tissue nutrient concentrations (Li et al., 2015b; Lü et al., 2011; Yuan & Chen, 2015). This result also supports the explanation above that plants in the old N addition plots benefitted from residual N from previous years of fertilization. Senesced tissue N concentrations were highest in both the new N addition plots and the old N addition plots, which coincides with the findings of a previous meta-analysis, in which N addition increased N in senesced leaves (Su et al., 2021; Yuan & Chen, 2015). The increased senesced tissue N concentration also corresponded with low N resorption proficiency (NRP) in the fertilized plots. This negative response of NRP to N addition coincides with the results from previous studies (Huang et al., 2008; Li et al., 2015b), and confirms that NRP is more sensitive to N addition than is nutrient resorption efficiency (NRE) (Huang et al., 2008; Killingbeck, 1996; Li et al., 2016a).

The new warmed plots had higher green tissue N content than the old plots; however, warming did not result in significant increases in the N content of senesced tissue. My results therefore contradict the findings from a study in which experimental warming (specifically, in long-term 4-year plots) decreased N concentration in both green and senesced tissue (An et al., 2005). The increase in the new warmed plots may have been explained by warming increasing plant available N in the soils by increasing microbial activity (i.e., through increased N mineralization (Shaw & Harte, 2001)).

Contrary to my prediction, in addition to NRE not differing significantly between the new and old N addition plots, there was no significant difference in NRE between the N addition and control plots. Overall, the lack of a significant N addition effect on NRE suggests that the N addition plots have not reached saturation, given that decreased NRE rates are indicative of the latter (Tian et al., 2016). While my result contradicts recent meta-analyses that found N addition decreased NRE by 12% (Yuan & Chen, 2015) and 13% (You et al., 2018) on average, my results are consistent with individual studies that have reported N addition had no effect on
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ant NRE (Aerts et al., 2007; Chapin & Moilanen, 1991; Li et al., 2015b; Wang et al., 2014a). On average, the control (no N addition) plots had a NRE of 43% (new control plots had a NRE of 42%, while old control plot NRE was 44%), and the N addition plots had a NRE of 45%. Similarly, Zong et al., (2018) reported an NRE of 45% for their dominant graminoid species.

However, global meta-analyses have reported much higher rates of NRE, with 74% (Vergutz et al., 2012) and 61% for graminoids (Wang et al., 2018b). Climatic factors can influence NRE, with NRE being negatively correlated with mean annual precipitation and temperature (Vergutz et al., 2012; Wang et al., 2018b; Yuan & Chen, 2009). Differences in methodologies among studies also can contribute to inconsistent results; for example, nutrient concentrations that are expressed without a mass loss correction factor (I used that of Vergutz et al., 2012) underestimate resorption by up to 20% (van Heerwaarden et al., 2003).

4.4 Nitrogen addition and warming effects on litter decomposition

My prediction that N addition would increase the mass loss of grass litter (pooled P. pratensis and B. inermis) was consistent with findings from a previous study done at the field site (Moise & Henry, 2014). This effect was primarily driven by the increase in mass loss in B. inermis leaf litter, and may be explained by high tissue N content of the senesced leaves, which could indicate they are of higher quality for microbial decomposition (Gong et al., 2015). Although I only assessed tissue N content for P. pratensis (as it was found in every plot), as described above, it is likely that B. inermis leaves also would have increased in N concentration. Interestingly, the decomposition rates of neither B. inermis stems nor P. pratensis tillers were affected significantly by N addition. However, individual species can vary substantially in decomposition rates (Cornelissen, 1996), with the latter often driven by factors other than N content (e.g., lignin concentration) (Aerts, 1997). Moreover, within plant communities, responses of decomposition to added N can be species-specific and time-scale dependant (Moise & Henry, 2014). However, in the absence of large treatments effects on the relative abundances of species, plot level decomposition responses would have been driven primarily by treatment effects on decomposition within species and functional groups.
Increasing temperature can have positive effects on decomposition rate by directly stimulating microbial activity (Belay-Tedla et al., 2009) or indirectly by improving litter quality (Cornelissen, 1996). *Cirsium arvense* exhibited an interaction with warming and plot age, whereby new warmed plots had the highest total mass loss and old warmed plots had the lowest total mass loss. Aerts (2006) observed among studies that warming (heating lamps) stimulated litter decomposition on average in cold biomes, provided that sufficient soil moisture was present. Another short-term study also found that warming (with high soil moisture) increased the rate of decomposition (Butenschoen et al., 2011). However, my results contradict a study that compared short- vs. long-term impacts of warming, where soil taken from the old warmed plots (14 years of warming) and incubated with litter had a decomposition rate 40% higher than that of ambient plots, while short-term warming in the laboratory increased decomposition by only 12% (Stuble et al., 2019). Furthermore, it can be a challenge to differentiate long-term shifts of a microbial community from the short-term impacts of warming on litter decomposition (Stuble et al., 2019). However, such acclimation/and or shifts in the microbiome associated with climate change may drive changes in litter decomposition (Hawkes and Keith, 2015). Moreover, these changes may cycle over time, by going through phases of increased and ambient decomposition (Melillo et al., 2017). As found by Stuble et al. (2019) climate-mediated structuring of microbial communities has the potential for longer lasting effects on labile carbon turnover.

*Cirsium arvense* also exhibited an interaction between N addition and warming in the old plots, whereby total mass loss rates were low in warmed plots when N addition was not present. This trend was driven by the response of *C. arvense* leaves. These results differ from that of a previous study in which the combined warming and N treatments increased the rate of decomposition (Gong et al., 2015).

### 4.5 Potential study limitations and future directions

A potential limitation of my productivity measurements was that with a single destructive harvest, the measurements would not account for mass loss prior to the harvest via herbivory by various fauna such as snails, meadow voles, slugs and insects. A previous study at the site found there were variable effects of different herbivore taxa on plant biomass responses to treatments (Moise & Henry, 2012). In addition, my measurements would not have accounted
for the pulse of additional growth of the cool season grasses that occurs in the late summer and early fall, although it was demonstrated previously that the July biomass peak provides a good estimate of annual productivity (Hutchison & Henry, 2010).

As for belowground productivity, I measured root responses destructively once, coinciding with peak aboveground biomass. Given that I did not examine root turnover, my root biomass measures cannot provide accurate estimates of belowground productivity (Stewart & Frank, 2008). To measure root turn-over, root length, and density non-destructively, as well as more accurately, minirhizotrons would need to be used (Murphy et al., 1994). It is also possible that mycorrhizal associations (which can be responsible for a substantial portion of a plant’s nutrient uptake) may have been directly influenced by the treatments. For example, a meta-analysis found that mycorrhizal abundance decreased by 15% under N fertilization (Treseder 2004), but neither the total amount of N or duration of fertilization played a significant factor. Another meta-analysis found a general trend of increased mycorrhizal abundance and deceased mycorrhizal activity under warming (Mohan et al., 2014).

The effects of other important global change drivers, such as elevated atmospheric carbon dioxide and increased precipitation, may interact with warming and increased N deposition to affect plant productivity (Henry et al., 2005). Moreover, extreme events such as floods, severe heatwaves and droughts are predicted to increase under climate change (IPCC, 2021), and the effects these climate events could have on productivity may differ considerably compared to those caused by a mean temperature increase. Finally, as discussed above, while 1 m² plots may have been sufficient for characterizing changes in the diversity, structure and composition of herbaceous species (Li et al., 2016), the spatial scale of the current field experiment was not adequate for observing treatments effects on ecological succession stages involving large clonal shrubs and trees.

While my study compared the short- and long-term plant responses to the effects of warming and N addition, there have been many additional research questions answered over the span of the 15-year study. This begs the question as to whether there are other long-term study questions that should be examined. With recent IPCC reports, there has been a push to reduce rates of atmospheric N deposition. Despite atmospheric N deposition reducing species diversity and well as community composition (Bobbink et al., 2010; Clark & Tilman, 2008;
Yue et al., 2020), little is still known about how reversible both the short- and long-term effects of atmospheric N deposition are. Some studies have found that 10- and 20-years after cessation of N addition, plant diversity does not recover to control levels (Clark & Tilman, 2008 and Isbell et al., 2013, respectively). However, species richness (Pallett et al., 2016) and relative species numbers (Clark & Tilman, 2008) have been found to increase after N addition is ceased. Furthermore, productivity has been found to decrease immediately after cessation, and biomass yields less than that of the levels recorded prior to fertilization after a decade after ceased N addition (Pallett et al., 2016).

4.6 Conclusions

Overall, N addition plots exhibited increases in aboveground biomass, and increased rates of both green and senesced tissue N, which in turn increased litter quality and subsequent decomposition rates. My biomass results differed from those that were observed 15 years previously by Hutchison and Henry (2010) which suggests that experimental initiation year plays an important role in treatment responses (Vaughn and Young, 2010). Moreover, the initial species composition of the non-native C₃ grasses Poa pratensis and Bromus inermis and their ability to outcompete and dominate other species at the site has resulted in no detectable change in forb species composition over the last 15 years. Therefore, non-native C₃ grasses appear to not only impede the stages of old-field succession but also suppress long-term aboveground responses of plant productivity and species composition to global change.
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**Curriculum Vitae**

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