

Effects of *Bacillus thuringiensis* subsp. *kurstaki* application on non-target nocturnal macromoth
biodiversity in the eastern boreal forest, Canada.

by

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Abstract

Lepidoptera, including butterflies and moths, play vital roles as herbivores, pollinators, and food sources, but also include species considered forest pests. The impact of *Bacillus thuringiensis* subs. *kurstaki* (Btk), a widely used bio-insecticide for controlling forest pests like the spruce budworm, on non-target lepidoptera in Canada remains uncertain. To address this, I established a replicated field study to evaluate the effects of Btk on non-target nocturnal macromoth communities in the eastern boreal forest of western Newfoundland, Canada. Over two years, I sampled moths across four groups: north treatment, north control, south treatment, and south control. My analysis focused on species diversity, abundance, and composition. Results showed no significant differences in total abundance or species composition between treatment and control groups. In 2022, control sites had significantly higher Hill numbers for Shannon and Simpson diversity compared to treatment sites. In 2021, differences in Hill numbers were only observed between north controls and treatments. These findings indicate that after multiple years of treatment, there can be shifts in the relative abundance of certain species, but without significant changes in species richness, total abundance, or composition between control and treatment groups. These results suggest that Btk can lead to stand-level shifts in relative abundance but does not substantially alter community structure during the early stages of treatment. The responses of species are idiosyncratic, likely influenced by differences in phenology and voltinism. Monitoring the impacts of Btk on non-target lepidoptera is crucial to effectively manage forest pests while minimizing unintended consequences for non-target species.

General Summary

Moths and butterflies (Lepidoptera) fulfill crucial roles in Canadian forests as herbivores, pollinators, and prey for other animals. The spruce budworm, a notorious forest pest, inflicts substantial damage on fir and spruce trees. *Bacillus thuringiensis* subs. *kurstaki* (Btk), an insecticide exclusively used for targeting Lepidoptera, is commonly used to control spruce budworm. With this study I aimed to evaluate the impact of Btk on local non-target moth species in western Newfoundland, Canada (NL) through moth collection from Btk-treated and untreated sites. While no significant differences of total abundance or community structure were observed between treated and untreated sites, relative abundance of species differed between treated and untreated sites. Community structure did not vary between Btk-treated and untreated stands, suggesting limited impact during the early stages of treatment. However, after multiple years of treatment, shifts in the relative abundance of certain species became apparent. These findings contribute to our understanding of pest management practices in Canada, aiding in the refinement of such strategies.

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Table of Contents

Abstract	iii
General Summary	iv
Acknowledgments.....	v
Table of Contents.....	vi
List of Figures	viii
List of Tables	ix
List of Appendices	x
Chapter 1: Literature Review.....	1
1.1 Lepidoptera	1
1.2 Forest pests.....	3
1.3 <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> (Btk).....	5
1.4 Btk on non-target lepidoptera	8
Chapter 2: Effects of <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> application on non-target nocturnal macromoth diversity in the eastern boreal forest, Canada.....	12
2.1 Abstract.....	12
2.2 Introduction.....	13
2.3 Materials and Methods.....	15
2.3.1 Study location	15
2.3.2 Spray Program	18

2.3.3 Collection Methods.....	19
2.3.4 Statistical Analyses.....	20
2.4 Results.....	21
2.4.1 Abundance.....	22
2.4.1.1 Abundance in 2021.....	22
2.4.1.2 Abundance in 2022.....	24
2.4.2 Diversity.....	29
2.4.2.1 Diversity in 2021.....	29
2.4.2.2 Diversity in 2022.....	29
2.4.3 Species Composition.....	32
2.5 Discussion.....	34
2.5.1 Abundance.....	34
2.5.2 Diversity.....	37
2.5.3 Species Composition.....	39
2.6 Conclusions.....	41
References.....	43
Appendices.....	55

List of Figures

Figure 1 a) Newfoundland, Atlantic Canada. b) Location of study location in western Newfoundland. c) Location of the study sites in relation to Gros Morne National Park. Black squares represent control sites and red circles represent Btk treated sites. d) Light trap set up for moth collection.....**Error! Bookmark not defined.**

Figure 2. Variation of total moth abundance from four treatment types, across two years, a) 2021 and b) 2022. 2021 treatment sites had been sprayed with Btk for one (South Treatment) and two (North Treatment) consecutive year(s). 2022 treatment sites had been sprayed with Btk for two (South Treatment) and three (North Treatment) consecutive years. Comparisons tested using a generalized linear model (GLM), fitted with a negative binomial distribution. Letters a, b, and c indicate significant differences between treatment types based on post-hoc testing (95% confidence)..... 28

Figure 3 Diversity indices, characterized by effective number of species (\pm 95% confidence interval), for each treatment group for a) 2021 and b) 2022. Values calculated from Hill number order $q = 0$ (species richness), $q = 1$ (Shannon diversity), and $q = 2$ (Simpson diversity), for each treatment type..... 31

Figure 4. Nonmetric multidimensional scaling of species composition across treatment types in a) 2021 and b) 2022. Species abundance pooled across all dates and log transformed ($x' = \log(x+1)$). Bray-Curtis dissimilarity with 95% confidence ellipses. Each individual treatment on the plot represents a replicate sample site for north control, north treatment, south control, or south treatment..... 33

List of Tables

Table 1. Total raw moth abundance for the five most abundant species in 2021 and 2022.

Collection days amalgamated by year and treatment type. Control/treatment pairs that appear in a box are significantly different. NC = North control, NT = North treatment, SC = South control, ST = South treatment. 27

List of Appendices

Appendix 1. Raw site data.	55
Appendix 2. Total raw moth abundance for 2021 collection year. Collection days amalgamated per month and sites amalgamated by treatment type. * Indicates groups that could not be identified to species or genus level due to extensive damage.....	56
Appendix 3. Total raw moth abundance for 2022 collection year. Collection days amalgamated per month and sites amalgamated by treatment type. * Indicates groups that could not be identified to species or genus level due to extensive damage.....	60

Chapter 1: Literature Review

1.1 Lepidoptera

Moths and butterflies belong to the order Lepidoptera, one of the most diverse orders of insects, with approximately 158,000 described species (Powell et al., 1998; van Nieukerken et al., 2011). Of these, Pohl et al. (2018), reported a total of 5455 species in 81 families in Canada, with species composition and diversity being influenced by latitudinal gradients and ecoregional differences (Kerr et al., 2014; Summerville et al., 2003). Butterflies are more well known than moths, however the number of moth species outnumber butterfly species by at least ten to one (Pohl et al., 2019). Moths are more taxonomically diverse than butterflies and are associated with all terrestrial plant communities from subpolar regions to the equator (Wagner et al., 2021). Both moths and butterflies play a critical role in ecosystem dynamics in the northern boreal forest by serving as defoliators, pollinators, and prey to other animals (Hammond & Miller, 1998; Holmes et al., 1979).

Wild pollinator populations are declining globally and there is therefore an increased interest in understanding and conserving their populations (Potts et al., 2010). Although bees are considered primary pollinators, butterflies and moths also play an important role in the provisioning of pollination services (Alison et al., 2022; Anderson et al., 2023; Macgregor et al., 2015; Schmitt, 1980). Alison et al. (2022) found that a single species of nocturnal macromoth, *Noctua pronuba* (Linnaeus; Noctuidae), provided 34% of visits to *Trifolium pratense* (red clover), not an insignificant amount. Another study by Anderson et al. (2023) found that 83% of all visits to *Rubus fruticosus* (European blackberry) were during the day from a variety of taxa; however, pollen deposition rates were significantly higher during the night. Nocturnal pollination was almost exclusively by moths, suggesting that moths are more efficient pollinators than

diurnal insects (Anderson et al., 2023). Moth species also differ substantially in their flight phenology (time that they are flying around as adults). Species that emerge as adults early in the season are likely to pollinate different plants than those that emerge later in the season. The loss of pollinators would have a detrimental impact on wildflower populations as well as crop production, as 80% of wild plant species and 75% of crops used for human food are dependent on insect pollination (Potts et al., 2010).

Aside from pollination, moths are an important source of prey for insectivorous vertebrates, particularly birds and bats (Wagner et al., 2021). Previous research has found a link between decreased caterpillar biomass and decreased nesting attempts in the black-throated blue warbler, suggesting that Lepidoptera larvae are a critical food source (Rodenhuse & Holmes, 1992). However, it is crucial to consider that spatial variation in prey density may not directly impact bird performance but rather attract birds to areas with readily available food resources (*sensu* bird-feeder effects). Hence, it is necessary to examine how these findings extend to broader ecological aspects such as breeding success, population dynamics, and other related factors. Bullington et al. (2021) found that Lepidoptera dominated the diets of both nocturnal birds and bats, with certain taxa being more frequently consumed by bats, whereas others are predominantly targeted by birds. Hammond and Miller (1998) found that diurnal birds are less likely to encounter flying moths but may search for perched adults and larvae, whereas bats are more likely to encounter flying moths. This highlights the importance of understanding the differences in phenology and physiology between taxa when trying to understand the role of different Lepidoptera species in ecosystems.

1.2 Forest pests

Although moths provide many forest ecosystem services, some Lepidoptera species are pests and are of particular concern ecologically and economically. Several hundred species are considered forest or agricultural pests in Canada and cause extensive defoliation damage during outbreaks (Munroe, 1979). Although many Lepidoptera are defoliators and remain at low, stable population levels, a small percentage are prone to outbreak (Johns et al., 2016). The reason behind some species reaching outbreak levels while others do not is still not fully understood; however, outbreak species tend to be either spring feeders that overwinter as eggs, or gregarious summer feeders (Hunter, 1991). Outbreak species also tend to exhibit high fecundity, polyphagy, and strong defences against natural enemies (Hunter, 1991). Johns et al. (2016) reviewed a century of reports on tree defoliation in Canada and, when they restricted the list to include only those that have significant long-term economic or ecological impacts on Canadian forests, most belonged to the order Lepidoptera. These included spruce budworm (*Choristoneura fumiferana* Clemens; Tortricidae), western spruce budworm (*Choristoneura freeman* Rozowski; Tortricidae), tent caterpillars (*Malacosoma disstria* Hubner; Lasiocampidae), and hemlock looper (*Lambdina fiscellaria* Guenée; Geometridae). Not surprisingly, all of these lepidopteran forest pests are moths.

In North American boreal forests, the spruce budworm (*Choristoneura fumiferana*, SBW) is the main defoliator, causing extensive ecological and economic damage (Chang et al., 2012; Maclean & Ostaff, 1989). The host trees are balsam fir (*Abies balsamea*), white spruce (*Picea glauca*), red spruce (*Picea rubens*), and black spruce (*Picea mariana*) (Fuentelba et al., 2015). SBW have one generation per year. Eggs are laid in July and August, in clusters, under the needles of shoots (Miller, 1975). Once hatched, the larvae move towards the crown of the tree in search of a suitable overwintering site and construct a silk hibernaculum (Mathers, 1932). The

larvae go through six instars and overwinters in the second (Miller, 1975). The fifth and sixth instars are the most damaging, as they feed voraciously on new foliage, causing extensive defoliation and reducing tree growth and productivity (Mathers, 1932). The adults are small grey-brown moths that live for approximately one week (Natural Resources Canada, 2018).

SBW outbreaks are cyclical, occurring approximately every 30-40 years and last for 6-10 years in a given region, although the severity and extent of outbreaks can vary between regions and over time (Blais, 1983; Irland, 1980). Outbreaks also appear to be occurring more frequently, with 23 outbreaks being recorded in the past 80 years as opposed to 9 in the preceding 100 years (Mattson et al., 1998). Mature balsam fir and white spruce stands experience the most extensive damage (Natural Resources Canada, 2018). 90% of fir and spruce stands in Canada are affected by SBW, and more than 50% of the annual loss of volume caused by insect damage is attributed to SBW (Natural Resources Canada, 1994). Stand mortality rates are high after multiple years of severe defoliation, especially vulnerable mature fir stands, which may suffer 100% mortality (Irland, 1980; Mattson et al., 1998). Over a period of 4 to 5 years, mature fir stands demonstrate a pronounced onset of mortality, whereas immature fir stand mortality is delayed slightly until approximately 7 to 8 years of defoliation (MacLean, 1984). Subsequently, after 12 years of defoliation, mature stands exhibit a mortality rate nearing 100%, while immature stands display varying levels of mortality ranging from 30% to 70% (MacLean, 1984).

The devastating impact of SBW outbreaks on the forestry industry is evident from historical records and economic assessments. Three major outbreaks (1910, 1940, and 1970) in eastern Canada spanned 10, 25, and 55 million hectares, respectively (Blais, 1983). 44 million m³ of wood losses per year could be attributed to the last SBW outbreak in Canada (Fuentelba et al., 2015). As expected, the negative impacts that SBW outbreaks have on the forestry industry

are immense. Fir and spruce trees are the preferred raw materials for major pulp products such as newsprint, market pulp, and printing papers (Irland, 1980). They are also the most susceptible to SBW damage. Chang et al. (2012) modelled the potential economic impacts of a SBW outbreak on 2.8 million hectares of land in New Brunswick, Canada, and found that a moderate to severe outbreak between 2012-2041 would result in the loss of CDN\$3.3 billion to \$4.7 billion, respectively.

Alongside the economic impacts, SBW plays an important and complex role in forest ecosystems and has an influence on carbon storage and landscape vegetation dynamics. Using modelling techniques, Dymond et al (2010) found that SBW outbreaks, at a similar magnitude of historical outbreaks, could cause stands to transform from net carbon sinks to carbon sources due to growth loss and tree mortality which decreases net primary production. Chagnon et al. (2022) also found that SBW outbreaks induced changes in the understory vegetation. Tree defoliation and mortality induced by SBW increases canopy openness, leading to increased sunlight reaching the understory. Consequently, the increase in light availability facilitates the growth of early successional vegetation. Not only does this alter the plant community composition, but it could also influence the fauna in the area by changing the availability of foraging materials and/or altering the predation risk (Chagnon et al., 2022). Due to these ecological and economic changes associated with spruce budworm outbreaks, the development and implementation of appropriate management strategies has been an important research topic for decades.

1.3 *Bacillus thuringiensis* subsp. *kurstaki* (Btk)

Historically, lepidopterous forest pests have been managed using chemical pesticides and/or natural enemies (Miller, 2000). However, since the mid-1980s, the bio-insecticide Btk has been used for managing and mitigating outbreaks in North America (Boulton & Otvos, 2004).

Bacillus thuringiensis (Bt) is a naturally occurring aerobic gram-positive soil bacterium that is characterized by its ability to produce proteinaceous crystals during sporulation (Hofte & Whiteley, 1989). Sporulation is a process that involves multiple cell differentiation events, ultimately leading to a highly resistant, dormant spore that can germinate when the conditions become favourable (De Hoon et al., 2010). The proteinaceous crystals, which are released into the environment at the end of the Bt sporulation process, can be consumed by insects and result in their death, giving it insecticidal properties (Lambert & Peferoen, 1992). The crystals are comprised of one or more proteins called Cry or Cyt toxins (Alejandro Bravo et al., 2007). There are many different strains of Bt, each producing different variations of the protein crystals that are specifically toxic to their targeted insect host (Alejandro Bravo et al., 2007; Lambert & Peferoen, 1992). The *Bacillus thuringiensis* Toxin Nomenclature Committee was established to sequence and classify these crystal protein genes of various Bt strains (Crickmore et al., 1998). Cry proteins are the largest group of proteins produced, with 73 different types (Cry1-Cry73) (Palma et al., 2014). These proteins have well-documented toxicity against lepidopterans, coleopterans, hemipterans, dipterans, nematodes, and some snails (Palma et al., 2014). Depending on the Bt strain, the number and structure of released proteins can vary widely; providing the variation in insecticidal properties based on strain (Schünemann et al., 2014). This host specificity of Bt is beneficial as it allows for precise and targeted control of specific pest populations without harming non-target insect orders.

Bacillus thuringiensis subs. *kurstaki* (Btk) produces four Cry proteins (Cry1Aa, Cry1Ab, Cry1Ac, and Cry2Aa), all of which are specifically toxic to lepidopteran larvae upon ingestion, making it an effective insecticide (Schünemann et al., 2014). Once ingested, Cry toxins become solubilized and activated by the alkaline pH and enzymes of the digestive system (Bravo et al.,

2011). The activated Cry toxins then bind to specific protein receptors on the membrane of the gut epithelial cells (Sanahuja et al., 2011). This mechanism enables the toxins to insert themselves into the plasma membrane and create pores, which are permeable to small molecules (Bravo et al., 2007; Kirouac et al., 2002). These pores eventually lead to cell lysis due to the disruption of the cellular ionic gradient and a large influx of solutes (Knowles & Ellar, 1987).

With SBW feeding primarily on new growth, the goal for many control programs is to protect the trees current year foliage to ensure survival (Fuentelba et al., 2019). To do so, commercial formulations of Btk are sprayed aerially on forest stands (van Frankenhuyzen, 1990). In regards to dosage, Bauce et al. (2004) found that when SBW larval densities were moderate (less than 30 larvae per 45cm branch), the protection of foliage was comparable between one or two applications of 30 billion international units per hectare (BIU/ha). However, if larval densities exceeded 30 larvae per branch, two consecutive applications of 30 BIU/ha resulted in a noteworthy improvement in foliage protection. Increasing the dosage beyond 30 BIU/ha did not result in superior protection of foliage and currently, in Canada, this is the dosage that is approved for controlling SBW (Bauce et al., 2004; Boulton et al., 2002). Btk is an effective method for mitigating the negative effects of SBW defoliation and protecting new growth when applied early enough (Fuentelba et al., 2015, 2019).

Furthermore, the short half-life of Btk, which is largely dependent on environmental factors such as exposure to sunlight, temperature, and pressure (Surgeoner & Farkas, 1990), can also be viewed as a positive attribute. In general, Btk loses 50% of its insecticidal activity in 1-3 days, with some reports of longer residual activity (i.e. 10 days) (Surgeoner & Farkas, 1990). This allows for repeated and targeted applications to manage pest populations while potentially decreasing the exposure time for non-target organisms.

1.4 Btk on non-target lepidoptera

With Btk being highly specific to Lepidoptera, it is consequently less hazardous to non-lepidopteran organisms than broad spectrum insecticides (Boulton & Otvos, 2004). Numerous studies have been conducted with the aim of assessing the efficacy of Btk as an insecticide (Bauce et al., 2004; Sanahuja et al., 2011). Nonetheless, it is crucial to complement these investigations with studies that document the potential impacts of this insecticide on non-target species. By doing so, it will be possible to comprehensively evaluate the ecological and economic implications of its use.

Btk has been shown to have an overall negative effect on the abundance and richness of non-target Lepidoptera (Miller 1990, Boulton et al. 2002, Boulton 2004). Miller (1990) monitored leaf-feeding caterpillars on Garry oak (*Quercus garryana*) in Oregon, USA, after one year of treatment with Btk and found that species richness and total caterpillar abundance was significantly reduced. Similarly, Boulton et al. (2002) assessed the effects of Btk on non-target caterpillars on wax currant (*Ribes cereum*) in British Columbia, Canada, and found a decline in all species that were feeding during the time in which the plants were treated. Miller (2000) found a decrease in caterpillar richness, abundance, and live mass in sprayed sites after just one year of treatment in Oregon, USA. Boulton and Otvos (2004) and Boulton (2004) both found a decline in non-target caterpillar richness and abundance in sprayed sites in British Columbia, Canada. Rodenhouse and Holmes (1992) found a significant reduction in caterpillar biomass after treatment in New Hampshire, USA. Furthermore, Wagner et al. (1996) also found that total caterpillar abundance and relative abundance of common species in Virginia, USA, were lower in treated sites, however these results were not statistically significant. Contrastingly, Glaus et al. (2023) found that Btk application had no significant impact on abundance or species richness of non-target Lepidoptera in New Brunswick, Canada.

Although most studies have focussed on non-target Lepidoptera larvae, few have included adults. Sample et al. (1996) collected moth larvae as well as adults in West Virginia, USA. They found that larval abundance declined in all treatments and the effects on the adults mirrored those of the larvae, however the effect lagged by one year. They hypothesized that this happened because larvae are the life stage affected by Btk and the collection of adults is two life stages removed from the treatment effect (Sample et al., 1996). Therefore, a lag between the time of treatment and the expression of treatment effect among adult Lepidoptera may be expected. Similarly, Strazanac et al. (2005) found a significant decrease in moth counts in some species after the second treatment year in Virginia and West Virginia. Contrastingly, Manderino et al. (2014) found that total moth abundance and diversity did not differ significantly between treated and non-treated sites in Virginia.

One commonality in previous research is that Lepidoptera taxa are not equally affected by Btk. Some appear to be more or less sensitive than others, however it is difficult to generalize when predicting the susceptibility of native species. It appears that both life history strategies and intrinsic sensitivities influence the effect of Btk on non-target Lepidoptera (Boulton et al., 2002; Boulton & Otvos, 2004; Leza et al., 2021; Manderino et al., 2014; Miller, 1990, 2000; Peacock et al., 1998; Strazanac et al., 2005; Wagner et al., 1996). Species with larval stages during the same time in which Btk is applied (i.e. early season phenology) tend to be more strongly affected than others (Boulton et al., 2002; Miller, 1990; Strazanac et al., 2005; Wagner et al., 1996). Miller (1990) and Wagner et al. (1996) found that univoltine moths were more sensitive, as species with one generation per year would not be capable of responding to local extinction as quickly as those with multiple generations (i.e. multivoltine). However, this was contrasted by Boulton et al. (2002) who found that univoltine species with an extended larval period are

unlikely to be harmed if enough of the larvae feed outside the time when residues are active.

Boulton (2004) also found that species that have an early phenology appear to be less affected, as they reach the pupal stage before exposure to Btk.

Aside from emergence timing, feeding strategy also appears to affect the susceptibility of some taxa. Species with caterpillars that form shelters such as leaf rolls, folds, or fascicles may be protected from lethal doses if they create shelters before application and successfully avoid consuming treated foliage by only consuming material within their shelter (Boulton, 2004; Wagner et al., 1996). However, it is important to note that SBW are also proficient shelter builders via silk hibernaculum (Miller, 1975). The efficiency of Btk as a control measure against SBW remains apparent, therefore the efficacy against shelter builders may be dependent on other factors. Generalist herbivore species may also experience different mortality rates on different host plants due to variation in their habitat and diet (Boulton et al., 2002).

There is evidence to suggest that some taxa are less sensitive to Btk application than others, possibly due to phenology. Peacock et al. (1998) evaluated the effects of Btk on 42 lepidopteran species (4 butterflies and 38 moths) in a laboratory setting. All 4 butterfly and 10 moth species were considered highly sensitive. They found Xylenine Noctuids to be the least sensitive to Btk application. Boulton and Otvos (2004) and Manderino et al. (2014) found the Geometridae family to be less sensitive, even when higher than normal doses of Btk were used. Leza et al. (2021) found the Drepanidae family to be less sensitive and Arctiidae family to be more sensitive. Accordingly, these idiosyncratic responses make it necessary to consider a substantial number of taxa to predict the outcome of Btk application on non-target lepidoptera.

Previous studies also suggest that Btk may have positive effects on some genera. According to Sample et al. (1996), although Btk application appears to have short-term negative impact on

non-target Lepidoptera, the long-term effects may be positive due to the decreased presence of target pest species, resulting in decreased competition. The findings of Manderino et al. (2014) concurred with this. They found that Btk may have had an overall protective effect for the Geometridae family by reducing target pest numbers which prevented competitive exclusion.

Although these studies show that Btk can have a negative effect on the abundance and richness of non-target Lepidoptera, the variation in susceptibility underscores the difficulty of predicting their response. There are particular knowledge gaps surrounding the effects of Btk on adult nocturnal macromoths, particularly in Canada. Of the studies that do exist, most focus on the effects after just one year of treatment and there is a lack of long-term research. Ongoing research is necessary to improve our understanding and implementation of appropriate management strategies for SBW outbreaks that take non-target species into consideration.

Chapter 2: Effects of *Bacillus thuringiensis* subsp. *kurstaki* application on non-target nocturnal macromoth diversity in the eastern boreal forest, Canada.

2.1 Abstract

Lepidoptera, including butterflies and moths, play vital roles as herbivores, pollinators, and food sources, but also include species considered forest pests. The impact of *Bacillus thuringiensis* subs. *kurstaki* (Btk), a widely used bio-insecticide for controlling forest pests like the spruce budworm, on non-target lepidoptera in Canada remains uncertain. To address this, we established a replicated field study to evaluate the effects of Btk on non-target nocturnal macromoth communities in the eastern boreal forest of western Newfoundland, Canada. Over two years, we sampled moths across four groups: north treatment, north control, south treatment, and south control. Our analysis focused on species diversity, abundance, and composition. Results showed no significant differences in total abundance or species composition between treatment and control groups. In 2022, control sites had significantly higher Hill numbers for Shannon and Simpson diversity compared to treatment sites. In 2021, differences in Hill numbers were only observed between north controls and treatments. These findings indicate that after multiple years of treatment, there can be shifts in the relative abundance of certain species, but without significant changes in species richness, total abundance, or composition between control and treatment groups. These results suggest that Btk can lead to stand-level shifts in relative abundance but does not substantially alter community structure during the early stages of treatment. The responses of species are idiosyncratic, likely influenced by differences in phenology and voltinism. Monitoring the impacts of Btk on non-target lepidoptera is crucial to effectively manage forest pests while minimizing unintended consequences for non-target species.

2.2 Introduction

The order Lepidoptera (moths and butterflies) is highly diverse and well represented in boreal forests. While butterflies are more well known, moths are 10 times more taxonomically diverse (Wagner et al., 2021). They provide numerous important ecosystem services such as defoliation, pollination, and prey to other animals (Hammond & Miller, 1998; Holmes et al., 1979). As pollinators, nocturnal moths have been found to be more efficient than daytime pollinators (Anderson et al., 2023). Moths also differ substantially in their flight phenology, meaning that early season flyers are likely to pollinate a different cohort of plants than those that emerge later in the season. Moths are also an important food source for many vertebrates such as birds and bats (Wagner et al., 2021). Moths are classified into micro- and macromoths based on their taxonomy and size. Micromoths, which have an older evolutionary history, are generally characterized by their smaller size (Beadle & Leckie, 2012).

The Tortricidae is a family of micromoths that contains numerous species of economic and ecological concern in forests. One of them, the spruce budworm (*Choristoneura fumiferana*, Clem., SBW), has a particularly large impact on fir and spruce forests across Canada and the United States (Chang et al., 2012; Maclean & Ostaff, 1989). SBW outbreaks occur cyclically approximately every 30-40 years and often last for many years (Blais, 1983). During these outbreaks, stands are heavily defoliated which leads to reduced growth and eventual tree mortality (MacLean, 1984). Historically, there has been three major outbreaks in Canada, resulting in millions of hectares of damage (Blais, 1983). These outbreaks have both economic and ecological consequences, therefore appropriate management strategies is imperative for forest health (Chang et al., 2012; Dymond et al., 2010).

Bacillus thuringiensis subsp. *kurstaki* (Btk) has been used to treat this irruptive pest since the mid-1980s (Boulton & Otvos, 2004). Btk is a naturally occurring soil bacterium that produces proteinaceous crystals, endowing it with insecticidal properties (Lambert & Peferoen, 1992). *Bacillus thuringiensis* subsp. *kurstaki* is a subspecies that releases crystals that are specifically harmful to Lepidoptera (Schünemann et al., 2014). These crystals are released into the environment and once consumed by larval stages, leads to their death by causing cell lysis (Knowles & Ellar, 1987; Lambert & Peferoen, 1992). Btk is applied aerially onto forest stands with the goal of protecting the current years foliage or reducing the outbreak spread and magnitude (Fuentealba et al., 2019; Johns et al., 2019). Previous studies have shown that Btk is an effective insecticide for lepidopterous pests (Bauce et al., 2004; Sanahuja et al., 2011).

With Btk being Lepidoptera specific, it can negatively impact non-target moth species. Previous studies have shown an overall negative effect on abundance and richness of non-target species (Boulton et al., 2002; Boulton, 2004; Miller, 1990; Sample et al., 1996). However, other research has shown that the impact on non-target species is not linear and taxa are not equally affected, with life history strategies and intrinsic sensitivities playing a role (Boulton et al., 2002; Boulton & Otvos, 2004; Leza et al., 2021; Manderino et al., 2014; Miller, 1990, 2000; Peacock et al., 1998; Strazanac et al., 2005; Wagner et al., 1996). There is even evidence to suggest that Btk may have a positive effect on some genera due to decreased competition in sprayed areas (Manderino et al., 2014; Sample et al., 1996). The variation in susceptibility makes it difficult to predict the non-target effects of Btk application and highlights the knowledge gaps that exist in the present research.

The objective of this study was to quantify the effect of Btk usage on non-target lepidopteran species. Forest management techniques that negatively impact lepidopteran

communities can have significant trophic implications both up and down the food chain, by either decreasing prey availability to higher trophic levels, or modifying the richness of consumers of primary production (Summerville, 2011). As lepidopterans are crucial pollinators and an essential food source, the consequences of such practices can reverberate throughout the ecosystem. As one of the most functionally significant taxa in forest ecosystems, nocturnal macromoths were selected for this study due to their diversity, abundance, and ease of capture and identification (Alison et al., 2022; Burford et al., 1999; Holmes et al., 1979; Young, 2005). Monitoring this group was straightforward since they could be captured and monitored in large numbers.

Using a replicated design, I made use of an ongoing “Early Intervention Strategy” (Johns et al., 2019) to manage SBW in western Newfoundland, Canada, to investigate how the aerial application of Btk in forests affects the community structure (i.e. diversity, abundance, and composition) of nocturnal macromoths. I predicted that:

1. Control sites would have higher moth abundance than the Btk treated sites.
2. Control sites would have higher species richness and diversity indices than Btk treated sites.
3. Community composition would differ between Btk treated sites and control sites.

2.3 Materials and Methods

2.3.1 Study location

I conducted this study over a two-year period (2021 and 2022) on the west coast of Newfoundland. The study sites were divided into two locations: north and south of Gros Morne National Park, Newfoundland and Labrador, Canada (Figure 1).

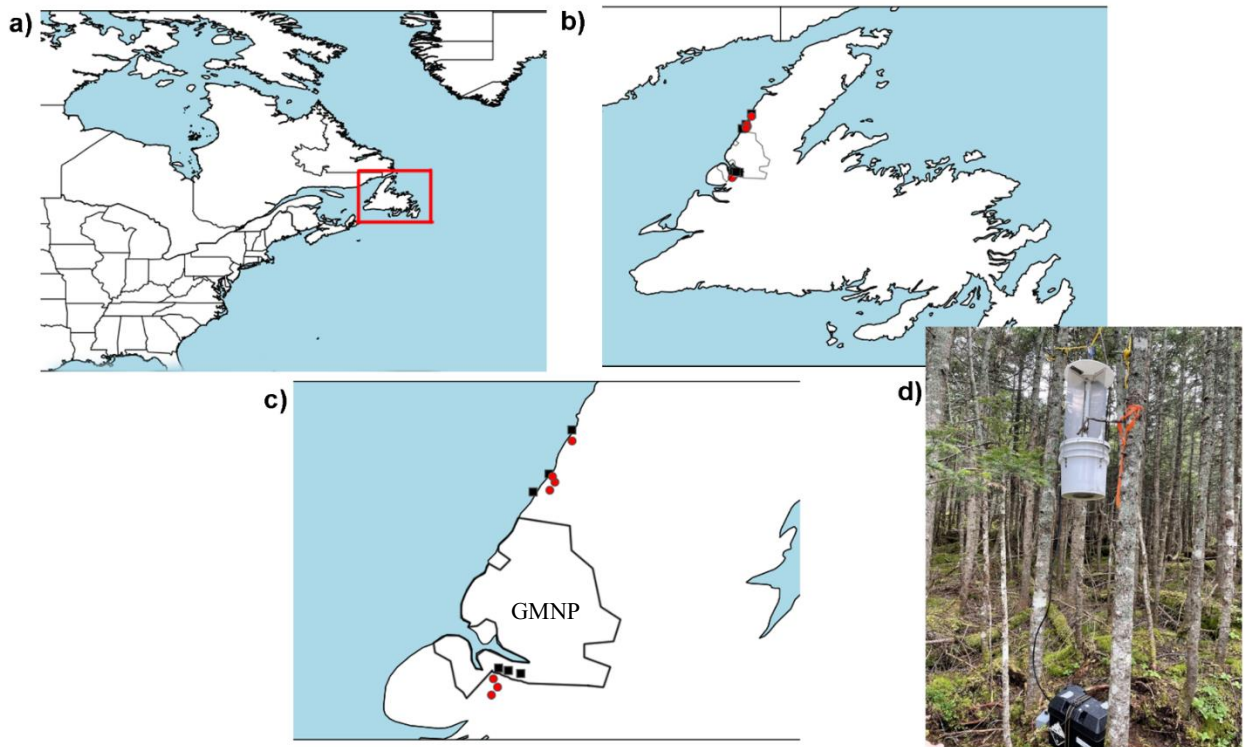


Figure 1. a) Newfoundland, Atlantic Canada. b) Location of study location in western Newfoundland. c) Location of the study sites in relation to Gros Morne National Park. Black squares represent control sites and red circles represent Btk treated sites. d) Light trap set up for moth collection.

The northern sites were located in the Northern Peninsula Forest ecoregion (Protected Areas Association of Newfoundland and Labrador, 2008a). The northern latitude (50°N) of this ecoregion results in the cool summers, long winters, and some of the coldest temperatures on the island. It has annual rainfall of 1300-1500mm and mean July temperatures of 13-15 $^{\circ}\text{C}$. Based on CanESM2 climate modelling, the average temperatures for June, July, and August are 11.2 $^{\circ}\text{C}$, 16.8 $^{\circ}\text{C}$, and 17 $^{\circ}\text{C}$, respectively. The Northern Peninsula Forest ecoregion has large portions of low-lying plateau bogs with some sections of boreal forest. The vegetation in this ecoregion is dominated by mountain avens (*Dryas octopetala*), swamp birch (*Betula alleghaniensis*), red bearberry (*Arctostaphylos uva-ursi*), dwarf willow (*Salix herbacea*), purple saxifrage (*Saxifraga*

oppositifolia), Greenland primrose (*Primula egaliksensis*), northern green orchids (*Platanthera aquilonis*), oxytropis (*Oxytropis* sp.), and sedges (Cyperaceae) (Protected Areas Association of Newfoundland and Labrador, 2008a). The forested areas in which I sampled are dominated by balsam fir (*Abies balsamea*). The cold temperatures of this ecoregion limit the growth of many species that can be found growing in the south.

The southern sites were located in the Western Newfoundland Forest ecoregion (Protected Areas Association of Newfoundland and Labrador, 2008b). This ecoregion experiences warm summers and cold winters. It has annual rainfall of 1200mm and mean July temperatures of 14-16 °C. Based on CanESM2 climate modelling, the average temperatures for June, July, and August are 12°C, 17.1°C, and 16.9°C, respectively. The vegetation in this ecoregion is dominated by boreal forests with balsam fir and floor coverage of fern (Protected Areas Association of Newfoundland and Labrador, 2008b). Black spruce (*Picea mariana*) is sometimes present but not as common. Alder (*Alnus* sp.) swamps with goldenrod (*Solidago* sp.) or bracken fern (*Pteridium* sp.) are also characteristic of this ecoregion. Some vegetative species found in this southern zone, such as yellow birch (*Betula alleghaniensis*), white pine (*Pinus strobus*), red maple (*Acer rubrum*), and trembling aspen (*Populus tremuloides*), reach their northern limits in this ecoregion (Protected Areas Association of Newfoundland and Labrador, 2008b). Corresponding with the northern sites, all collections took place in balsam fir dominated boreal forest stands.

I sampled a total of 13 sites (Appendix 1). In 2021, I collected moths from 12 sites in a paired design of treatment and control sites. In the south, there were three sites that had been sprayed with Btk for one year (2021) and three control sites. In the north, there were three sites that had been sprayed for two consecutive years (2020 and 2021) and three control sites. In 2022,

I sampled most of these sites again, with an extra consecutive year of Btk treatment: in the south, the treatment sites had been sprayed for two consecutive years (2021 and 2022) and in the north, the treatment sites had been sprayed for three consecutive years (2020, 2021, and 2022). The control sites remained the same. In 2022, one northern treatment site had to be replaced as it was no longer included in the 2022 spray program, and I wanted to maintain consistent spray parameters across all sites. I chose a new site approximately 4.5 kilometers away from the previous site. As with all northern treatment sites, this one had been sprayed for three consecutive years (2020, 2021, and 2022).

2.3.2 Spray Program

The Spruce Budworm Early Intervention Strategy was developed by the Government of Canada with the goal of identifying potential spruce budworm outbreak locations and proactively curtailing the growth of budworm populations with application of Btk. Newfoundland and Labrador began their treatment in 2020 actioned by their Department of Fisheries, Forestry, and Agriculture (Government of Newfoundland and Labrador, n.d.).

Btk was used in 2020, 2021, and 2022 to protect 35 583, 138 950, and 142 474, respectively (Government of Newfoundland and Labrador, n.d.). This largely took place in the Great Northern Peninsula using one or more treatments. All aerial applications were applied when weather conditions and larval development were optimal for Btk treatment. In 2020, all spraying took place between June 26th and July 16th. In 2021, all spraying took place between June 7th and July 12th. Finally, in 2022, all spraying took place between June 13th and July 8th. It is important to note that in 2020 all spraying was limited to areas north of Gros Morne National Park and consequently our study sites south of the park received one year less of treatment than the northern sites.

2.3.3 Collection Methods

At each site I used one light-flight intercept trap to capture adult nocturnal macromoths. Each trap was equipped with a 12W black-light fluorescent tube light that was powered by lead-acid automotive batteries. 5-gallon buckets were attached to hold collected moths and three insecticide strips as a killing agent (Hercon® Vaportape II™). The traps were also equipped with photocell light sensors that turned the tube lighting on at dusk and off at dawn.

I placed the traps approximately 2 meters off the ground by tying them to available trees. Sampling took place over two summers (2021 and 2022) with three collections (June, July, and August) per year. Although this does not encompass the breeding times of all species, this is when the majority are active (Pinkson et al., 2021). I chose sampling dates based on the lunar cycle, with the new moon being targeted due to a lessened influence of moonlight (Yela & Holyoak, 1997). Sampling took place June 15-17 2021, July 13-15 2021, August 10-12 2021, June 28-30 2022, July 27-29 2022, and August 23-25 2022 for a total of nine trapping nights over three sampling periods per year. During each sampling event, the traps were set up for three days and emptied daily into cardboard boxes to allow for adequate air flow to promote drying and avoid mold growth. Once collected, samples were sorted to retain only moths that belonged to families that are considered macromoths (i.e., Drepanidae, Geometridae, Saturniidae, Sphingidae, Notodontidae, Erebidae, and Noctuidae). Moths were collected and identified to lowest possible taxonomic ranking, using a variety of resources (Beadle & Leckie, 2012; John, n.d.; *Moth Photographers Group*, n.d.) A voucher collection was created and is stored with Natural Resources Canada, Canadian Forest Service, Corner Brook, Newfoundland and Labrador, Canada. This voucher collection was sent to Dr. Christian Schmidt with the Canadian National Collection of Insects, Arachnids and Nematodes to confirm the species identification.

2.3.4 Statistical Analyses

I completed all data analysis using R (version 4.2.2, R core team). I separated the data by collection year (2021 and 2022) and treatment type (control or Btk application). This separation aimed to ensure that the collection year and treatments remained distinct and were not confounded with each other.

Abundance - I ran a generalized linear model to assess the effect of treatment type on moth abundance. A distribution model was created using the *fitdistrplus* (Delignette-Muller & Dutang, 2015) package to assess the distribution of the data. I pooled the data over month as seasonal differences in moth abundance were both expected (Leza et al., 2021) as well as not the focus of my study. I removed singletons to eliminate the influence of rare species. I ran this generalized linear model using the *glm.nb* function in the *MASS* package (Ripley et al., 2023) which is fitted with a negative binomial distribution as this was best suited for my data based on distribution tests. The dependent response variable was the absolute moth abundance per site and the independent predictor variable was the site type (north treatment, north control, south treatment, south control). In case of significant difference ($p < 0.05$), I used the post hoc (pairwise Tukey's analysis) using the package *emmeans* (Lenth et al., 2022) to determine how abundance differed between treatment types. Using this same methodology, I analysed the effect of treatment type on each of the top five most abundant species per year, as they accounted for $\geq 35\%$ of the collection each year.

Diversity - I calculated diversity indices using Hill numbers ($q = 0$; species richness, $q = 1$; Shannon diversity, $q = 2$; Simpson diversity) with the *iNext* package (Chao et al., 2014; Hsieh et al., 2016). Hill numbers are calculated into units of effective number of species, i.e. the

number of equally common species needed to achieve the same diversity value in a community (Chao et al., 2014).

Composition - I used nonmetric multidimensional scaling (NMDS) ordination to assess any visual differences in species composition between site types. I created a species-by-site matrix using transformed counts ($x' = \log(x+1)$) and removed singletons to eliminate the influence of rare species in the ordination. I completed this using the function *MetaMDS* in the R package *Vegan* (Oksanen et al., 2019) with Bray-Curtis dissimilarity scores. I chose Bray-Curtis dissimilarity scores as it allows for quantitative comparison between ecological abundance data. The ordination was plotted using the function *ggplot* in the R package *ggplot2* (Wickham et al., 2016). Two dimensions were chosen as it allowed for easy visualization and interpretation. To assess for any significant differences between treatments, I performed a permutational multivariate analysis of variance (PERMANOVA) using the function *adonis2* in the R package *vegan* (Oksanen et al., 2019).

2.4 Results

I collected and identified a total of 11,802 macromoths and they comprised 174 species belonging to seven families (Drepanidae, Erebidae, Geometridae, Noctuidae, Notodontidae, Nolidae, and Sphingidae). Of these, I identified 95.7% to the species level and the remaining 4.3% to family. In 2021, I collected 6,507 individuals. Geometridae was the most abundant family, accounting for 59% of the collection, followed by Noctuidae (38%), Drepanidae (2%), Notodontidae (0.75%), and Erebidae (0.25%). In 2022, I collected 5,295 individuals. Noctuidae was the most abundant, accounting for 51.9% of the collection, followed by Geometridae (45.7%), Drepanidae (1.3%), Erebidae (0.57%), Notodontidae (0.49%), and Nolidae and Sphingidae (0.019% each).

2.4.1 Abundance

2.4.1.1 Abundance in 2021

The site type had a significant effect on overall moth abundance ($p < 0.001$; Table 1, Figure 2). However, I found no significant difference in the abundance of moths between the treatment and control groups within the north or south regions. The south control sites had a higher number of individuals compared to the north control or north treatment sites ($p < 0.001$ and $p = 0.0121$, respectively), but it did not differ from the south treatment. I found more individuals in the south treatment sites than the north control sites ($p = 0.0017$), but I detected no significant difference between the south treatment and the north treatment. It is noteworthy that, although not significant, the mean abundance was higher in the north treatment group than the north control group, and higher in the south control group than the south treatment group.

The five most abundant species collected in 2021 accounted for 39% of the entire collection and each had more than 175 individuals, and included: *Dysstroma citrata* (Linnaeus; Geometridae) ($n = 1183$), *Macaria signaria* (Hübner; Geometridae) ($n = 613$), *Caripeta divisata* (Walker; Geometridae) ($n = 381$), *Syngrapha viridisigma* (Grote; Noctuidae) ($n = 178$), and *Anaplectoides pressus* (Grote; Noctuidae) ($n = 178$). I also assessed the top five most abundant species collected in 2022, where two remained the same as 2021 (*Macaria signaria* and *Dysstroma citrata*) but three were different from 2021 (*Xestia badicollis* (Grote; Noctuidae) ($n = 152$), *Syngrapha rectangula* (Kirby; Noctuidae) ($n = 94$), *Phlogophora periculosa* (Guenée; Noctuidae) ($n = 133$)). Although these three species were not among the top five most abundant in 2021, I assessed any changes in abundance for this year as well as 2022 so I could properly compare any changes in abundance across years.

There were significant differences among sites for *Syngrapha viridisigma* ($p < 0.001$). I found significantly more individuals in the control than treatment site in the south ($p = 0.0273$), but no significant difference between treatment and control in the north. Although not a significant finding, there were also more individuals found in the north treatment than the north control. There were significant differences among sites for *Macaria signaria* ($p < 0.001$), with more individuals found in the treatment than controls in the north ($p = 0.0221$). I found no significant differences between the treatment and control in the south, but there were more individuals found in the south control than the south treatment. *Dysstroma citrata* differed among site type ($p < 0.001$), with there being a significant difference between the north and south but not a significant difference between treatment and controls within a region. Although not a significant finding, the north treatment supported more individuals than the north control and the south control supported more individuals than the south treatment. Furthermore, *Caripeta divisata* differed among site type ($p < 0.001$). I found more individuals in the north treatment than the north control ($p < 0.001$). I found more individuals in the south control than the south treatment; however, this was not a significant difference. The abundance of *Anaplectoides pressus* did not differ significantly among site types, although it did trend higher in the north and south control sites than the north and south treatment types.

For the three species that were more abundant in 2022, in 2021 I found a significant difference in the abundance of *Xestia badicollis* ($n = 152$, $p = 0.037$) and *Phlogophora periculosa* ($n = 133$, $p < 0.001$) in terms of site type. *Syngrapha rectangula* ($n = 94$) did not differ significantly between site types, but I found more individuals in the north treatment than the north control and more in the south control than the south treatment. I found significantly more individuals of *Xestia badicollis* in the south control than the north control ($p = 0.037$); however, I found no other

significant differences. Although not a significant finding, I found more individuals in the north treatment than the north control and more individuals in the south control than the south treatment. I found more individuals of *Phlogophora periculosa* (n=133) in the south treatment than the south control (p<0.001). Although not significant, I found more individuals in the north treatment than in the north control.

2.4.1.2 Abundance in 2022

Treatment type had a significant effect on total moth abundance (p=0.0208; Table 1, Figure 2). Like the previous year, there was no significant differences found between the treatment and control groups within the north and south regions. The only significant difference was between the north control sites and south control sites, in which more individuals were found in the south control sites (p=0.0467). However, the mean abundance was higher in the north treatment group than in the north control group and higher in the south control group than in the south treatment group.

The 5 most abundant species in 2022 accounted for 35% (vs. 39% in 2021) of the collection and had over 140 individuals. Of these species, two remained the same as in the previous year, namely *Macaria signaria* (n=333) and *Dysstroma citrata* (n=869), while the other three were *Xestia badicollis* (Grote; Noctuidae) (n=306), *Syngrapha rectangula* (Kirby; Noctuidae) (n=185), and *Phlogophora periculosa* (Guenée; Noctuidae) (n=142). The top five most abundant species from the previous year (2021) were re-evaluated in 2022 to identify any changes. There was a decrease in abundance across all five species. They accounted for only 28% of the total collection for this year, with each having more than 60 individuals. The counts for these species were as follows: *Anaplectoides pressus* (n=113), *Macaria signaria* (n=333), *Dysstroma citrata* (n=869), *Syngrapha viridisigma* (n=68), and *Caripeta divisata* (n=74).

The abundance of *Dysstroma citrata* ($p < 0.001$), *Xestia badicollis* ($p < 0.001$), *Syngrapha rectangula* ($p = 0.0213$), and *Phlogophora periculosa* ($p < 0.001$) varied among the four site types. By contrast, the abundance of *Macaria signaria* did not exhibit any significant differences across the treatment groups, however it did trend higher in the north treatment compared to north control and higher in the south control compared to south treatment. More individuals of *Xestia badicollis* were found in the south control than in the south treatment ($p = 0.0487$). Although not significant, there were more individuals in the north treatment than the north control. For *Syngrapha rectangula*, the only significant difference in abundance was between the south control and south treatment sites, with the controls supporting a higher number of individuals ($p = 0.013$). Although the difference was not statistically significant, there was a higher number of individuals observed in the north control compared to the north treatment. Although *Dysstroma citrata* differed significantly among site types, I found no differences between the treatment and controls within each region. Despite lacking statistical significance, the north treatment and south treatment supported more individuals than their paired controls. Similarly, *Phlogophora periculosa* differed among site type; however, I found no differences between the treatment and controls within each region. Again, although not a significant difference, there were more individuals found in the north treatment than the north controls and more in the south treatment than the south controls.

The abundance of *Dysstroma citrata* and *Caripeta divisata* differed significantly between sites ($p < 0.001$ each). The abundance of *Syngrapha viridisigma*, *Macaria signaria*, and *Anaplectoides pressus* did not. Despite a lack of significant results, more individuals of *Syngrapha viridisigma* were found in the north treatment and south treatment when compared to the north control and south treatment, respectively. Similarly, I found more individuals of

Macaria signaria in the north treatment than the north control; however, I found more in the south control than the south treatment. Also not a significant finding, but I found more individuals of *Anaplectoides pressus* in the north control than the north treatment and more in the south control than the south treatment. Although *Dysstroma citrata* differed significantly among site type, I found no significant differences between the treatment and controls within each region. Although not significant findings, the north treatment and south treatment supported more individuals than their paired controls. Similarly, *Caripeta divisata* differed significantly among site types; however, I found no differences between treatment and controls within each region. Again, I found non-significant differences with more individuals in the north treatment than the north controls. I found the same number of individuals in the south control and south treatment.

Table 1. Total raw moth abundance for the five most abundant species in 2021 and 2022. Collection days amalgamated by year and treatment type. Control/treatment pairs that appear in a box are significantly different. NC = North control, NT = North treatment, SC = South control, ST = South treatment.

Species	Top 5 year	2021						Overall sig.	2022					
		N	NC	NT	SC	ST	N		NC	NT	SC	ST	Overall sig.	
<i>Anaplectoides pressus</i>	2021	178	37	26	67	48	NS	118	41	23	25	29	NS	
<i>Caripeta divisata</i>	2021	381	36	173	115	57	*	90	17	51	11	11	*	
<i>Dysstroma citrata</i>	2021/22	1183	70	123	585	405	*	967	98	255	260	354	*	
<i>Macaria signaria</i>	2021/22	613	61	271	154	127	*	356	47	169	78	62	NS	
<i>Phlogophora periculosa</i>	2022	135	21	20	24	70	*	158	8	48	23	79	*	
<i>Syngrapha rectangula</i>	2022	94	9	14	62	9	NS	204	43	59	80	22	*	
<i>Syngrapha viridisigma</i>	2021	178	18	26	109	25	*	74	24	25	19	6	NS	
<i>Xestia badicollis</i>	2022	152	1	5	135	11	*	312	4	20	207	81	*	

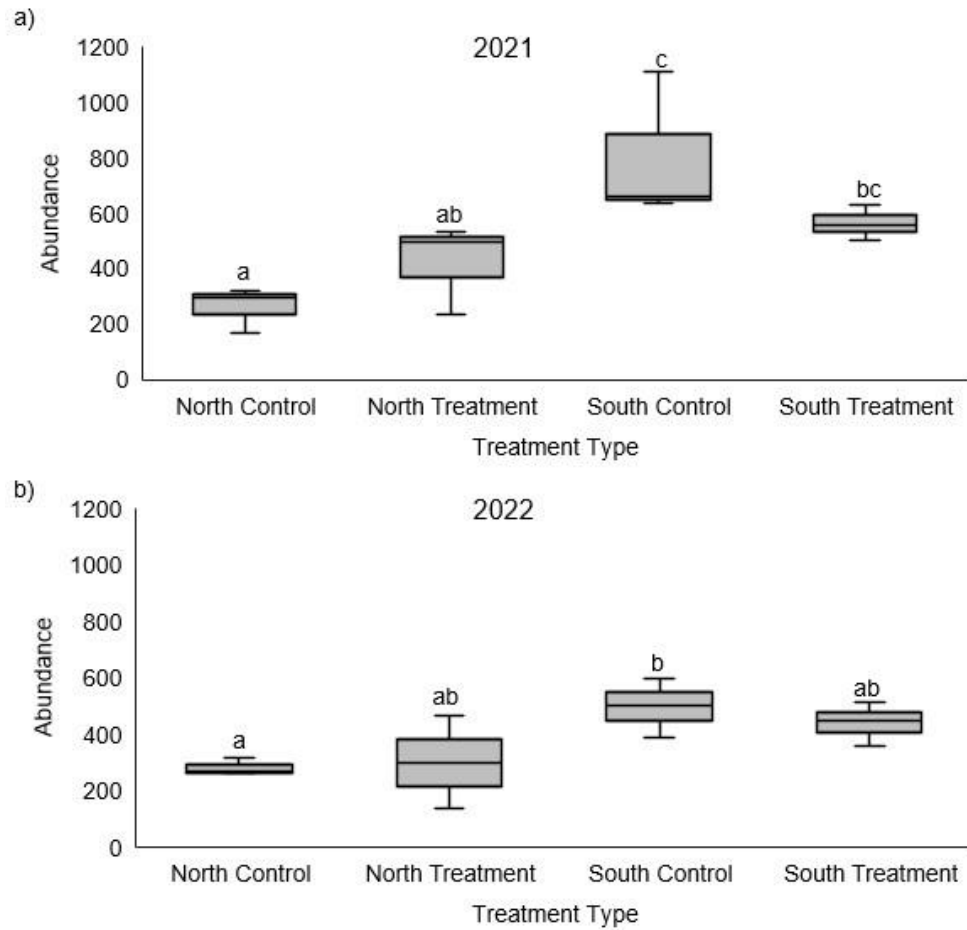


Figure 1. Variation of total moth abundance from four treatment types, across two years, a) 2021 and b) 2022. 2021 treatment sites had been sprayed with Btk for one (South Treatment) and two (North Treatment) consecutive year(s). 2022 treatment sites had been sprayed with Btk for two (South Treatment) and three (North Treatment) consecutive years. Comparisons tested using a generalized linear model (GLM), fitted with a negative binomial distribution. Letters a, b, and c indicate significant differences between treatment types based on post-hoc testing (95% confidence).

2.4.2 Diversity

2.4.2.1 Diversity in 2021

I detected a significant difference in Shannon diversity (Hill number, $q=1$) and Simpson diversity ($q=2$) between the north control and treatment sites (Figure 3), but not species richness ($q=0$). I detected no differences between the south control and treatment sites. While higher in the controls, the species richness confidence intervals overlapped between the north control and treatment, as well as between the south control and treatment. However, the confidence intervals did not overlap between the north and south sites, indicating a difference between the two regions, with the south sites having a higher richness.

Shannon diversity was similar in the south control and south treatment sites. There was no overlap in confidence intervals between the north control and north treatment sites, indicating a difference in diversity, with the north control having a higher Shannon diversity value. There was also no overlap in confidence intervals between the north and south sites, with the south supporting an intermediate diversity. Simpson diversity was similar in the south control, south treatment, and north treatment sites, but was higher in the north control sites.

2.4.2.2 Diversity in 2022

In 2022, Shannon diversity ($q=1$), and Simpson diversity ($q=2$) differed between treatment types (Figure 3) as indicated by non-overlapping confidence intervals. Although the confidence intervals overlapped, species richness was higher in the north controls than the north treatments and higher in the south controls than the south treatments. However, there were differences when comparing the north and south regions, with the south control sites having the highest species richness.

Regarding Shannon diversity, there was a lack of overlap in the confidence intervals between the south control and south treatment sites, as well as between the north control and north treatment sites, with the controls having a higher Shannon diversity in both regions. When comparing the Shannon diversity between the north and south regions, the north treatment, south treatment, and south control were similar, while north control sites supported a higher diversity. Lastly, Simpson diversity was higher in the north control group than the north treatment group and higher in the south control group than the south treatment group. The north sites had a higher Simpson diversity than the south sites.

Between years, there were some differences in Simpson and Shannon diversity, but not in species richness based on overlapping confidence intervals. Simpson diversity differed between years for the north sites, with diversity increasing from 2021 to 2022 in the treatment sites and decreasing from 2021 to 2022 in the control sites. There were no differences in Simpson diversity found between years in the southern sites. Shannon diversity increased from 2021 to 2022 in the north treatment sites but decreased from 2021 to 2022 in the south treatment and north control sites. There was no difference between years in the south control sites.

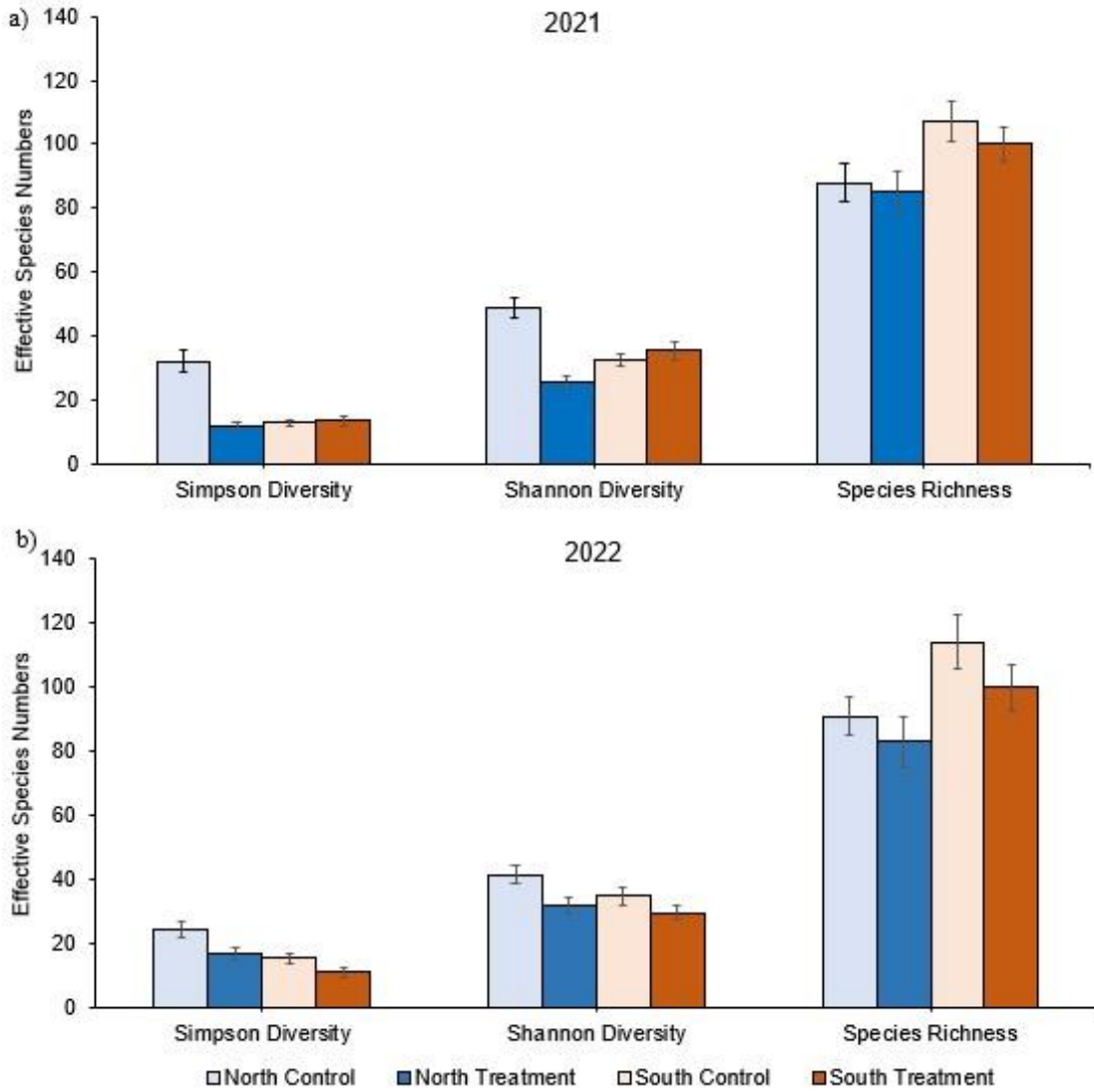


Figure 2 Diversity indices, characterized by effective number of species ($\pm 95\%$ confidence interval), for each treatment group for a) 2021 and b) 2022. Values calculated from Hill number order $q = 0$ (species richness), $q = 1$ (Shannon diversity), and $q = 2$ (Simpson diversity), for each treatment type.

2.4.3 Species Composition

NMDS ordination indicated that species composition did not differ among site types in either year (stress = 0.096 and 0.078 in 2021 and 2022, respectively; Figure 4). In both years, there was a clear grouping into collection month. In 2021, there was an outlier in one of the north control sites: only 79 individuals were captured in June, compared to 150+ in the other north control sites for the same month. PERMANOVA confirmed that species composition did not differ significantly among site types (202: $F=0.9504$, $p=0.486$; 2022: $F=0.8787$, $p=0.529$).

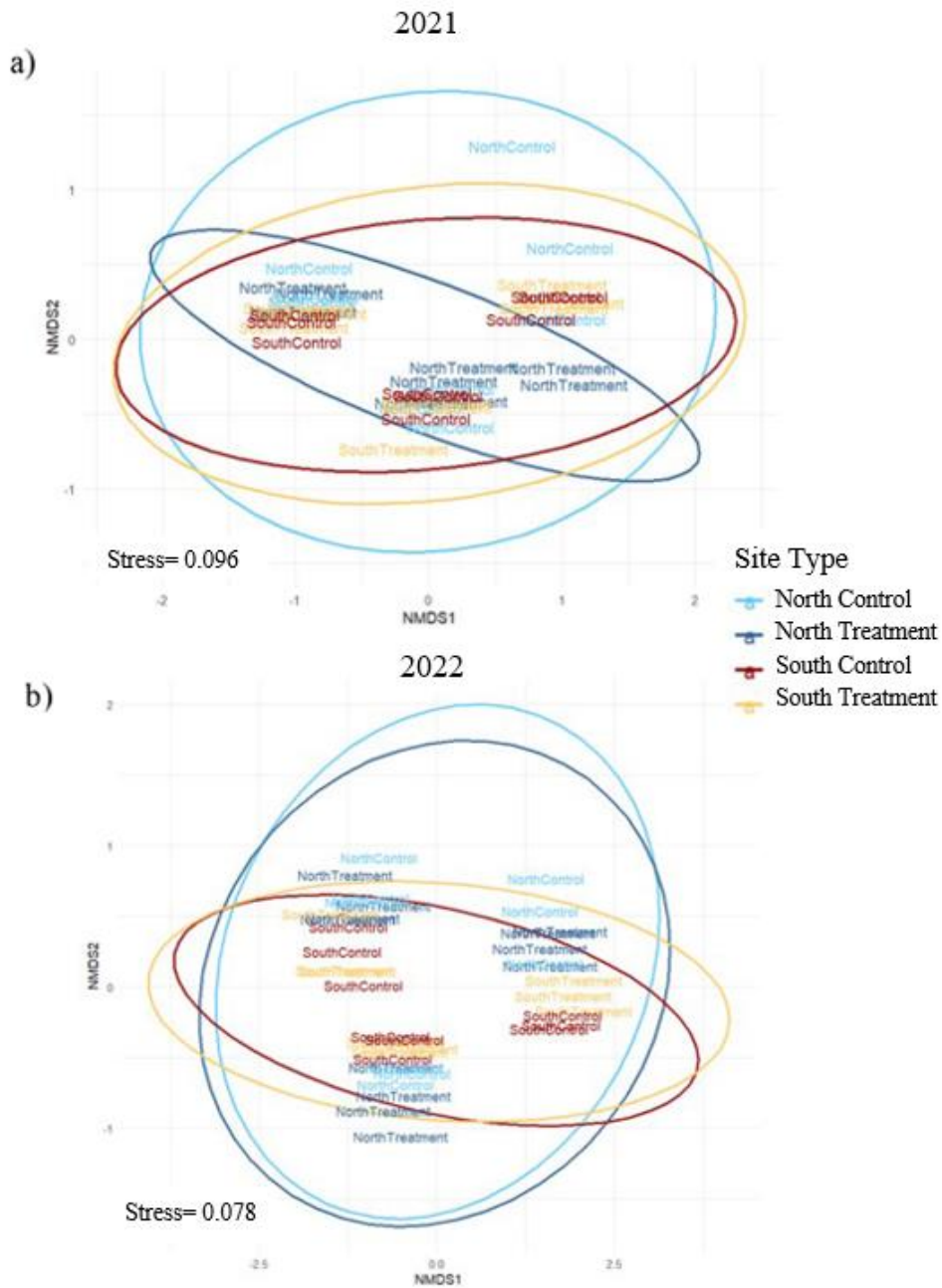


Figure 3. Nonmetric multidimensional scaling of species composition across treatment types in a) 2021 and b) 2022. Species abundance pooled across all dates and log transformed ($x' = \log(x+1)$). Bray-Curtis dissimilarity with 95% confidence ellipses. Each individual treatment on the plot represents a replicate sample site for north control, north treatment, south control, or south treatment.

2.5 Discussion

Contrary to my predictions, overall moth abundance and richness were not higher in the control sites, nor did community composition differ. However, patterns of changing relative abundance among species were reflected in the Shannon and Simpson diversity indices. Although species richness did not differ significantly, it was consistently higher in control sites. These complex results point to the need for a subtler assessment of Btk impacts on non-target taxa.

2.5.1 Abundance

Although I predicted that control sites would support the highest moth abundance, I found that the abundance of macromoths did not differ between Btk treated and control (untreated) sites. My findings are in contrast to those of Boulton (2004) who observed a decline in non-target caterpillar abundance after Btk application. Similarly, Sample et al. (1996) found a decrease in abundance of both adult and larvae non-target macromoths following Btk application. Wagner et al. (1996) also found abundance of common species to be lower in treated sites, however these results were not statistically significant. My findings are congruent with those of Glaus et al. (2023) who found that the abundance of non-target Lepidoptera did not differ between Btk treated areas and non-treated areas.

These results may be explained by the five most abundant species in each year, which accounted for 39% and 35% of total macromoths in 2021 and 2022, respectively. These species were the primary drivers of total macromoth abundance. Upon closer examination, it became apparent that although not significant, several of these species exhibited a higher abundance in the treatment sites, particularly in the north. In 2021, six of the eight most common species across both years (*Dysstroma citrata*, *Macaria signaria*, *Caripeta divisata*, *Syngrapha*

viridisigma, *Xestia badicollis* and *Syngrapha rectangula*) were more abundant in the north treatment than the north control, and in 2022 five of the eight (*Dysstroma citrata*, *Macaria signaria*, *Caripeta divisata*, *Xestia badicollis*, and *Phlogophore periculosa*) were more abundant. This is a stark contrast to the findings of Wagner et al. (1996) who found the abundance of the most common species to be decreased in the treated sites, although his findings were not statistically significant. In the south for 2021, only one of the dominant species (*Phlogophora periculosa*) was more abundant in the treatment than the control. In 2022, three of the eight species (*Dysstroma citrata*, *Anaplectoides pressus*, and *Phlogophora periculosa*) were more abundant in the south treatment than the south control. My findings suggests that while the overall abundance did not differ significantly between the treatment and controls, the increased abundances of these particular species may have offset any potential differences in the total moth population.

The increased abundance of certain species in the treatment sites presents an unexpected pattern, which allows for further questioning. There could be a variety of factors contributing to the disparity, which may encompass differences in phenology and/or intrinsic resistance to Btk. This intricate pattern of abundance allows us to consider the ecological dynamics that might underlie these observed differences.

Differences in phenology between species is a compelling avenue that can help explain the observed pattern. Among Lepidoptera groups, sensitivity to Btk application often aligns with life history characteristics (Peacock et al., 1998). Species that have an early phenology, are multivoltine, or univoltine with a long larval period appear to be less sensitive to Btk (Boulton, 2004; Boulton et al., 2002). Although comprehensive phenological data are lacking for all prevalent species in this study, noteworthy patterns emerge. For instance, the activity window of

the bivoltine *Macaria signaria*, which spans from May to August, suggests that it would have surpassed its larval stage prior to Btk application. *Anaplectoides pressus*, *Caripeta divisata*, and *Syngrapha rectangula*'s emergence around June aligns with their pupal stage occurring prior to Btk application. Contrastingly, the flight times of *Dysstroma citrata*, *Syngrapha viridisigma*, *Phlogophora periculosa*, and *Xestia badicollis* begin in July, potentially coinciding with the SBW's flight, the primary target of Btk application.

Intrinsic lack of sensitivity to Btk is another aspect that may be influencing the observed pattern. Certain species may inherently exhibit reduced sensitivity to Btk (REFS), potentially contributing to the absence of significant differences in abundance between treatment and control sites. This may explain why *Dysstroma citrata* and *Phlogophora periculosa* were slightly more abundant in the treatment sites. This also may explain why *Xestia badicollis* and *Syngrapha viridisigma* were more abundant in the treatment sites in the north.

The difference in abundance between the north and south was likely due to the spatial variation between sites. Moth diversity and composition has been shown to be influenced by latitudinal gradients and ecoregional differences (Kerr et al., 2014; Summerville et al., 2003). Although all of the stands were paired in a similar region and were all of similar composition (i.e. balsam fir dominated stands), other factors, such as proximity to the coast or microclimatic variations, which could potentially contribute to the observed difference in abundance, were not directly investigated in this study. The more southerly latitude and differences in the ecoregion of the south sites may be directly or indirectly promoting a slightly higher abundance of macromoths than in the north. Sample et al. (1996) suggested that environmental factors, such as weather, may have equal or even greater impacts on non-target Lepidoptera than Btk application.

This was beyond the scope of my research and data collection; however, it is important that this be taken into consideration in future investigations in this region.

2.5.2 Diversity

I predicted that the control sites would exhibit the highest levels of both diversity and richness as previous research has demonstrated a significant decrease in the richness of adult macromoths (Sample et al., 1996), as well as reduction in Shannon and Simpson's diversity indices for nocturnal Lepidoptera (Leza et al., 2021), in response to Btk application. However, my results were not in agreement with the previous research.

My findings revealed mixed outcomes between the north and south. In the north, for both 2021 and 2022, Shannon and Simpson diversity were greater in the control groups than in the treatment groups. Richness was consistently higher in controls, although did not differ significantly. This suggests that although the number of species remained relatively consistent across the treatment types, there was a shift in the relative abundance of different species within the community, leading to an uneven distribution of species abundance in the treated sites and ultimately creating differences in diversity. I found that the shift in relative abundance can be attributed to the presence of the most dominant species. Upon examining species with low and intermediate abundance, I observed minimal differences between the treatment and control groups. However, when focusing on the dominant species, more pronounced and contrasting differences became evident between the treatment types. The abundance of several dominant species (*Dysstroma citrata*, *Macaria signaria*, *Caripeta divisata*, *Syngrapha viridisigma*, *Xestia badicollis* and *Syngrapha rectangula*, and *Phlogophore periculosa*) was higher in the north treatment compared to the north control. Although this was not a significant finding for many of these species, the higher abundance in the treatment sites could have an influence on the

evenness. Consequently, this decreased evenness likely explains the observed difference in diversity, where the control sites exhibited a higher diversity due to a more equitable distribution of abundance across species.

In the south, there were few differences in richness and diversity between treatment and control groups in 2021; however, in 2022, diversity decreased in the treatment sites. Interestingly, in 2021 only one of the dominant species (*Phlogophora periculosa*) was more abundant in the treatment sites but in 2022 three of the eight dominant species (*Dysstroma citrata*, *Anaplectoides pressus*, and *Phlogophora periculosa*) were more abundant in the treatment sites. In 2021, richness remained similar among treatment types; however, it was slightly higher in the control sites. In 2022, Shannon and Simpson diversity were greater in the control group than the treatment group, while richness was similar between them. This finding further reinforces the notion that the shift in relative abundance, particularly among the most dominant species within the treatment sites is a key driver behind the observed shifts in diversity.

In 2021, the treatment sites had been sprayed with Btk for only one year (2021) whereas in 2022 they had been sprayed for two consecutive years (2021 and 2022). The lack of difference in diversity in 2021, followed by a decrease in 2022, may be a lagged effect of adult macromoth responses to Btk application and suggests that continued and repeated treatment of Btk could eventually have impacts on the assemblage. Boulton et al. (2007) sampled moths over a period of four years following Btk application and found the most significant impacts on the community occurred 12 to 14 months post spray. Sample et al. (1996) observed the effects of Btk application on adult Lepidoptera in the year following application rather than the year of, with reduced abundance and richness. The change in diversity from 2021 to 2022, following the second year of spraying, is indicative of a lagged affect as observed by Sample et al (1996).

My findings suggest that the treatment sites benefit some species, leading to a decrease in diversity because their abundances are creating less evenness in these sites. Manderino et al. (2014) and Sample et al. (1996) both found Btk to have an overall positive effect on some macromoths taxa. The reason why some species prevail in treated sites is likely due to a multitude of factors, including differences in phenology (Boulton et al., 2002; Miller, 1990; Strazanac et al., 2005; Wagner et al., 1996), intrinsic differences in susceptibility to Btk (Peacock et al., 1998), and/or environmental conditions (Kerr et al., 2014; Sample et al., 1996; Summerville et al., 2003). My data highlights the importance of long-term monitoring for understanding the dynamics of species response and assessing the long-term effects of Btk treatments on macromoth assemblages.

2.5.3 Species Composition

I predicted that species composition would differ between treated sites and control sites as changes in relative abundance have been recorded after exposure to Btk (Wagner et al., 1996). Although the diversity analyses indicated a shift in relative abundance of species, the ordination showed no difference in species composition between treatment types in either year. This contrasting evidence may be explained by the magnitude of the change in relative abundance. It is possible that, while the impact of Btk was significant enough to create a difference in diversity, it was not enough to cause turnover in species composition. If the application of Btk resulted in an increase in abundance of less sensitive species, this could potentially lead to changes in the species composition of non-target Lepidoptera. However, the magnitude of these changes and their impact on the overall species composition would depend on the specific species involved and the extent of the changes in their relative abundances. Overall, the relative abundance of some species may have been affected by Btk application; however, the changes were not significant enough to alter the overall community composition.

There were clear differences in the composition of moth species based on the month of collection. However, these findings were not unexpected, as seasonal patterns in diversity are common (Leza et al., 2021) due to differences in individual species phenology. The varying phenologies of moth species can be attributed to differences in their life histories, such as differences in developmental/emergence rates and reproductive timing. As such, the observed differences in species composition based on collection month are likely driven by these underlying differences in life history strategies and highlight the importance of considering temporal dynamics when studying the composition and structure of Lepidoptera communities.

2.6 Conclusions

Application of *Bacillus thuringiensis* subsp. *kurstaki* in the eastern boreal forest of western Newfoundland did not significantly affect moth species community structure in the first few years of the treatment program for spruce budworm in the region. However, non-treated sites did exhibit higher diversity. Southern sites did support a higher abundance of moths, likely due to environmental factors related to the latitudinal differences. I propose that continued treatment in the region over time may reveal changes in the macromoth community and/or that multiple application of Btk in a given stand in the same year may more significantly impact the non-target moth community – a future avenue of investigation. The results of this study suggest that the response of non-target nocturnal macromoths to Btk are somewhat idiosyncratic in that species likely have different thresholds to Btk as witnessed by the changes in relative abundance. The observed variable responses among species are likely attributed to variations in phenology and/or intrinsic sensitivities, thereby warranting further investigation in this area. Although previous research has highlighted the importance of phenology in contributing to differential sensitivities to Btk, the specific life history strategies that allow some species to be more or less sensitive to Btk remains unclear.

Although I did not find any evidence of strong negative effects of Btk on non-target Lepidoptera, it is important to note that the application of Btk in this region has not been occurring long and previous research has highlighted that Btk can impact moth assemblages over time and under varying applications, with some contrasting responses (Boulton et al., 2002; Boulton & Otvos, 2004; Miller, 1990; Rodenhouse & Holmes, 1992; Sample et al., 1996; Wagner et al., 1996). With Lepidoptera providing important ecosystem services such as herbivory, pollination, and being a food source for other species (Hammond & Miller, 1998), it

is imperative that we continue to research and understand the impacts of Btk on non-target communities under different conditions (e.g., application frequency, concentration, different environments and climates etc.). This study highlights the importance of managing forest pests while minimizing unintended consequences on non-target species. This research contributes evidence towards forest pest management strategies with aims to conserve biodiversity of boreal forest ecosystems.

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Appendices

Appendix 1. Raw site data.

Latitude	Longitude	Site Name	Type	Years of Btk Treatment as of 2021	Years of Btk Treatment as of 2022
50.15	-57.63	PCreek	North Control	None	None
50.31	-57.55	WP09	North Control	None	None
50.09	-57.69	S19-13	North Control	None	None
50.12	-57.61	26004	North Treatment	2020 & 2021	2020, 2021, & 2022
50.27	-57.54	23500	North Treatment	2020 & 2021	2020, 2021, & 2022
50.09	-57.63	26002	North Treatment	2020 & 2021	N/A
50.14	-57.62	26002-2	North Treatment	N/A	2020, 2021, & 2022
49.42	-57.73	TR3-2	South Control	None	None
49.43	-57.78	49006	South Control	None	None
49.44	-57.81	49007	South Control	None	None
49.40	-57.83	49078	South Treatment	2021	2021 & 2022
49.37	-57.82	49079	South Treatment	2021	2021 & 2022
49.34	-57.84	49062	South Treatment	2021	2021 & 2022

Appendix 2. Total raw moth abundance for 2021 collection year. Collection days amalgamated per month and sites amalgamated by treatment type. * Indicates groups that could not be identified to species or genus level due to extensive damage.

Species	June				July				August			
	NC	NT	SC	ST	NC	NT	SC	ST	NC	NT	SC	ST
<i>Acronicta fragilis</i>		1	6	1			113	4	4	5	7	2
<i>Acronicta grisea</i>				1			39	1	4		1	1
<i>Acronicta innotata</i>					1		8					
<i>Agrotis ipsilon</i>												1
<i>Agrotis venerabilis</i>										13		
<i>Amphiphyra tragopoginis</i>											2	
<i>Amphipoea americana</i>											1	
<i>Anaplectoides prasina</i>					2	1	3		7	7	26	5
<i>Anaplectoides pressus</i>	14				37	11	60	43		1	7	5
<i>Anticlea vasiliata</i>			4	2				2				
<i>Apamea alia</i>				3		1						
<i>Apamea amputatrix</i>		1										
<i>Apamea cogitata</i>		3			2	2	8	15				1
<i>Apamea commoda</i>									3		2	17
<i>Apamea inebriata</i>								1				
<i>Apamea sordens</i>					1							
<i>Apamea vultuosa</i>		3			7	8	1	9				
<i>Aplectoides condita</i>					4		17	8	4	3	1	
<i>Arctia parthenos</i>					2	2						
<i>Autographa ampla</i>					2		1		1		4	1
<i>Autographa bimaculata</i>									3	3	7	5
<i>Autographa flagellum</i>								8				1
<i>Autographa mappa</i>		1			7	4	15	8				
<i>Autographa precautionis</i>						1						
<i>Autographa pseudogamma</i>	2		1		1			1	1	2	2	2
<i>Besma quercivoraria</i>		1	8	1			1					
<i>Biston betularia</i>			1				1					
<i>Cabera erythemaria</i>			2		1	5	6	3				
<i>Campaea perlata</i>					2	1	21	41	10		2	1
<i>Caripeta divisata</i>	3	59	5	4	33	114	110	52				1
<i>Catocala unijuga</i>									1			1
<i>Cecrita biundata</i>		1			3	2	30	2				
<i>Chrysanympha formosa</i>							1				13	8
<i>Cisseps fulvicollis</i>										1		
<i>Cladara limitaria</i>			15									

<i>Coenophila opacifrons</i>										1		20
<i>Cryptocala acadensis</i>						1	1		9	8	4	6
<i>Cyclophora pendularia</i>	4		7	22	3	2	10	3				
<i>Dargida diffusa</i>			1									
<i>Diachrysia aereoides</i>						2			1		1	
<i>Diachrysia balluca</i>										1	1	
<i>Diarsia jucunda</i>		3			4	4	15	5	2	10	2	5
<i>Diarsia rosaria</i>					3	2		34	1			1
<i>Digrammia neptaria</i>			1									
<i>Drepana arcuata</i>					1	1	4	1	1	1		
<i>Drepana bilineata</i>			3	1	2		3		1		1	
<i>Dysstroma citrata</i>		16	1		33	48	330	251	37	59	254	154
<i>Ecliptopera silaceata</i>	7	2	2		11		6	5	3			1
<i>Ectropis crepuscularia</i>		5	1	5			15	2				
<i>Eosphropteryx thyatyroides</i>												1
<i>Eueretagrotis perattentus</i>					4	1	5	1	1		2	
<i>Eulithis propulsata</i>					1				11		2	2
<i>Euphyia intermediata</i>	3	3	2	1	28	34	10	9			1	
<i>Eupithecia lariciata</i>	4	2	35		4	6		3				
<i>Eupithecia russeliata</i>	15	16	35	17	3	7	1					
<i>Euplexia benesimilis</i>	4	4	3	2	4	3	1	1	14		2	
<i>Eurois stricta</i>						2	5	9	1	1	1	7
<i>Eurois occulta</i>		1				3	9	1	9	5	17	4
<i>Euxoa comosa</i>						1					1	
<i>Feraliacom stocki</i>	6	7	1	4			1					
<i>Habrosyne scripta</i>		25	1		5	27	12	9		2		
<i>Hemipachnobia monochromatea</i>				2								
<i>Homochlodes fritillaria</i>			4	7								
<i>Hydriomena exculpata</i>	6	15	40	30	5	8	37	27		9		
<i>Hydriomena furcata</i>												2
<i>Hydriomena renunciata</i>			7				20					
<i>Hyppa xylinoides</i>		28			28	29	25	12	4	6		
<i>Iridopsis larvaria</i>	1	7		6			2			2		
<i>Lacanobia nevadae</i>			1	2				1				
<i>Lacanobia radix</i>				4								
<i>Lacinipolia olivacea</i>									30	11		1
<i>Lacinipolia renigera</i>					2							
<i>Leucania multilinea</i>					4							
<i>Lomographa vestaliata</i>					1	1		2				
<i>Lophocampa maculata</i>					1							
<i>Lycophotia phyllophora</i>	1	2			16	22	28	52		1		1

<i>Macaria notata</i>				1			4	17	2				
<i>Macaria oweni</i>	1			3	2	12	36	2	1				
<i>Macaria signaria</i>	13	78	63	45	48	193	91	81					1
<i>Melanchra adjuncta</i>				1									
<i>Melanchra pulverulenta</i>		1	1	4	4	2	4	12					
<i>Melanolophia signataria</i>					5								
<i>Mesoleuca ruficillata</i>				2	3			1					
<i>Metanema determinata</i>								1					
<i>Metarranthis duaria</i>				2	20								
<i>Mythimna oxygala</i>						3	1	2	8	2	2		
<i>Nadata gibbosa</i>				1			1	9					
<i>Nephelodes minians</i>										2	2	5	2
<i>Noctua pronuba</i>						1				17	6	1	2
<i>Ochropleura implecta</i>				1	2	5				7			
<i>Oreta rosea</i>								22	5			1	3
<i>Panthea acronyctoides</i>				1				3					
<i>Perizoma alchemillata</i>					8	2	9	8	2				
<i>Pero morrisonaria</i>	2			1	1								
<i>Phlogophora iris</i>		1	2	1	3	1	2	3				1	2
<i>Phlogophora periculosa</i>								6		21	20	18	70
<i>Plagodis phlogosaria</i>	5	1	12	9							1		
<i>Plagodis pulveraria</i>				1	14								
<i>Plusia putnami</i>						2					2	4	
<i>Probole amicaria</i>	3			7	20								
<i>Protoboarmia porcelaria</i>		8	13	3	36	1	34	26	1				1
<i>Pseudeva purpurigera</i>										7	5	12	9
<i>Pseudohermonassa tenuicula</i>									1	1	1		
<i>Pyrrhia exprimens</i>		1											
<i>Rheumaptera prunivorata</i>						3	1						
<i>Scoliopteryx libatrix</i>	1			2	1								
<i>Scopula frigidaria</i>							2						
<i>Selenia alciphearia</i>	2	1	4	16				1					
<i>Sicya macularia</i>										1	1	6	2
<i>Spaelotis clandestina</i>						2				3	2	3	2
<i>Spargania luctuata</i>		1				6	1	2	1				
<i>Spiramater lutra</i>					2	2							
<i>Sutyna privata</i>	3										9		
<i>Sympistis dinalda</i>													7
<i>Syngrapha abstrusa</i>							3		3	4	1	18	
<i>Syngrapha epigaea</i>									1		5	9	5
<i>Syngrapha octoscripta</i>						1	1	2		7	3	12	20

<i>Syngrapha rectangula</i>	1					1	1		9	12	61	9
<i>Syngrapha viridisigma</i>				2	3	1			15	25	109	23
<i>Tacpasia atropunctata</i>	2											
<i>Trichodezia albovittata</i>			1		8	1						
<i>Triphosa haesitata</i>			1									
<i>Venusia cambrica</i>			1		3		18	22				
<i>Xanthorhoe abrasaria</i>				5			19	14				11
<i>Xanthorhoe decoloraria</i>				2			3	2				
<i>Xanthorhoe ferrugata</i>	4	6	10	14	2		13	5				
<i>Xanthorhoe labradorensis</i>		3	2		22	28	36	5				
<i>Xestia badicollis</i>									1	5	135	11
<i>Xestia c-nigrum</i>	2				2				5		2	1
<i>Xestia dilucida</i>								3			1	1
<i>Xestia mixta</i>						6		3		18	30	27
<i>Xestia oblata</i>					4	2		24	1		1	
<i>Xestia perquiritata</i>								1	4	15	44	35
<i>Xestia smithii</i>		1			1		2	2	7	16	24	50
<i>Zale aeruginosa</i>	1	1	1									
Unknown Geometrid*		15	97	74	4	37	28	49	36	12	5	30
Unkown Noctuid*		3	1						2			

Appendix 3. Total raw moth abundance for 2022 collection year. Collection days amalgamated per month and sites amalgamated by treatment type. * Indicates groups that could not be identified to species or genus level due to extensive damage.

Species	June				July				August			
	NC	NT	SC	ST	NC	NT	SC	ST	NC	NT	SC	ST
<i>Acronicta fallax</i>							1					
<i>Acronicta fragilis</i>		1	79	5			2					
<i>Acronicta grisea</i>	5	3	14	8		1						
<i>Acronicta innotata</i>	3		1	1	2		2					
<i>Acronicta insita</i>			1									
<i>Acronicta radcliffei</i>				1								
<i>Agrotis ipsilon</i>									3			
<i>Agrotis venerabilis</i>									3			
<i>Amphiphyra tragopoginis</i>									5	4	4	
<i>Amphipoea americana</i>											3	
<i>Anaplectoides prasina</i>					27	22	31	5	1	1	12	1
<i>Anaplectoides pressus</i>					40	23	25	29	1			
<i>Anticlea vasiliata</i>			2									
<i>Apamea amputatrix</i>									1			
<i>Apamea cogitata</i>					5	1	4	10	8	10	6	2
<i>Apamea commoda</i>		2			75		34	15	3		6	
<i>Apamea impulsa</i>									2			
<i>Apamea sordens</i>	7	2				1						
<i>Apamea vultuosa</i>		1					1	1				
<i>Aplectoides condita</i>	5	8	21	5		5						
<i>Autographa ampla</i>					7	1	3	3				
<i>Autographa bimaculata</i>					2	3	1		1		3	2
<i>Autographa flagellum</i>			1				3		1			
<i>Autographa mappa</i>	2					2	6	1				
<i>Baileya doubledayi</i>						1						
<i>Besma quercivoraria</i>	3	3										
<i>Biston betularia</i>			1	1								
<i>Cabera erythemaria</i>			1	8		1						
<i>Campaea perlata</i>					19	18	8	13				
<i>Caripeta divisata</i>	12	34	9	8	5	17	2	3				
<i>Catocala blandula</i>										1	5	
<i>Cecrita biundata</i>		2	2	1		4	2			1		
<i>Chersotis juncta</i>							1					
<i>Chrysanympha formosa</i>							55	60				2
<i>Cisseps fulvicollis</i>					3	1						

<i>Cryptocala acadensis</i>					27	41	3	4	1	1				
<i>Ctenucha virginica</i>					1	2								
<i>Cyclophora pendularia</i>			4	16					1					
<i>Diachrysis aereoides</i>					3	14	2		1	1				
<i>Diarsia jucunda</i>					37	53	18	31	1					
<i>Diarsia rosaria</i>				6										
<i>Drepana arcuata</i>	1		5	1	1	1								
<i>Drepana bilineata</i>	1	1	4		1		1							
<i>Dysstroma citrata</i>					6	14	75	83	92	241	185	271		
<i>Ecliptopera silaceata</i>	3	2	7	6		2								
<i>Eosphropteryx thyatyroides</i>						2	4		1		4	1		
<i>Eueretagrotis perattentus</i>	1	5				1	2	3						
<i>Eulithis explanata</i>					7	10								8
<i>Eulithis testata</i>									3	5	2	1		
<i>Euphyia intermediata</i>	3	21	2	1		2								
<i>Eupithecia lariciata</i>			8											
<i>Eupithecia russeliata</i>	8	35		24			1							
<i>Euplexia benesimilis</i>	9	30	3	17		1				2				
<i>Eurois stricta</i>					1	3	10	31						
<i>Eurois occulta</i>	2	2	2	1	10	1	11	4	7	2	3	1		
<i>Euxoa comosa</i>									1					
<i>Euxoa ochrogaster</i>									2					
<i>Feralia comstocki</i>	3	2		1		1								
<i>Fishia illocata</i>											4			
<i>Graphiphora augur</i>					9	6	3	2						
<i>Habrosyne scripta</i>	4	6	1		4	7	6	2		1				
<i>Hemipachnobia monochromatea</i>		3		3										
<i>Homochlodes fritillaria</i>			18	7										
<i>Hydraecia micacea</i>												1		
<i>Hydriomena exculpata</i>	8	6	22	46										
<i>Hydriomena renunciata</i>			31											
<i>Hypocoena inquinata</i>						1		3						
<i>Hyppa xylinoides</i>	16	23	3	2	14	3	6	3						
<i>Idia aemula</i>			1											
<i>Iridopsis larvaria</i>		1	1	2		2								
<i>Lacanobia nevadae</i>				4										
<i>Lacanobia radix</i>				3										
<i>Lacinipolia olivacea</i>	1		1		1	2	2		6	4	7	2		
<i>Lacinipolia renigera</i>					2	1								
<i>Lambdina fiscellaria</i>									4	8	6	1		
<i>Leucania multilinea</i>	1						3	2						

<i>Lithomoia germana</i>									5	7	2	5
<i>Lomographa vestaliata</i>			7									
<i>Lophocampa maculata</i>	1			1					1			
<i>Lycophotia phyllophora</i>	1	1										
<i>Macaria anataria</i>					1							
<i>Macaria notata</i>			2	3	1				1			
<i>Macaria oweni</i>			1	5	1							
<i>Macaria pustularia</i>									5			
<i>Macaria signaria</i>	47	124	78	61		45				1		
<i>Maliattha concinnimacula</i>	1		1			1						
<i>Melanchra adjuncta</i>									1			
<i>Melanchra pulverulenta</i>			3	4	8					1		
<i>Melanolophia signataria</i>					2							
<i>Mesoleuca ruficillata</i>					1							
<i>Metanema determinata</i>				1	2							
<i>Metarranthis duaria</i>				3	1							
<i>Mythimna oxygala</i>						12	8	1	3	1		
<i>Nadata gibbosa</i>			14									
<i>Nephelodes minians</i>										1		2
<i>Noctua pronuba</i>						2	4	2	1	9	10	
<i>Ochropleura implecta</i>			1	3		1			1			
<i>Oligia bridghamii</i>										5		
<i>Oligia minuscula</i>										1	2	14
<i>Oreta rosea</i>								18	1			
<i>Palthis angulalis</i>					1							
<i>Panthea acronyctoides</i>			1									
<i>Papaipema harrisii</i>										10	35	5
<i>Papaipema unimoda</i>												1
<i>Perizoma alchemillata</i>			43			2	1					
<i>Pero morrisonaria</i>	3	2	4	9		3						
<i>Phlogophora iris</i>	5	13	1	1								
<i>Phlogophora periculosa</i>						7	48	21	75	1		2
<i>Plagodis alcoolaria</i>				1								
<i>Plagodis phlogosaria</i>	4		3									
<i>Plagodis pulveraria</i>	1		2	2								
<i>Plusia putnami</i>						2	2	1				
<i>Plusia venusta</i>											1	
<i>Probole amicaria</i>	3	3	17	20		1						
<i>Prochoerodes lineola</i>												8
<i>Protoarmia porcelaria</i>	1		16	5	1	22			8			5
<i>Protodeltote albidula</i>			1	14								

<i>Pseudeva purpurigera</i>					10	13	19	2			12	21
<i>Pseudohermonassa tenuicula</i>						1			1			
<i>Pseudothyatira cymatophoroides</i>			1									
<i>Rheumaptera prunivorata</i>							2					
<i>Scoliopteryx libatrix</i>	2		3	1								
<i>Scopula frigidaria</i>			3									
<i>Selenia alciphearia</i>			1	1								
<i>Sicya macularia</i>									3	5	5	13
<i>Spaelotis clandestina</i>		1										
<i>Spargania luctuata</i>	4	3	1	1								
<i>Spargania magnoliata</i>		1										
<i>Sphinx poecila</i>				1								
<i>Spilosoma virginica</i>			3		1	1						
<i>Spiramater lutra</i>	1	2		1		1						
<i>Striacosta albicosta</i>						1			1			
<i>Sutyna privata</i>									1			
<i>Sympistis dinalda</i>							1	1				1
<i>Syngrapha abstrusa</i>					1	4	11	6				
<i>Syngrapha epigaea</i>						6				3	1	8
<i>Syngrapha octoscripta</i>					8		8	3	17	16	4	2
<i>Syngrapha rectangula</i>					42	52	60	17	1	7	20	5
<i>Syngrapha viridisigma</i>					19	8	14	3	5	17	5	3
<i>Trichodezia albovittata</i>		1	3									
<i>Triphosa haesitata</i>										1	2	
<i>Venusia cambrica</i>	1		12	8								
<i>Xanthorhoe abrasaria</i>	1		1				1	7	6			
<i>Xanthorhoe ferrugata</i>		5	1	7								
<i>Xanthorhoe labradorensis</i>	5											
<i>Xestia badicollis</i>									4	20	207	81
<i>Xestia c-nigrum</i>		1			2	1	3	1			1	
<i>Xestia dilucida</i>								1	8	4		8
<i>Xestia mixta</i>						15	2	15				
<i>Xestia oblata</i>							1	11				
<i>Xestia perquiritata</i>				1	14	17	19	14				
<i>Xestia smithii</i>					8	22	4	9	1	4	9	23
<i>Xylotype arcadia</i>										6	4	8
<i>Zale horrida</i>			1									
Unknown Geometrid*	1	7	24	20	39	53	47	62	3			
Unkown Noctuid*												2