Université du Québec en Abitibi-Témiscamingue

IMPACT DES SITES MINIERS VÉGÉTALISÉS AVEC DES PLANTES À FLEURS INDIGÈNES SUR LES COMMUNAUTÉS D'INSECTES POLLINISATEURS DANS LE CONTEXTE FORESTIER BORÉAL

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IMPACT OF MINING SITES REVEGETATED WITH NATIVE FLOWERING PLANTS ON COMMUNITIES OF POLLINATOR INSECTS IN THE CONTEXT OF THE BOREAL FOREST

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DEDICATION

To those who started over during the COVID-19 pandemic, who hit rock bottom and didn’t know how to start over, and to those who went back to university later in life; thank you for daring to do something new. You inspired me.

EPIGRAPH

We delight in the beauty of the butterfly but rarely admit the changes it has gone through to achieve that beauty. – Maya Angelou

FOREWORD

This master's thesis is presented in the form of a manuscript. A forthcoming article will be submitted to the journal "Restoration Ecology" with the authors Shawna Ann O'Flaherty, Marie Guittonny and Julia Mlynarek. I am the principal researcher responsible for the study, for the collection of data, data analysis, and writing the article. My research director, Marie Guittonny, and my research co-director, Julia Mlynarek, conceived of the study and assisted me in the interpretation of the results. They have also assisted with the constructive critique of the content of the article, in addition to this manuscript.

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LIST OF ACRONYMS AND ABBREVIATIONS

COSEWIC: Committee on the Status of Endangered Wildlife in Canada
GOL: Goldex Mine
LAM: Eldoraldo Gold Lamaque Mine
LAP: Lapa Mine
LAR: LaRonde Mine
MAN: Manitou
MCM: Mine Canadian Malartic
HRN: Horne Smelter
NOR 5-1: Noranda 5-1
NOR 5-2: Noranda 5-2
IUCN: International Union for Conservation of Nature
MERN: Ministère de l'Énergie et des Ressources naturelles
MLMP: Monarch Larva Monitoring Project
MRNF: Ministère des Ressources naturelles et des Forêts
UQAT: Université du Québec en Abitibi-Témiscamingue
U.S.: United States
USDA: United States Department of Agriculture

LIST OF SYMBOLS AND UNITS

ppm: Parts per million
dS/M: decisiemens per metre
µg/g: micrograms per gram
µg/L: micrograms per litre

RÉSUMÉ

La végétalisation des sites miniers est une obligation légale au Québec, ainsi que dans plusieurs provinces canadiennes et aux États-Unis. Le but est de retourner un site minier à un état satisfaisant après des efforts de restauration. Les objectifs antérieurs des approches de végétalisation consistaient principalement à reverdir les surfaces et à limiter les effets de l'érosion. Dernièrement, les efforts se sont axés sur la capacité des sols dégradés, ce qui inclut des sols miniers végétalisés, à fournir des services écosystémiques supplémentaires. Ce projet met l'accent sur les services écosystémiques de création d'habitats pour les insectes pollinisateurs en utilisant des plantes à fleurs indigènes pour effectuer la végétalisation des sites miniers. L'étude a été effectuée dans le contexte de l'écosystème de la forêt boréale en Abitibi-Témiscamingue dans l'ouest du Québec.

Premièrement, le projet a examiné l'*Asclepias syriaca*, une des espèces d'asclépiades qui sert de plante hôte pour le papillon monarque, *Danaus plexippus*, une espèce en voie de disparition. Nous avons testé l'hypothèse que *D. plexippus* aura un taux de survie semblable sur les sites miniers végétalisés avec cette espèce et sur des sites témoins en bord de route.

Le deuxième volet est axé sur les communautés d'insectes pollinisateurs présents sur la verge d'or du Canada, *Solidago canadensis*, une plante pionnière polyvalente utilisée sur des sites miniers végétalisés. L'abondance et la diversité d'insectes pollinisateurs ont été comparées sur des sites miniers végétalisés et des sites témoins en bord de route.

Le plan expérimental impliquait 9 dispositifs en blocs complets aléatoires sur 8 sites miniers en Abitibi-Témiscamingue. Huit sites témoins ont été choisis avec *A. syriaca* ou *S. canadensis*. Les suivis sur le terrain ont été effectués pendant les étés 2021 et 2022. Les variables mesurées pour *D. plexippus* et *A. syriaca* incluent : la température, la hauteur des plantes, le nombre de feuilles, le nombre d'œufs et chenilles de *D. plexippus*, ainsi que les concentrations des éléments traces et nutriments dans le sol. Les variables pour *S. canadensis* incluent : la hauteur et densité des plantes, les concentrations des éléments traces et nutriments du sol, ainsi que le nombre d'insectes issus de la capture avec un filet et de la capture passive avec des pièges bols.

Les connaissances sur la présence de syrphes (Syrphidae) et diverses familles d'abeilles (Apidae, Halictidae, Megachilidae, etc.) sont largement concentrées dans les grands centres de population du sud du Québec. Les résultats de cette étude montrent une abondance et une diversité importante d'insectes pollinisateurs sur les sites miniers végétalisés avec la verge d'or du Canada, avec une plus grande abondance sur les sites miniers par rapport aux sites témoins. Le nombre d'individus d'insectes pollinisateurs augmentait de façon linéaire avec le nombre cumulé de tiges de *S. canadensis*. Cette étude apporte de nouvelles informations sur la diversité d'insectes pollinisateurs qui visitent la verge d’or du Canada sur les sites miniers végétalisés dans le contexte de l’écosystème forestier boréal d’Abitibi-Témiscamingue.

La limite nordique de la migration de *D. plexippus* se situe en Abitibi-Témiscamingue. Pendant la saison 2021, des chenilles de *D. plexippus* ont survécu jusqu’aux stades mobiles de développement larvaire (stades 4 et 5) sur 5 des 8 sites miniers investigués, tandis qu’en 2022, la présence de *D. plexippus* a été observée sur 1 site minier. Le facteur avec la plus grande influence sur le nombre d’individus de *D. plexippus* était la hauteur totale cumulée des tiges d’*A. syriaca*, où une taille totale plus élevée de tiges correspondait au plus grand nombre d’individus de *D. plexippus*. Il n’y avait aucune indication que les concentrations des éléments traces et nutriments du sol avaient un impact sur le nombre d’individus ou le taux de survie de *D. plexippus* sur les sites miniers.

Mots clés **:** Végétalisation minière, *Danaus plexippus*, papillon monarque, *Asclepias syriaca*, asclépiade commune, *Solidago canadensis*, verge d’or du Canada

ABSTRACT

The revegetation of mining sites is a legal requirement in many Canadian provinces and American states; the goal is that a mining site may return to a satisfactory state after reclamation efforts. Previous objectives in revegetation practices consisted of greening the space and limiting erosion. More recent efforts have focused on the capacity for anthropogenic soils, including revegetated mines, to provide additional ecosystem services. This project focuses on ecosystem services by creating suitable habitats for communities of indigenous pollinator insects by selecting indigenous flowering plants to use in mine site revegetation. It takes place in the context of the boreal forest ecosystem in Abitibi-Témiscamingue in western Québec.

First, the project examined *Asclepias syriaca*, one of the host milkweed plant species for the endangered monarch butterfly, *Danaus plexippus*. Concentrations of nutrients such as nitrogen were examined in *A. syriaca* leaves and trace elements of heavy metals were measured. I tested the hypothesis that *D. plexippus* will have a comparable survival rate on revegetated mining sites compared with roadside control locations.

The second portion of the project focused on communities of pollinator insects on *Solidago canadensis*, a polyvalent pioneer forb used on revegetated mining sites. To compare feeding habitat quality between mining sites and roadside control sites, and the pollinator insect abundance and diversity was compared.

Nine experimental settings (randomized complete block design) were used on 8 different mining sites in Abitibi-Témiscamingue. A total of 8 roadside control sites were chosen as they have *A. syriaca* or *S. canadensis* growing. Fieldwork was conducted during the summers of 2021 and 2022. Variables measured for *D. plexippus* and *A. syriaca* include temperature, height of *A. syriaca,* number of leaves, the numbers of *D. plexippus* eggs and caterpillars, as well as chemical trace element and nutrient concentrations of soil. *Solidago canadensis* variables included temperature, wind level using the Beaufort scale, height of plants, patch density, trace element and nutrient analysis of the soil, timed focal flower observations of insect visitors, as well as active capture using net sweeps and passive capture using pan traps.

Existing knowledge regarding the presence of flower flies (Syrphidae) and diverse families of bees (Apidae, Halictidae, Megachilidae, etc.) are mostly concentrated in the large population centres of southern Québec. The results of this study show that the abundance and diversity of pollinator insects are important on mine sites revegetated with Canadian goldenrod, with a greater abundance on mine sites compared to the control sites. This study brings new information on the diversity of pollinator insects visiting Canadian goldenrod on revegetated mine sites in the context of the boreal forest of Abitibi-Témiscamingue.

The northern limit of *D. plexippus'* migration lies within Abitibi-Témiscamingue. During the 2021 season, *D. plexippus* caterpillars survived to the mobile stages of larval development (stages 4 and 5) on 5 of the 8 mine sites, while in 2022 survival was observed on 1 mine site. The strongest influence on survival to the mobile stages of development was the pooled total height of *A. syriaca* stems of a plot, with higher pooled height having a higher rate of survival. There was no indication that soil trace element and nutrient analysis impacted survival rates of *D. plexippus* on mine sites.

Keywords**:** Mining revegetation, *Danaus plexippus*, monarch butterfly, *Asclepias syriaca*, common milkweed, *Solidago canadensis*, Canadian goldenrod

INTRODUCTION

Human impacts have modified the landscape through fragmentation, degradation and destruction of natural habitats and the creation of new anthropogenic habitats (Kremen et al., 2007).

Mining extraction and exploitation leave behind a legacy of scarred landscapes stripped of vegetation. An ecosystem that may have existed for millennia is now altered; vegetation was removed and replaced with a hole hundreds of metres deep built with the purpose of resource extraction. When these primary resources are depleted, the mine is decommissioned, and restoration projects are employed to assist in returning the area to a naturalized state. Revegetation is an important part of the reclamation plan of the mining operation, which is a legal requirement in Québec (Ministère de l'Énergie et des Ressources naturelles, 2020, 2022) in order to obtain exploitation permits.

This research project explores mining revegetation efforts underway in Abitibi-Témiscamingue, Québec. This approach is important for scientific efforts as it highlights the value of ecological restoration covered under Goals A and B of the Kunming-Montreal Global Biodiversity Framework for 2050 (Convention on Biological Diversity, 2022). The studied region of western Québec is dominated by the boreal forest natural ecosystem (Figure 1 and 5.2.1 Study sites, Figure 2). It is a sparsely populated region where employment in the primary sectors makes up 14.3% of the region's employment sector (Ministère de l'Économie et de l'Innovation, 2021). Primary mining resource extraction focuses on copper and gold, resulting in a mix of open pit and underground active mines, and decommissioned mines. The experimental revegetated mine sites used in this research include examples of this mix. The mining sites are covered in human-made substrates called technosols, including tailings and waste rock storage facilities (Guittonny, 2020). Sometimes, mineral soil with minimal organic content also needs to be revegetated as well on mine sites or are used to cover the mine wastes. Degraded ecosystems such as mine sites or roadsides often require substantial intervention to compensate for the loss of the natural recovery potential (Gann et al., 2019) towards the reference ecosystem.

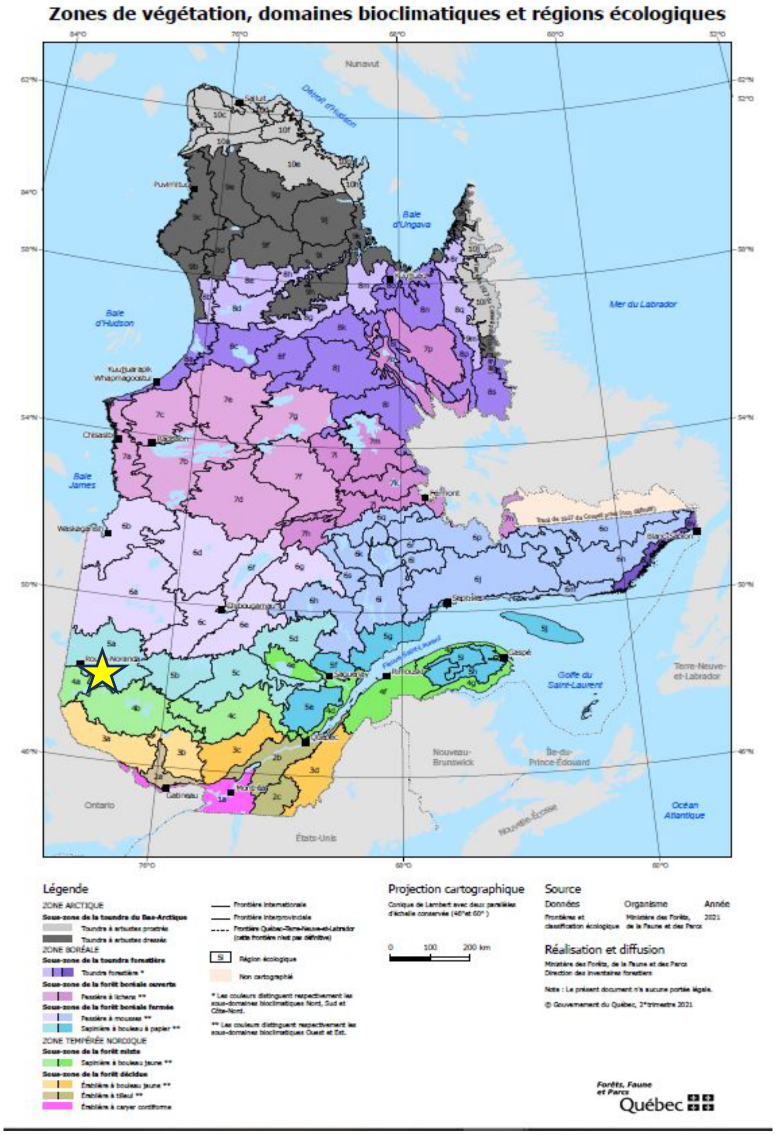


Figure 1
Bioclimatic domains and vegetation zones of Québec
Source: Direction des inventaires & Morneau, 2021. Map of the bioclimatic domains and vegetation zones of Québec. Abitibi-Témiscamingue, where this study was conducted, is situated in subzone 5a: closed boreal forest (Ministère des Forêts de la faune et des Parcs, 2021). Yellow star indicates the localization of the study.

The harsh mineral soil conditions of mining sites (Guittonny, 2020) can potentially be revegetated using native pioneer plants that thrive in hostile growing conditions (Winterhalder, 1995). Flowering pioneer plants can potentially be used to kickstart vegetation growth (Winterhalder, 1995, 1996; Norris et al., 2008; Gann et al., 2019) and attract insect communities (Steffan-Dewenter & Tscharntke, 2001). Two native pioneer plants that thrive in hostile growing conditions such as these are *Asclepias. syriaca* (Committee on the Status of Endangered Wildlife in Canada, 2016; Zalucki et al., 2016) and *Solidago. canadensis* (Holl, 1995; Winterhalder, 1995; Czortek et al., 2020, 2023). These species could serve as nurse plants facilitating the succession of other colonizing plants.

This research is divided into two specific problems:

Can *Danaus plexippus* L. 1758 (monarch butterfly) be found up to and survive until the final stages of larval development on *Asclepias syriaca* L. 1753 (common milkweed) that was used to revegetate mine sites?

Are pollinating insect families (bees and hoverflies) visiting *Solidago canadensis* L. 1753 (Canadian goldenrod) on revegetated mine sites?

Declining population numbers of pollinator insects have been documented since the 1990s (Allen-Wardell et al., 1998; Kearns et al., 1998). The loss of pollinator insects has the potential to fundamentally disrupt biological communities and impair ecosystem functions locally, regionally or even globally (Zylstra et al., 2021). This loss from a biotic community may not be easily reversible (Allen-Wardell et al., 1998). Pollinator insect population declines impact pollination ecosystem services. This loss of biodiversity has far-reaching and long-lasting implications on global food security. At least 87 of the world's major food crops critically depend on insect pollination, which accounts for 30-35% of the world food production volume (Kremen et al., 2007; van der Sluijs, 2020). These crops include a range of vegetables, fruits, nuts, edible oils and proteinaceous crops such as legumes, among others (van der Sluijs & Vaage, 2016). There are approximately 5.5 million insect species globally and approximately 90% have not been named, nor have their roles in ecosystems been mapped (van der Sluijs, 2020) – the potential biodiversity loss of pollinator insects is staggering.

Pollination services are a crucial ecosystem service provided by wild, free-living organisms (chiefly melittophily by bees, but also psychophily by many butterflies and moths, myophily by flies, entomophily by beetles and wasps, and pollination by selected other invertebrates, birds and mammals), and by commercially managed bee species – primarily *Apis mellifera* L. 1758. Managing pollination services requires planning for mobile organisms and a consideration of both the local scale where the services are delivered, as well as a larger landscape scale that reflects the spatial distribution of resources, as well as the foraging and dispersal movements of the organisms themselves (Kremen et al., 2007). The disruption of pollination systems, and declines of certain types of pollinators, have been reported on every continent except Antarctica (Kearns et al., 1998). The decline of pollinator insects impacts the world economy, global health, and the delicate balance of the interconnected networks of every species.

One such vulnerable pollinating insect species is *Danaus plexippus*. Fluctuating population figures (International Union for Conservation of Nature, 2022; Walker et al., 2022; International Union for Conservation of Nature Standards and Petitions Committee, 2023) of the emblematic eastern subspecies *D. plexippus* landed this once iconic lepidopteran on the International Union for Conservation of Nature's (IUCN) Red List as a vulnerable species after the most recent amended version of the IUCN Red List in September 2023 (Walker et al., 2022; International Union for Conservation of Nature Standards and Petitions Committee, 2023). The decline of this species is due to a range of factors, including loss of larval host plants (Holl, 1995), habitat decline (Thogmartin, Wiederholt, et al., 2017), deforestation in the overwintering range in

Central America, exposure to bacteria, herbicides and insecticides, predation (Wilcox et al., 2019) and the increasing prevalence of parasites such as *Ophryocystis elektroscirrha* (OE) (Pocius et al., 2021).

The goal of this thesis was to determine if revegetated mine sites can sustain pollinator insect communities, where pioneer flowering plants could serve as their breeding and feeding habitats. This research project examined whether mining sites revegetated with indigenous flowering plants are a suitable habitat for 1- the establishment and survival of the monarch butterfly and 2- the broader pollinator insect communities.

1. LITERATURE REVIEW

1.1 Ecosystem services

Revegetation projects can be classified under the umbrella concept of ecosystem services restoration (Costanza et al., 1997; Vanbergen & Initiative, 2013). According to Costanza et al. (1997), ecosystem services or functions can be defined as the overall ecosystem functions that refer variously to habitat, biological, system properties or processes of ecosystems. Ecosystem goods include food and waste assimilation functions which human populations benefit from, either directly or indirectly (Costanza et al., 1997). Approaching ecology issues through the lens of ecosystem services has become a population approach in assigning cultural and ecological values (Kremen et al., 2007; M'Gonigle et al., 2015; Kütt et al., 2016; Sybertz et al., 2017) defending seemingly intangible benefits of the ecosystem.

Ecosystem services provided by insects are wide ranging and the economic benefits are not always measurable in dollar amounts. Insect ecosystem services range from dung burial, parasites and pest fly control, and pollination services by native insects (Losey & Vaughan, 2006; Kremen et al., 2007; van der Sluijs, 2020). There is also the potential for spillover onto crops, where bees initially attracted to wildflowers planted adjacent to farmland will also potentially forage on neighbouring crop flowers, increasing revenue for farmers through greater pollination, particularly if the wildflower plantings are established in areas unsuitable for crops (Perkins et al., 2024).

Pollination services by native insects are often lumped together with managed bee species such as *A. mellifera*, *Megachile rotundata* (Fabricius 1987) and various *Bombus* spp. Thus the proportion of crops pollinated by native species and variation between cultivars of the same species become harder to quantify (Losey & Vaughan, 2006).

1.2 Revegetation of mining sites in Québec

Current provincial regulations within the province of Québec (Beaulieu, 2021) include legal frameworks that govern redevelopment and restoration work on mining sites. Notably, restoration work must begin within three years of the cessation of mining operations with the aim of returning the site to a satisfactory condition (Ministère de l'Énergie et des Ressources naturelles, 2022). A closure plan, including revegetation works, must be developed before a mining lease permit (Ministère des Ressources naturelles, 1997; Ministère de l'Énergie et des Ressources naturelles, 2016, 2022) for exploitation is granted. MERN (2022) defines satisfactory conditions as the following:

- Eliminating unacceptable health hazards and ensuring public safety;
- Limiting the production and spread of contaminants that could damage the receiving environment and, in the long term, aiming to eliminate all forms of maintenance and monitoring;
- Returning the site to a condition in which it is visually acceptable (Ministère de l'Énergie et des Ressources naturelles, 2022) (visually acceptable is not further defined by the MERN 2022 guidelines);
- Returning the infrastructure areas (excluding the tailings impoundment and waste rock piles) to a state that is compatible with future use (rehabilitation as defined by Chapin and Young (Chapin et al., 2012; Young et al., 2022)).

According to Tordoff et al. (2000), it is only by using vegetation to stabilize mine wastes that complete long-term rehabilitation be achieved. Successful revegetation can be an inexpensive, permanent, and visually attractive solution. Restoration projects following mining usually considers vegetative cover as a means of stabilizing the substrates (Martínez-Ruiz & Fernández-Santos, 2005) and preventing erosion from occurring. Older revegetation frameworks from Tordoff et al. (2000) recommend the use of native plant species in the revegetation plan, as these species are ecologically adapted to the local climatic conditions. More recent laws in Québec (Ministère de l'Énergie et des Ressources naturelles, 2022) stipulate that the revegetated area can control erosion; that once planted, vegetation must be hardy, viable in the long term, and able to grow without the use of fertilizer or maintenance. The guidelines for mine closure in Québec further recommend the use of indigenous plants, herbaceous plants or shrubs (Ministère de l'Énergie et des Ressources naturelles, 2022).

1.3 Constraints related to mining soils

There is considerable heterogeneity among the different types of mine sites in Abitibi-Témiscamingue, with each type of site presenting unique challenges and constraints for the revegetation on different substrates (Guittonny, 2020). Metalliferous mining waste typically consists of either very coarse waste rock with a diameter of 2-20 cm or tailings consisting of fine-grained deposits (<2 mm) from the final stage separators in the plant processing the ore (Tordoff et al., 2000). Metalliferous mine wastes generally cover large surface areas and lack the building blocks of vegetation: organic matter, nutrients and soil organisms (Burger & Zipper, 2002; Larchevêque et al., 2014). In addition to lacking organic composition, mining soils generally lack surface roughness (Macdonald et al., 2015) that contribute to physical structure (Larchevêque et al., 2014) necessary for the establishment of plant life, can exhibit extreme pH values (Canadian Council of Ministers of the Environment, 2007), as well as possibly contain levels of salts and heavy metals that may be beyond phytotoxic thresholds (Tordoff et al., 2000; Larchevêque et al., 2014). Furthermore, metals and metalloid elements can be detected in foliar tissues of plant species growing on the wastes (González & González-Chávez, 2006). The LaRonde site consist of waste rock material while the Manitou and Lamaque Eldorado Gold sites consist of tailings.

High levels of salinity are characteristic of gold mine tailings (Tordoff et al., 2000; Fashola et al., 2016; Guittonny, 2020). Tailings soil is further characterized by other poor soil physical properties such as poor aggregation, low hydraulic conductivity, homogeneous fine texture and limited cohesive ability leading to a high moisture content (Fashola et al., 2016). In addition to the natural weathering of minerals due to atmospheric conditions, the salinity of tailings can be increased due to chemical additives used in the metal extraction process (Guittonny, 2020) and thus requires selection of plants tolerant to elevated salinity (Norris et al., 2008). The Lamaque Eldorado Gold site is an example of a tailings facility in this study, while the Manitou site is a revegetated site on tailings material.

Arsenic is a toxic element commonly found in mining wastes (Cooke & Johnson, 2002; Fashola et al., 2016), and until the 1970s it was associated with the manufacturing of pesticides (Kabata-Pendias, 2011). Arsenic minerals and compounds are also readily soluble (Kabata-Pendias, 2011); the high temperature processing of metals during smelting can emit metals such as As in both particulate and vapour forms (Nagajyoti et al., 2010). The presence of As in deposits is closely associated with several metals and metalloids and generally recovered from sludge and flue dust during the smelting of several heavy metals, including copper, zinc, lead, gold and silver ores (Kabata-Pendias, 2011); this study includes an experimental site on the grounds of the Horne Smelter that is not part of the active smelting operations.

1.4 Pioneer plants

The first group of plant species to colonize an ecosystem during primary succession (Chazdon, 2008) is pioneer plants. Primary succession is an ecological concept (Clements, 1916) that can be defined as the species change on substrates where the disturbance has left a scant biological legacy (Clements, 1916; Walker & Moral, 2009), while secondary succession occurs on more fertile and stable substrates (Walker & Moral, 2009). Without human assistance, primary succession on raw substrates can take several centuries to complete (Bradshaw, 2000).

The role of pioneer plants is to stabilize the soil and ameliorate its condition by adding a first layer of organic soil over bare rock or hardpan, improve soil pH levels, and increase its nitrogen content (Connell & Slatyer, 1977). Much of the existing body of literature on mining revegetation focuses on pioneer tree species such as *Salicacae* spp, *Betula* spp and *Populus* spp (Norris et al., 2008; Larchevêque et al., 2014; Boulanger et al., 2017; Guittonny-Larchevêque & Lortie, 2017). On the disturbed landscape of volcanoes, pioneer stages of succession tend to be dominated by perennial herbs rather than lower plants: colonization occurs by seed dispersal of forbs (Tsuyuzaki & del Moral, 1995).

Species selection for assisted primary succession should take into account the goals of restoration, the position of the disturbed sites on environmental gradients, and possible sources of diaspores of species (Prach & Hobbs, 2008) of a disturbed site, or the reference ecosystem (Gann et al., 2019).

An additional benefit of primary succession plants is their potential for phytoremediation of metal mine wastes (Cooke & Johnson, 2002). Phytoremediation makes use of the uptake, accumulation and breakdown of contaminants in plants (seedlings, grasses or shrubs), (Song et al., 2019) and their associated microbiota, possibly using soil amendments, or agronomic techniques (Wong, 2003).

1.4.1 Pioneer plants: *Asclepias syriaca*

A pioneer plant native to western Québec (Flockhart et al., 2013) that can thrive in hostile growing conditions is *Asclepias syriaca* (Winterhalder, 1995), and the plant also performs well in more natural environments. The plant thrives in anthropogenic soils such as those found on mining sites or roadside habitats (Kasten et al., 2016; Mitchell et al., 2020). *Asclepias syriaca* tolerates salt concentrations up to 2500 ppm, soil pH ranging from 2 to 12, and most soil textures (Evetts & Burnside, 1972). It is most prevalent in well-drained, loamy soils and in areas with 30% to full sunlight (Berkman, 1949).

In more natural settings, *A. syriaca* can be found in farmers' fields and pastures, as well as roadsides, backyard gardens, and natural areas such as state, provincial and national parks as well as other nature preserves (Kasten et al., 2016). The plant's range is from Texas to Saskatchewan, central Canadian provinces through Québec and the Maritimes (not Newfoundland) and south to Georgia (Stevens, 2000). *Asclepias syriaca* is invasive outside of its range, such as in European grasslands (Kelemen et al., 2016; Bakacsy & Bagi, 2020). *Asclepias syriaca* colonization can be facilitated by anthropogenic disturbances of the soil, and thrives in well-drained sandy or sandy-loess soils (Bakacsy & Bagi, 2020) whose shoots die back every autumn, but due to the perennial clonal nature of the plant, it can resprout in the same place for extended periods thanks to rhizomatic roots (Bakacsy & Bagi, 2020).

Climate change is impacting the flowering date of *A. syriaca* (Howard, 2018), causing earlier incidences of inflorescence. Howard (2018) demonstrated that each average degree Celsius of temperature increase saw a mean flowering date decrease by 3.93 days between 2011-2016. Many plant species' flowering times have occurred earlier in the year, which has many negative consequences for the plants including phenological mismatches and temporal shifts between their blooming periods and their pollinators' flight periods (Howard, 2018; Freedman et al., 2020; Boyle et al., 2023; Mohl et al., 2023).

Asclepias syriaca began experiencing an overall decline starting in 1970, particularly on agricultural land (Hartzler & Buhler, 2000; Hartzler, 2010; Pleasants & Oberhauser, 2013; Pleasants, 2017; Boyle et al., 2019). The plant was on the Ontario Noxious Weed list from 1990-2014 (Cowbrough, 2014; Lalonde et al., 2022), which mandates that plants identified as noxious weeds in the province of Ontario be destroyed if they negatively impact agricultural lands (Lalonde et al., 2022). *Asclepias syriaca,* along with all species of *Asclepias,* was removed from the Weed Control Act in 2014 on the basis of the plant's use as an important habitat and larval food source for *Danaus plexippus* (Cowbrough, 2014).

In statistical modelling by Boyle et al. (2019), every selected model of *A. syriaca* abundance was negatively correlated with the number of farms, declining over the period 1950 to 2006 as smaller farms consolidated in favour of larger scale monoculture commercial farms. During that time, *A. syriaca* declined and was largely eradicated from Midwestern U.S. croplands largely due to widespread herbicide use (Lalonde et al., 2022). Various *Asclepias* spp. are the host plants for the egg and larval stages of *D. plexippus* (Urquhart, 1960; Walker et al., 2022) and its decline has implications on declining pollinator insect numbers (Allen-Wardell et al., 1998; Kearns et al., 1998) as a wide range of pollinator insects consume *A. syriaca*.

There appears to be a negative correlation between the number of lanes in the road and the density of adjacent *A. syriaca* clusters (Lalonde et al., 2022). There are multiple factors influencing *A. syriaca* density and abundance: usage frequency, traffic volume, higher human population density, higher pollutant concentrations and herbicide applications as a potential predictor of milkweed density (Lalonde et al., 2022). The distribution and abundance of milkweeds in randomly surveyed sites (Kasten et al., 2016) suggest that these habitats are important for monarchs, but that roadside milkweeds have lower per plant use than milkweeds in other habitats (Kasten et al., 2016).

Asclepias syriaca bioaccumulates metals in the plant material. Cadmium ions bioaccumulate in the foliar parts of *A. syriaca* (Stingu et al., 2012), and the most commonly found toxic elements in mining wastes are arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, and zinc (Cooke & Johnson, 2002; Fashola et al., 2016).

1.4.2 Pioneer plants: *Solidago canadensis*

The pioneer plant *Solidago canadensis* is known for its ability to grow in disturbed anthropogenic soils (Melville & Morton, 1982) with elevated levels of trace metals (Królak et al., 2020). *Solidago canadensis* bioaccumulate metals in the plant material, and it is known to bioaccumulate lead and zinc in both the underground and aboveground parts of the plant (Bielecka & Królak, 2019).

A native Asteraceae plant indigenous to North America (Ustinova & Lysenkov, 2020), *S. canadensis* can be found in great abundance in Ontario and Québec across the territory in all but the far north of both provinces, and it displays a wide range of polymorphism in morphological characteristics and genetic diversity (Melville & Morton, 1982). In successful habitats, it colonizes early during plant succession (Hartnett & Bazzaz, 1983). *Solidago canadensis* can clonally reproduce from rhizomes and buds at the base of the stem (Huang et al., 2007). In such plants, an extensive dormant bud bank can be activated, resprouted and made able to colonize an empty niche or re-establish monospecific strands after a fresh disturbance (Bakacsy & Bagi, 2020). It is to be noted that outside of its North American natural range, in Europe and Asia, *S. canadensis* has highly invasive characteristics and is considered an invasive exotic species (Czortek et al., 2020).

Solidago canadensis is a widely documented early-successional plant species (Reed & Hall, 1993; Milam et al., 2018) commonly found along roadsides in addition to other disturbed habitats (Heinrich, 1976) and may function in habitat filtering, niche portioning, and with river buffer zones (Czortek et al., 2023).

More recent distribution data from the USDA PLANTS Database (Pavek, 2012) shows that *S. canadensis* is widely distributed in every Canadian province and territory except for Nunavut (Pavek, 2012), located in the north of the country. Melville and Morton (1982) listed sites located in Nipissing, Timiskaming, and Cochrane districts which straddle the Ontario side of the Ontario/Québec border along the Cadillac Fault Zone (Dubé et al., 2007; Bedeaux et al., 2017).

Solidago canadensis has shown promise as a pioneer plant on reclaimed mine soil (Holl & Cairns Jr., 1994; Holl, 1995) and is associated with higher plant-soil nutrient concentrations in nutrient-poor environments (Ye et al., 2019) is akin to the conditions found on revegetated mine sites. The perennial herb *S. canadensis* reproduces both sexually by seeds and vegetatively by rhizomes (McBrien & Harmsen, 1987). Individual clones of *S. canadensis* form dense clusters, composed of many ramets, depending on the age of a given clone (Bielecka & Królak, 2019). It often occurs in dense monospecific colonies, probably because of its high growth rate and its ability to spread locally by means of rhizomes (Groot et al., 2007).

Solidago canadensis propagates through wind-dispersed seeds (Bhowmilk & Bandeen, 1976) and asexually through its rhizomes. Rhizome connections remain intact for up to four seasons, becoming thicker and slightly woody with age. Clonal genets can be composed of one or several units with 10-20 morphologically interconnected ramets (Hartnett & Bazzaz, 1983). The optimal habitat for *S. canadensis* has fertile, moist clay soil with elevated nitrogen concentrations (Lu, 2005; Chen et al., 2013; Królak et al., 2020). Previous research on nectar resources and their influence on butterfly communities on reclaimed coal surface mines in Virginia noted the presence of *S. canadensis* was commonly found on reclaimed sites 5-12 years post reclamation (Holl, 1995).

1.5 The nature of revegetated mining sites vs. roadsides – potential ecological traps

The term "ecological trap" refers to negative outcomes of inappropriate habitat selection (Schlaepfer et al., 2002), where an environment has been altered suddenly by anthropogenic activity, leading to an organism making a maladaptive habitat choice based on formerly reliable environmental cues, despite the availability of a higher quality habitat (Schlaepfer et al., 2002). The concept of an ecological trap (Dwernychuk & Boag, 1972) refers to a habitat that is low in quality for reproduction and survival that cannot sustain a population. However it is used by the species over other available, higher quality habitats (Donovan & Thompson, 2001; Battin, 2004).

All types of disturbed habitats have the potential to be considered ecological traps that attract living organisms, however, due to their poor quality, they can negatively affect an organism's fitness, survival, and establishment. Mine sites could be an ecological trap due to the chemical composition of their soils and the human selection of primary succession plants used for revegetation. Another type of ecological trap is roads and their associated traffic; creating habitat fragmentation, mortality from collisions, increased human access to remote areas, and altered chemical ecology (Trombulak & Frissell, 2000; Gardiner et al., 2018; Mitchell et al., 2020).

The body of literature examining the presence of arthropods, invertebrates or other animals on revegetated mine sites is currently sparse. A 2019 review article found that most restoration articles focus on replanting native plant species or by removing invasive plant taxa (Hale et al., 2019). One study from the Sokolovsko region of the Czech Republic determined that the naturally restored post-mining areas from strip coal mines in Sokolovsko were an ecological trap for the insect order Odonata (dragonflies), with a high degree of variability in survival rates noted (Poskočilová et al., 2024).

Another example of an ecological trap for an arthropod is in a wetlands-restricted population of *Lycaena xanthoides* Boisduval (Severns, 2011), a butterfly found in western Oregon, USA. Severns (2011) found that the host plants *Rumex salicifolius* Weinm. selected for ovipositing shared similar habitat and attributes as plants intentionally seeded into the seasonally flooded restored wetlands, while a logistic regression revealed a strong relationship between oviposition probability and the surrounding vegetation to the host plant height ratio. The study concluded that the ecological trap was likely attributed to simultaneous devaluing of optimal reproductive habitat by exotic grass invasion and re-association of host plant appearance cues to inaccurately replaced host plants to seasonally flooded conditions (Severns, 2011).

As the literature on revegetated mine sites as ecological traps is sparse, we are comparing this type of ecological trap with a more thoroughly researched type of disturbed habitat: the common disturbance of roadside sites. The disturbances associated with roadsides can negatively impact plant populations in many ways. For example, dust deposition, nitrogen, chemical pollutants such as chromium and altered pH can modify the soil and water used by roadside plants (Forman et al., 2003; Kabata-Pendias, 2004; Coffin, 2007; Kabata-Pendias, 2011; Snell-Rood et al., 2014; Lalonde et al., 2022) which could make some roadside sites a hostile environment comparable to the mining sites we are examining. Roadsides are a site of natural colonization of *A. syriaca* and *S. canadensis* in the Abitibi-Témiscamingue region, and one of the few places where natural populations of *A. syriaca* can be found in the region. Although the roadside sites are also disturbed environments, they were the best available control sites we found for the study.

1.6 Danaus plexippus

The monarch butterfly (*D. plexippus*) is an easily identifiable species because of its bright orange and black wings, making *D. plexippus* a charismatic megafauna contender (Preston et al., 2021) in the insect world.

There are five distinct populations of *D. plexippus* in the Americas with different overwintering areas – however there is no discrete genetic differentiation or morphology differences between these populations (Freedman et al., 2021). These groups are comprised of the Eastern North American, Western North American, Southern Florida, Cuban and Central American populations. The Canadian population is comprised of two mostly disjunct populations: the large and widely distributed Eastern migratory population, which is the focus of this research, and the smaller Western population (Environment and Climate Change Canada, 2016). The migratory nature of *D. plexippus* sees adults migrating to central Mexico to overwinter, returning to Eastern Canada as far north as 50° latitude in Ontario, Québec and the Maritimes (Committee on the Status of Endangered Wildlife in Canada, 2016).

1.6.1 Development of *Danaus plexippus*

Danaus plexippus caterpillars feed on *Asclepias* spp., a genus of host plants high in cardenolides. Cardenolides are bitter tasting and can disrupt the ionic balance of a variety of animal cell types. *Danaus plexippus* instars and adults sequester cardenolides as a defence against predators – in fact monarchs are most effective at sequestering cardenolides from plants with low cardenolide content of less than 100 µg/0.1 g dry leaf weight (Malcolm, 1994).

All life stages of *D. plexippus* are temperature dependent (Barker & Herman, 1976). Warming spring and summer temperatures (above 12ºC) increase the developmental rate (Barker & Herman, 1976), although extreme heat is detrimental and even lethal (York & Oberhauser, 2002; Pocius et al., 2021).

Table 1
Life cycle of *Danaus plexippus*

Life stage	Size and appearance	Stage duration
Egg	- 1.2 mm length by 0.9 mm width - Off-white or yellow, marked by longitudinal ridges - Attached to the underside of leaves - Immobile	3 to 8 days
1st instar	- 2-6 mm length by 0.5-1.5 mm width - Pale green to greyish white, shiny and almost translucent - Remains on initial plant (immobile)	1-3 days
2nd instar	- 6-9 mm length by 1-2 mm width - Clear pattern of black or dark brown, yellow and white bands are visible - Remains on initial plant (immobile)	1-3 days
3rd instar	- 10-14 mm length by 2-3.5 mm width - Discernable tentacles on the front and rear - Remains on initial plant (immobile)	1-3 days
4th instar	- 13-25 mm length by 2.5-5 mm width - Distinct banding on thorax, first pair of legs visibly closer to head	1-3 days

	- Mobile, will leave birth plant for others nearby	
5th instar	- 25-45 mm length by 5-8 mm width - Vivid colours, black bands appear almost velvety - Mobile, will begin searching for a place to form chrysalis	3-5 days
Chrysalis	- Bright green, turning translucent before eclosure	9-15 days
Adult butterfly	- Bright orange with black lines. Males have a black dot on the lower portion of the wing. - Females lay 300-400 eggs during the life cycle	2-5 weeks, except for the generation overwintering in Mexico that survives up to 9 months

Source : The life cycle in Table 1 above is adapted from Monarch Joint Venture (2020) and Oberhauser & Kuda (1997), and describes the morphological and duration characteristics of each life stage.

1.6.2 Declining populations of *Danaus plexippus*: fluctuating conservation status

The conservation status of *D. plexippus* has experienced a great deal of fluctuations over the last few decades, much like the species' population numbers. There is no consensus on what the most accurate status is, according to conservation authorities and researchers across Canada, the United States and Mexico.

In the United States, *D. plexippus* is listed as vulnerable to extinction (Jepsen et al., 2015; U.S. Fish and Wildlife Service, 2020). The United States Fish and Wildlife Service last assessed the species in 2020 (U.S. Fish and Wildlife Service, 2020), and determined that listing the *D. plexippus* under the Endangered Species Act is warranted but it was precluded at that time by higher priority listing actions. *Danaus plexippus* is a candidate for future listing and, does not have U.S. federal protection at the time of writing.

The migratory subpopulation of the monarch butterfly, *Danaus plexippus* ssp. *plexippus* made international headlines when it was listed as Endangered on the Redlist of endangered species by the IUCN in July 2022 (International Union for Conservation of Nature, 2022; Walker et al., 2022). An amended version of this report was released in 2023, relisting the species as Vulnerable (International Union for Conservation of Nature Standards and Petitions Committee, 2023).

The Committee on the Status of Endangered Wildlife (COSEWIC) in Canada listed *D. plexippus* as endangered in 2016 (Committee on the Status of Endangered Wildlife in Canada, 2016; Environment and Climate Change Canada, 2016; Committee on the Status of Endangered Wildlife in Canada, 2017, 2022) and maintained that status as of the most recent assessment in 2022.

The overwintering sites for the migratory population of *D. plexippus* are situated in central Mexico along the border of Michoacán and the State of Mexico in an area known since 2000 as the Monarch Butterfly Biosphere Reserve (Rendón-Salinas, Alonso, et al., 2023; Rendón-Salinas et al., 2024). The reserve protects an area of 56 259 ha, of which 13 551 ha were designated as the core zone of overwintering *D. plexippus* (Rendón-Salinas, Alonso, et al., 2023; Rendón-Salinas, Fernández-Islas, et al., 2023).

There are several hypotheses to explain declining population numbers of *D. plexippus*. Wilcox et al. (2019) clearly lay out the broad threats into five main categories in their paper: (1) change in suitable abiotic environmental conditions; (2) deforestation in the overwintering range; (3) exposure to contaminants including the bacteria *Bacillus thuringiensis*, herbicides and insecticides; (4) loss of breeding habitat; (5) predation, parasitism, and species-specific pathogens such as OE. Threats one and four are addressed in the research of this thesis.

Change in suitable abiotic environmental conditions suggests that adverse weather patterns or events have a significant negative impact on *D. plexippus* (Brower et al., 2017; Hunt & Tongen, 2017; Wilcox et al., 2019), creating conditions that can impact *D. plexippus* at each stage of their life cycle (Hunt & Tongen, 2017). This may drive the northward expansion of the breeding range past the existing limit (Batalden et al., 2007; Lemoine, 2015; Wilcox et al., 2019; Hemstrom et al., 2022) with positive impacts on the total number of *D. plexippus*. It can also impact the Mexican overwintering grounds by raising temperatures beyond the conditions suitable for the annual diapause period of 4-5 months (Oberhauser & Peterson, 2003; Wilcox et al., 2019).

The loss of breeding habitat was a broad explanation for decreasing numbers of *D. plexippus* (Wilcox et al., 2019) that this thesis addresses. A loss of ≥ 1.3 billion stems of milkweed (various *Asclepias* spp.) was recorded in Canada, the U.S. and Mexico since 1999 (Pleasants, 2017; Thogmartin, López-Hoffman, et al., 2017), largely attributed to diminishing quantities in agricultural fields. For much of the twentieth century, a large majority of monarchs produced in the eastern U.S. likely originated from milkweed grown in agricultural fields. However, since the introduction of herbicide-tolerant corn and soybeans, milkweed has largely disappeared from these fields (Thogmartin, López-Hoffman, et al., 2017). The breeding habitat loss of milkweed (Wilcox et al., 2019) has detrimental impacts to *D. plexippus* as an unintended consequence. The presence of larval host plants such as various *Asclepias* spp. is necessary to the survival of *D. plexippus*, but this is only one factor in a suite of factors responsible for their survival, including additional nectar resources, that may determine habitat suitability (Holl, 1995). There needs to be enough potential food sources for adult *D. plexippus* to survive.

1.6.3 Finding *Danaus plexippus*

Migration timing of *D. plexippus* appears to be shifting in recent decades, hinting that there may be a resource mismatch in crucial species interactions (Culbertson et al., 2022). Between 1992 and 2020, the migration midpoint, average peak migration day and first peak migration day shifted between 16 and 19 days earlier in the season, an average of approximately six days per decade. This is problematic, as *D. plexippus* are known migratory animals that use both temperature and light-based triggers to determine migration departure and pace. This suggests that warmer global temperatures may affect their migratory behaviour (Culbertson et al., 2022). While both the *A. syriaca* and *D. plexippus* seem to have advanced their spring phenology, there appears to be a disruption to the match between the timing of its flowering and its pollinators flight season. Other pollinator insects may be able to benefit from the earlier flowering dates of *A. syriaca* in this changing phenology, but given the preference for *D. plexippus* ovipositing on younger *A. syriaca* stems (Haan & Landis, 2019) and overall higher survival rates on younger plants (Pocius et al., 2021), the phenology of the two species may no longer align in the near future.

Detecting the presence of larval host plants for *D. plexippus* is thought to be more difficult when surrounded by a high diversity of other plants (Pitman et al., 2018). Plant age influences *Asclepias* spp. attractiveness to ovipositing females. *Danaus plexippus* caterpillars often perform better on younger plants compared to those near senescence (Scriber & Slansky, 1981; Slansky Jr, 1993; Pocius et al., 2021). Furthermore, young *Asclepias* spp. or newly regenerated stems may also harbour fewer natural enemies of young larvae (Pocius et al., 2021). Monarchs prefer to lay their eggs on young *Asclepias* spp. stems (Haan & Landis, 2019), taller stems with intermediate cardenolide content (Cohen & Brower, 1982; Agrawal et al., 2021), and produce the highest egg counts in areas that contain a mix of milkweed species (Pocius et al., 2018; Pocius et al., 2021).

Density monitoring of *D. plexippus* ovipositing on roadside locations of *A. syriaca* was measured by Kasten et al. (2016). They found that female *D. plexippus* were using roadside habitats with *A. syriaca* to oviposit, but there was a lower per plant use on roadside plants compared to more naturalized areas known to contain milkweed (Kasten et al., 2016), supporting the ideal free distribution theory in relation to habitat availability (Fretwell & Lucas, 1969; Dreisig, 1995). The methodology employed by Fisher and Bradbury (2021) in their study on the influence of habitat quality found that *D. plexippus* butterflies were typically observed feeding within high- and low-density habitats, and most often observed resting in areas without resources. A high-density habitat was classified as any site (mosaic or roadsides) with a density of > 5 resources/m^2, while a low-density habitat was classified as any site (mosaic or roadsides) with a density of milkweed and blooming inflorescences < 3 resources/m^2 (Fisher & Bradbury, 2021). The mosaic was defined as a patchwork of habitat classes within 64-ha (Fisher & Bradbury, 2021).

Additionally, parasitic tachinid flies (Diptera: Tachnidae) are known to parasitize (Wilcox et al., 2019) the larval stages of *D. plexippus* (Oberhauser et al., 2017), with Oberhauser's 2017 study documenting parasitism by tachinids across life stages at 9.8%, and 17% of *D. plexippus* collected as fifth instars (Oberhauser et al., 2017; International Union for Conservation of Nature Standards and Petitions Committee, 2023). Evidence of herbivory and frass on *A. syriaca* indicates that *D. plexippus* instars were present but may have been eaten.

1.6.4 Measuring survival rates of *Danaus plexippus*

Cohorts of *D. plexippus* instars can overlap on their host plant, presenting challenges to measuring the survival rate. Furthermore, even with fastidious weekly verification of *A. syriaca* plants for the presence of *D. plexippus* eggs, it is possible that trained experts can miss the presence of eggs or stage 1 instar larvae (Oberhauser & Prysby, 2008). A model for measuring the survival rate of *D. plexippus* caterpillars was proposed by Nail et al. (2015). The model requires regular follow-ups of a given plot, with a minimum of 5 regularly timed visits per season recommended to try to capture multiple generations on the same plot. The original equation (Eq. 1) for larval survival (Nail et al., 2015):

$$Larval\ Survival_{i,t,y} = \frac{Number\ of\ fifth\ instars\ from\ time\ period_{i,t,y}}{(Number\ of\ eggs\ from\ same\ time\ period\ two\ weeks\ earlier_{i,t,y})}$$
$$where\ i = site, t = time\ period, and\ y = year$$

Equation 1 Larval survival

The methodology used by Nail et al. (2015) considers a two-week interval between the number of eggs and stage 5 caterpillars in the equation, and is focused on *D. plexippus* breeding locations in the United States and mainly southern Ontario identified by the Monarch Larva Monitoring Project (MLMP), accounting for up to five generations of *D. plexippus*. Our methodology was adapted as the original method (Nail et al., 2015) was not logistically feasible based on the missing data from certain weeks in 2021 and the different number of cohorts each year. Furthermore, Nail's methodology was adapted for this study to account for a lower number of eggs than expected. In our experiment, it was modified by pooling the number of stage 5 caterpillars per plot for the entirety of a season and dividing by the total number of eggs from the plot for the same duration. An adapted equation (Eq. 2) is in the methodology section 4.4.2.

1.7 Insect communities and Solidago canadensis

Solidago canadensis serves as a polyvalent late-summer food source for a range of pollinators (Blackwell & Powell, 1981; Ustinova & Lysenkov, 2020) in eastern and central Canada as far north as the James Bay region (Melville & Morton, 1982). It is typically found in disturbed ecosystems and fallow lands (Melville & Morton, 1982; Bielecka & Królak, 2019; Ustinova & Lysenkov, 2020).

We can expect these plants to be visited by a wide range of insects (Ustinova & Lysenkov, 2020). While there is no specific pollinator insect visitor set, *S. canadensis* was the plant species with the third-highest abundance of wild bees in New Hampshire (Millam et al. 2018). A range of pollinator insects can be expected according to the optimal foraging theory or ideal free distribution theory of insect foraging (Dreisig, 1995) which is an extension of the density assessment hypothesis (Fretwell & Lucas, 1969).

Several studies across the world: pan-Canadian (Werner et al. 1980), Ontario (Melville & Morton, 1982), Romania (Fenesi et al., 2015) and New Hampshire in the United States (Milam et al., 2018) documented a large variety of Hymenoptera (Colletidae, Melittidae, Halictidae, Andrenidae, Megachilidae, and Apidae), Diptera (Syrphidae), and Lepidoptera species, notably many native bees and the non-native *Apis mellifera* (European honeybees) present on *S. canadensis* (Melville & Morton, 1982; Ustinova & Lysenkov, 2020). For the purpose of our study, the term "communities of insects" is based on the definition offered by Holl (1995), which is the assemblage of adult insects observed in a given site during transect walks.

Flowers develop on *S. canadensis* later in the summer, and inflorescence can be reported until October if it is a particularly warm year (Blackwell & Powell, 1981). This is noteworthy as it is an important food source for migratory insects prior to their southern migration, including adult *D. plexippus*. Abundant visitors in Ohio included the non-native *A. mellifera* and a social species of wasp, *Polistes fuscatus* Fabricius (Blackwell & Powell, 1981).

Despite being a pioneer plant species that thrives in hostile growing conditions, *S. canadensis* is susceptible to environmental stressors that impact its growth and leaf traits. Dong and He (2019) found that climate, plant species diversity, soil, and insect interactions had inverse reactions in regulating leaf traits. There was a significantly negative correlation between leaf production potential and leaf stress-tolerance potential, and 65.7% of the total variation was explained by each other. For this trade-off between leaf number production and leaf stress tolerance, the per capita contribution followed the order: diversity (7.7%) > climate (6.9%) > interactions (6.2%) > soil (5.6%); the most key contributor was soil nutrients (11.0% among 16 variables the researchers examined) (Dong & He, 2019).

1.8 Diversity indices

Diversity indices can be used to measure alpha (α) diversity and beta (β) diversity, as well as the species relationships in the community (Whittaker, 1972). Alpha (α) diversity is the diversity of a defined assemblage or habitat; the property of a defined spatial unit (Magurran, 2004), while beta (β) diversity reflects biotic change or species replacement; a measure of the change in diversity between samples along transects or across environmental gradients or sampling units (Magurran, 2004).

Relative abundance data assumes that members of the community are divided into species; the relative abundance data describes the proportions in which they are present (Leinster & Cobbold, 2012). Identification was done at the family level due to logistical constraints and patterns of diversity differences can sometimes be seen that this level of resolution.

In one example in the literature, the Shannon diversity index was used to measure the response of different trophic levels of insects to the invasion of *S. canadensis* in Slovenia (Groot et al., 2007). The Shannon index was also used to measure bee species richness in reclaimed sand mines in Maryland, USA (Seitz et al., 2019), which is the most comparable published study to the subject of the *S. canadensis* research in this thesis.

1.9 Citizen science

Citizen science projects have gained in popularity over the last 15-20 years, enabling researchers to involve people who are not professional scientists in scientific research

(Oberhauser & Prysby, 2008). Monarch conservation efforts have benefited from the popularity of such programs, including Mission Monarch (Insectarium – Montréal Space for Life & Institut de recherche en biologie végétale, 2024), the Monarch Larva Monitoring Project (Monarch Joint Venture & University of Wisconsin–Madison Arboretum, 2024), Journey North (University of Wisconsin–Madison Arboretum, 2024) and Monarch Watch (Baum et al., 2024). These initiatives may compliment scientific research by contributing to our understanding of how *D. plexippus* choose specific overwintering sites and reveal how landscape features contribute to migration (Green II, 2023).

Additional citizen science initiatives such as Abeilles citoyennes (Gervais et al., 2024) combine community science and taxonomist expertise for a larger-scale monitoring of broader insect pollinator communities (Rondeau et al., 2023) while Bumble Bee Watch (Xerces Society for Invertebrate Conservation et al., 2024) allows users to track *Bombus* spp. sightings which are geotagged. More generalist mobile smartphone applications include iNaturalist (Canadian Wildlife Federation et al., 2024), Seek by iNaturalist (Canadian Wildlife Federation et al., 2024), and the plant-specific paid artificial intelligence application, PictureThis (Glority Global Group Limited, 2024), just to name a few popular ones as of 2024. Citizen science contributions through these websites and mobile apps allow experts to vet the observations or suggest improved taxonomic identification when photos are misidentified. There is a bias towards larger population centres in North America on these applications (Rondeau et al., 2023), as data are user-generated, as well as a bias towards self-selected methodologies (Solis-Sosa et al., 2019).

2. OBJECTIVES

Current attempts to revegetate mining sites in the context of the boreal forest include planned planting efforts to foster the growth of pioneer indigenous flowering plants such as *A. syriaca* and *S. canadensis*. The overarching objective of this research is to measure the impact that these plant species have on communities of native pollinator insects on revegetated mining sites. There are two components of this research that were conducted in the field on revegetated mining sites. The first component focused on *D. plexippus* establishment and survival on *Asclepias syriaca*. The second component focused on pollinator insect communities visiting *S. canadensis*.

2.1 Objectives of researching Asclepias syriaca and Danaus plexippus

The general objective is to assess whether revegetated mining sites compared with roadsides can sustain developing *D. plexippus* larvae. I answer the question: can *D. plexippus* eggs and caterpillars survive until the mobile instar stages (stages 4 and 5) on *A. syriaca* that is growing on different disturbed sites (revegetated mine sites and roadsides)? Analysis for this research ceased at the *D. plexippus* mobile instar larval stages as no chrysalids were observed during either year.

2.2 Objectives of researching Solidago canadensis and pollinator insect communities

The general objective is to compare the pollinator insect communities visiting *S. canadensis* on revegetated mine sites with those found on *S. canadensis* plants at roadside control sites. I answer the question: how do pollinating insect families differ between different disturbed habitats (revegetated mine sites and roadsides)?

3. RESEARCH HYPOTHESES

3.1 Danaus plexippus ovipositing hypothesis

I hypothesize that *D. plexippus* adults will oviposit on *A. syriaca* used to revegetate mine sites at the same rate as naturally occurring *A. syriaca* found on the disturbed ecosystem of the control roadside sites.

3.2 Danaus plexippus instar larvae survival hypothesis

I hypothesize that the survival rate to the mobile stages of instar larvae (stages 4 and 5) of *D. plexippus* will be similar between revegetated mining sites and the survival rate found on the disturbed ecosystem of the control roadside sites.

3.3 Pollinator insect community hypothesis

I hypothesize that mining sites revegetated in the boreal forest context with *S. canadensis* will experience a diminished species richness (number of families) in the community of pollinator insects visiting the plants, as well as a reduction in the number of individuals of each family visiting *S. canadensis* compared to control test sites situated on the side of roads, as stem density is expected to be lower at the mine sites.

3.4 Solidago canadensis hypothesis

I hypothesize that increased numbers of *S. canadensis* stems per plot will result in a higher abundance and diversity of pollinator insect individuals. We expect to find more *S. canadensis* stems on the control sites than on the mine sites as we expect the physicochemical soil properties of the mine sites to be too harsh to support the planted *S. canadensis* stems.

3.5 Soil trace metal hypothesis

I hypothesize that there will be a difference between the mine sites and the roadside control sites, with different total trace metal concentrations being found at the mine sites compared to the roadside sites. We expect to find higher total concentrations of heavy metal in the soil of mine sites, and a higher level of salinity on roadside sites.

4. METHODOLOGY

4.1 Profile of the studied region: Abitibi-Témiscamingue

The Abitibi-Témiscamingue region is a sparsely populated area of western Québec (Figure 2). The territory is situated on gold and copper deposits of the Archean Abitibi greenstone belt (Robert, 2001) characterized by quartz monzonite to syenite stocks and dikes (Bedeaux et al., 2017). Abitibi-Témiscamingue sits atop the 250 km-long east trending Cadillac Fault Zone (Dubé et al., 2007; Bedeaux et al., 2017) straddling the Ontario-Québec border between Timmins, ON and Val-d'Or, QC, consisting of orogenic gold deposits. The Abitibi-Témiscamingue region is involved in both primary extraction as well as secondary processing (milling, processing, refining, and waste disposal) (Barbour, 1994; Cooke & Johnson, 2002). Mining extraction of metals and minerals represent a wide range of economic activities characteristic of the region. A century of extraction in the region has left a spectrum of mines in various states of operation, from long defunct sites to active mining sites both in semi-urban (e.g., Mine Canadian Malartic) and rural settings (e.g., Mine LaRonde), with varying states of environmental degradation and reclamation works.

The ecoregion is marked by warm summers and cold snowy winters with a mean annual temperature of approximately 1°C (Ministère du Développement durable de l'Environnement et de la Lutte contre les changements climatiques, 2010). The climate is characterized by a mild subpolar, subhumid climate with a growing season that has a historic average of 181 days (Conseil pour le développement de l'agriculture du Québec, 2021). The region averages between 874.8 mm (Rouyn-Noranda) and 988.7 mm (La Vallée-de-l'Or) (Ministère du Développement durable de l'environnement de la Faune et des Parcs, 2014) of total annual precipitation.

Rouyn-Noranda had a mean temperature of 4.68°C and total precipitation of 747.4 mm in 2021 (Environment and natural resources Canada, 2021a) and a mean temperature of 2.83°C with a total precipitation of 1112.2 mm in 2022 (Environment and natural resources Canada, 2022a). Val-d'Or had a mean temperature of 3.67°C with a total precipitation of 673 mm in 2021 (Environment and natural resources Canada, 2021b), and mean temperature of 2.29°C with total precipitation of 946.8 mm in 2022 (Environment and natural resources Canada, 2022b), illustrated in Table 2.

Table 2
Mean temperatures and total precipitation for Rouyn-Noranda and Val-d'Or, QC for 2021 and 2022

	2021	**2022**	**Difference**
Rouyn-Noranda mean temp °C	4.68°C	2.83°C	**-1.85°C**
Rouyn-Noranda mean precip. mm	747.4 mm	1112.2 mm	**+364.8 mm**
Val-d'Or mean temp °C	3.67°C	2.29°C	**-1.38°C**
Val-d'Or mean precip. mm	673 mm	946.8 mm	**+273.8 mm**

Source : (Environment and natural resources Canada, 2021a, 2021b, 2022a, 2022b)

The region includes the eastern part of the northern clay belt of Québec and Ontario. Gray Luvisols and Gleysols found on the clayey lacustrine and loamy tills are the dominant soils in the ecoregion, with some poorly drained areas characterized by Mesisols and Fibrisols, Humo-Ferric Podzols and sandy deposits in the southern portion of the ecoregion (Ecological Stratification Working Group, 1995).

Boreal forest is the reference end-of-succession ecosystem (Gann et al., 2019) for the mining and control sites of this study, and it is the dominant natural ecosystem of Abitibi (Figure 1). The boreal forest of Abitibi corresponds to the bioclimatic domain of the western balsam fir-white birch forest: a mixed forest characterized by stands of

Picea glauca (Moench) Voss (white spruce), *Abies balsamea* (L.) Mill. (balsam fir), *Betula papyrifera* Marshall (paper birch) and *Populus tremuloides* Michx. (trembling aspen) (Ecological Stratification Working Group, 1995). Drier sites may have pure stands of *Pinus banksiana* Lamb. (jack pine) or mixtures of *P. banksiana*, *B. papyrifera*, and *P. tremuloides*. Wet sites are characterized by *Picea mariana* (Mill.) (black spruce) and *A. balsamea*.

4.2 Study sites and experimental setting

4.2.1 Study sites

The mining and control sites selected for this study are situated along the Trans-Canada Hwy 117 in the Abitibi portion of the region, between the cities of Rouyn-Noranda to the west and Val-d'Or to the east, and the sites consist of a mix of urban and peri-urban locations (Figure 2).

A total of 8 mining sites between the western limits of Rouyn-Noranda and the eastern limits of Val-d'Or were selected in 2019 to conduct a revegetation study focusing on the impacts of using native flowering plants on pollinator insects. A total of 8 control sites were also selected in 2021 or 2022 to be compared to mine sites. Descriptions of each mine site and control site can be found in Table 3 below, which shows a significant spatial autocorrelation. The location of the mining sites and control sites within Abitibi-Témiscamingue are illustrated in Figure 2. There were 2 sites installed on the Noranda 5 site, identified as Noranda 5-1 and Noranda 5-2. As the topography is different for the two experimental settings on the Noranda 5 site, they are treated as two unique sites for all data analysis.

Table 3

Mining sites and control sites for *A. syriaca* and *S. canadensis* for 2021-2022

Mining sites with GPS coordinates	Location	Site details
Horne Smelter (48.24959, -79.01109)	Rouyn-Noranda (urban)	Located adjacent to an urban residential neighbourhood in Rouyn-Noranda, at the southern edge of Canada’s only active copper smelter, the Horne Smelter (Fonderie Horne), close to the northern shore of Lake Osisko. Experimental setting is on the industrial site right next to the residential neighbourhood of Notre-Dame. Experimental setting is situated on the grounds of the smelter site but is separated from the smelter’s main activities by train tracks. The soil is fine and clayey, colonized by graminoids.
Noranda 5-1 and 5-2 (48.23926, -79.07767 and 48.23988, -79.0779)	Rouyn-Noranda (outskirts)	Mining operations ceased in the 1970s, now includes a tailings pond for the Horne Smelter. Experimental settings 5-1 and 5-2 are located on stripped clayey soils and are protected by boreal forest on 2 sides and are situated adjacent to each other and separated by a braided flood plain. Natural plant succession is occurring along the forest edges outside of the limits of the experimental setting. For 5-1, the fourth side is protected by a small hill and the experimental setting has a mostly flat topography. The soil is very fine and large puddles retain water after rainfall. For 5-2, the fourth side is open to a natural prairie setting and the experimental setting has an inclined

		topography that follows the incline of the braided flood plain. The soil is also very fine mixed with clay, with runoff towards the braided flood plain. The plots closer to the braided flood plain retain water for a longer duration after the rain.
Goldex (48.09378, -77.87118)	Val-d’Or (urban)	Active underground gold mine situated at the western limits of the city of Val-d’Or along Hwy 117. The experimental setting is located along Hwy 117 and is separated from the shoulder of the road by a chainmail fence. It is located on a former parking lot and the soil is sandy with gravel that quickly drains water after the rain.
Mine Canadian Malartic (48.10998, -78.12739)	Malartic (urban)	Active open pit gold mine. Canada’s largest open-pit gold mine, the open pit was at least 360 m deep during the time of the study. Dynamite blasting occurs 2x per day, 7 days per week. There are two open pits in active exploitation: Malartic and Barnat during the years examined. Experimental setting is situated on the western raise in the tailings facility, on waste rocks covered with overburden fine silt. The experimental setting is surrounded on 2 sides by untouched waste rock. The third side is adjacent to other experimental revegetation projects featuring willow trees and the fourth side is adjacent to a mining service road. The soil is a fine till.
LaRonde (48.256, -78.47692)	Preissac (rural)	Underground gold mine that uses longitudinal retreat with cemented paste backfill; and transverse open stopping with paste or unconsolidated backfill. Experimental site is surrounded on two sides by boreal forest and natural plant succession is

		occurring along the forest edges as well as the other 2 sides adjacent to the experimental site. The experimental site sits atop large pieces of waste rock that were deposited on natural soil to build the experimental setting, experimental plants are growing directly out of the spaces between large and small pieces of rock.
Eldorado Gold Lamaque (48.10801, -77.74262)	Val-d'Or (outskirts)	Active underground gold mine at the eastern limits of the city of Val-d'Or along Hwy 117. Experimental site is situated on the tailings management facility, where excavation works took place around the experimental setting in 2021. The experimental setting is elevated from its surroundings and it is surrounded by waste rock deposits on one side and the tailings facility on 3 sides. The soil is silty tailings.
Manitou (48.07509, -77.63897)	Val-d'Or (rural)	Situated southeast of the Val-d'Or city limits and only accessible along a remote gravel road. Previously mined for zinc and copper until mining operations ceased in the 1970s, Manitou now functions as a tailings facility for Goldex (desulfurized gold tailings). Boreal forest and a drainage stream surround the experimental setting on one side, a short access trail on one side, and natural plant succession is occurring along the other 2 sides adjacent to the experimental site. Experimental plots are distributed in 2 rows of 8 plots, over the tailings covered by a few cm of topsoil. Naturally occurring plant succession is outcompeting the 7 species selected for revegetation efforts in 2019 and 4 visits occurred before visitation ceased due to

		competition. Detection of the presence of host plants for *D. plexippus* is believed to be more difficult when surrounded by a high diversity of other plants (Pitman et al., 2018), which justifies removing this site in 2022. The soil consists of silty and sandy tailings covered with a few cm of topsoil. **Data from Manitou are not included in the overall data analysis for 2022.**
Lapa (48.22918, -78.28365)	Rivière-Héva (rural)	Underground gold and silver mine situated in the boreal forest that ceased operations in 2018 and is undergoing active revegetation initiatives through its parent company. The experimental setting is located on till deposits with a slight slope. This site was part of the initial plan for summer 2022, however, monitoring of the experimental setting ceased after 1 visit as the parent company could not guarantee safe access to the site nor could they guarantee that their revegetation initiatives would not be conducted on the experimental setting. The soil is a fine till. **Data from Lapa are not included in the overall data analysis for 2022.**
A. syriaca control site 1 (48.22608, -79.01019)	Boul. de l’Université, Rouyn-Noranda (urban)	Single control plot situated on the southwest corner of boul. de l’Université and av. de la Rivière in Rouyn-Noranda (urban portion of Hwy 117). Exposed rock borders one side of the repetition and boul. de l’Université borders a second side. Two sides are naturally occurring prairie with wildflowers along a ditch. There is an abundance of *A. syriaca* plants outside of the control setting. The soil is a till.

A. syriaca control site 2 (48.21576, -78.85018)	Pont Kinojévis, Rouyn-Noranda (rural)	Roadside control setting situated on the northwest side of Pont Kinojévis in a rural setting on the outskirts of Rouyn-Noranda along Hwy 117 near the regional airport. 3 plots were installed here. Exposed boulders and boreal forest surround the site on one side, and Hwy 117 surrounds the site on another. Two sides are surrounded by an abundance of *A. syriaca* plants outside of the control setting. The topography has a steep incline towards the Kinojévis River, and the control settings are inclined. The soil is a till.
A. syriaca control site 3 (48.07853, -77.80834)	Boul. Barrette, Val-d'Or (rural)	Roadside control setting situated on the southeast side of Val-d'Or, within the main city limits but along a rural stretch of boul. Barrette near the regional airport. Two plots were installed on the south side of the road, and 1 plot was installed on the north side of the road. Boreal forest surrounds the site and there is an abundance of *A. syriaca* plants outside of the control setting. The soil is sandy.
A. syriaca control site 4 (48.23822, -78.98725)	Maison de l'Envol, Rouyn-Noranda (urban)	Control setting with 2 plots situated on rue Perreault Est in a residential neighbourhood on the south side of Lac Osisko. The setting is a landscaped garden on private property. Landscaped grass surrounds the control repetitions and there is an abundance of *A. syriaca* plants outside of the control setting. The soil is topsoil.

S. canadensis control site 1 (48.20635, -78.85683)	Rang Kinojévis, Rouyn-Noranda (rural)	Single control plot situated on the west side of Rang Kinojévis, a rural route on the outskirts of Rouyn-Noranda. The site is surrounded on one side by boreal forest backing onto the Kinojévis River, natural prairie on two sides, and gravel road on one side. The soil is a mix of clay and sand.
S. canadensis control site 2 (48.24236, -79.03747)	Pizza Pizza boul. Rideau, Rouyn-Noranda (urban)	Single control plot situated in the parking lot behind Pizza Pizza location on boul. Rideau on an urban portion of Hwy 117. Control setting is in a small green space adjacent to 20$^{\text{ième}}$ rue and is surrounded by parking lot on 3 sides and a residential road on one side. The soil is a till.
S. canadensis control site 3 (48.23005, -79.00655)	UQAT parking lot #3, Rouyn-Noranda (urban)	Single control plot situated adjacent to parking lot #3 at the back of the UQAT campus on a strip of prairie land separating the university and CEGEP Abitibi-Témiscamingue campuses. The control setting is surrounded on 3 sides by prairie and one side by an access lane to the parking lots. The soil is a till.
S. canadensis control site 4 (48.24748, -79.06385)	Hwy 117 Évain, Rouyn-Noranda (outskirts)	Single control plot situated west of Rouyn-Noranda, on the south side of Hwy 117 between the junction of Hwy 117 and Hwy 101 and the roundabout for av. Davy, close to a Petro-Canada gas station. The control setting is surrounded on 3 sides by prairie and Hwy 117 on one side. The soil is sandy.

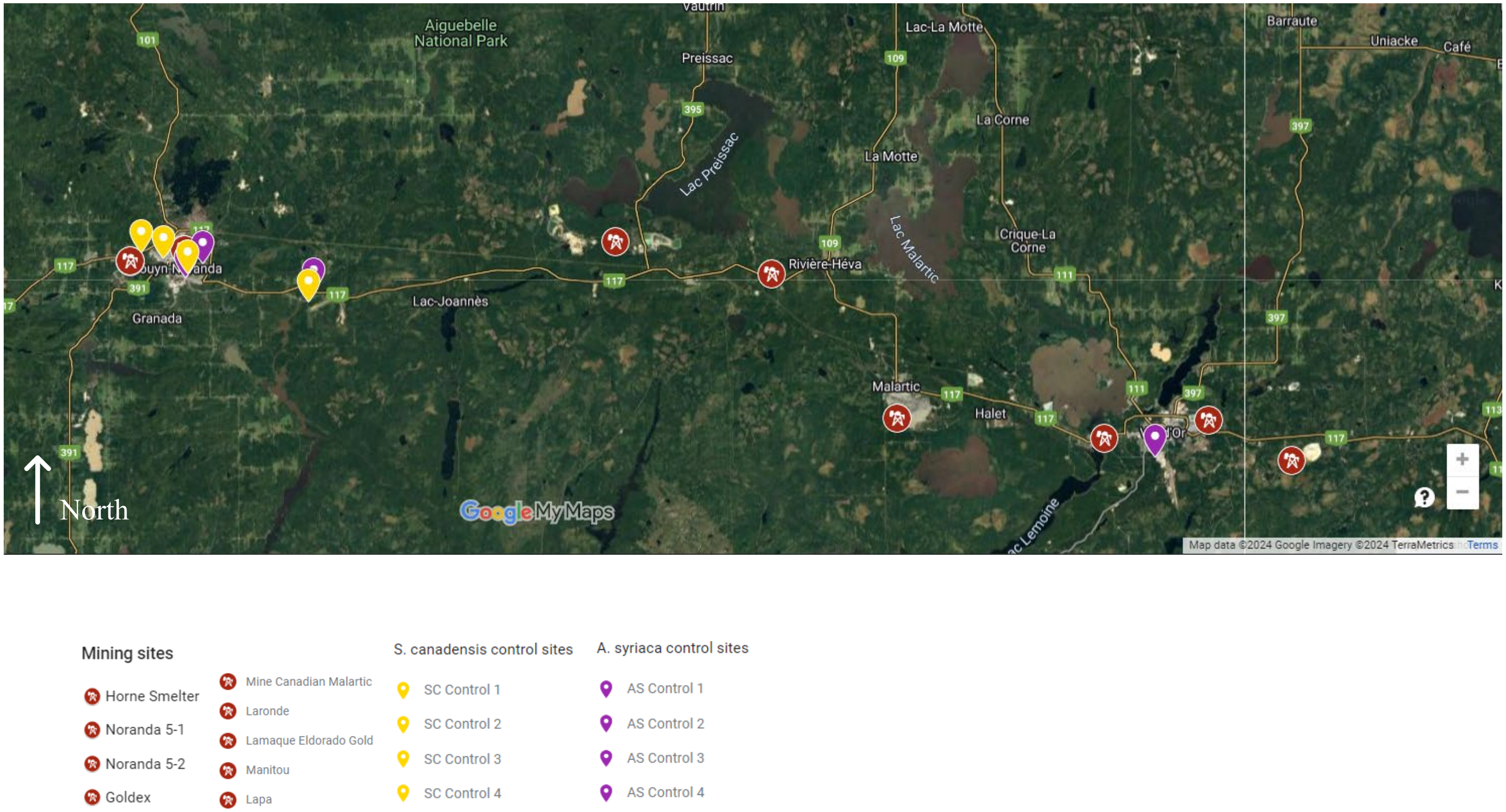


Figure 2

Map of revegetated mining sites and roadside control sites

Source: GoogleMaps, GoogleEarth. A map depicting the location of 8 revegetated mining sites (red icons) and 8 roadside control sites in Abitibi-Témiscamingue between the cities of Rouyn-Noranda in the west and Val-d'Or in the east. The A. syriaca roadside control sites are illustrated in purple, and S. canadensis roadside control sites are illustrated in yellow.

4.2.2 Experimental setting: Mine sites

Each mine site received one standardized experimental setting, except Noranda 5 site, where two experimental settings were installed. Experimental settings consisted of four treatments (T1-T4) with four repetitions (R1-R4) distributed in a randomized complete block design (Figure 3), for a total of 16 plots per experimental setting. The experimental setting at the Goldex site received only two repetitions for a total of eight plots due to space constraints. Each plot measured 5 m x 5 m with an untreated buffer zone of 1 m between plots.

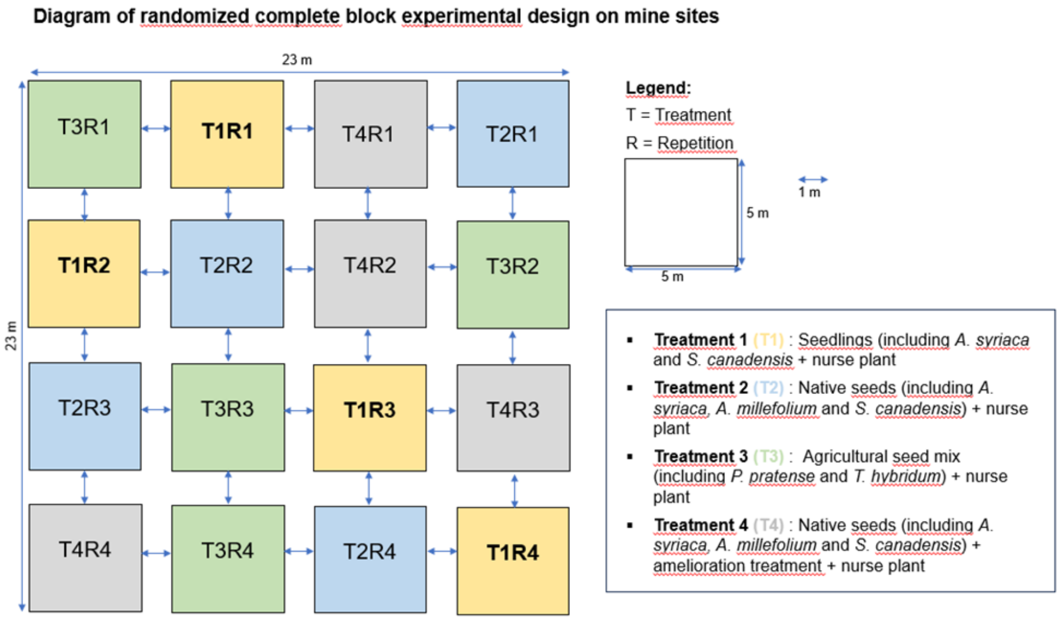


Figure 3
Sample diagram of revegetated mine settings
Four treatments (T1-T4) per experimental revegetated mine site, and each treatment is repeated 4 times. Treatments measured 5 m by 5 m, with a 1 m buffer between treatments. T1 treatments received 16 *A. syriaca* and *S. canadensis* seedlings in 2019. The T1 treatment is the focus setting of this research.

In the present study, only the T1 treatment plots were used, so they will be presented in detail below while the other treatments are briefly presented. Within the T1 treatments, seven species were planted, including *A. syriaca* and *S. canadensis* when the plots were created in 2019. Each plot from either mining sites or control sites constitutes an experimental unit.

T1 received planted seedlings and a seeded nurse plant, *Avena sativa* (Guittonny-Larchevêque et al., 2016). The T1 experimental settings were treated with a random dispersal of 16 plantings each of the seven species in 2019, as illustrated in Figure 3. Figure 4 illustrates that seedlings were planted in 4 rows of 4 seedlings each, spaced 40 cm apart. The seeded nurse plant, *A. sativa*, was seeded with a 200 kg/ha rate of seeds for the T1 treatment. Some T1 plots only received seedlings of 6 species instead of 7 seedlings if an insufficient number of *C. angustifolium* seedlings were available for one species. This scenario is also illustrated in Figure 4. Within the T1 experimental mine setting, we examined Sp 1 (*A. syriaca*) and Sp 7 (*S. canadensis*) (Figure 4) in 2021 and 2022.

The Noranda 5-1 and Noranda 5-2 mining sites in Rouyn-Noranda and the LaRonde mine in Preissac were selected for the portion of the research dedicated to investigating pollinator insect communities on *S. canadensis.*

The 7 species used were:

Sp.1: *Asclepias syriaca* L. (Common milkweed)
Sp.2: *Desmodium canadense* (L.) DC. (Showy tick trefoil)
Sp.3: *Astragalus canadensis* L. (Canadian milkvetch)
Sp.4: *Sanguisorba canadensis* L. (Canadian Burnet)
Sp.5: *Rudbeckia hirta* L. (Black-eyed Susan)
Sp.6: *Chamerion angustifolium* (L.) Holub (Fireweed)
Sp.7: *Solidago canadensis* L. (Canada goldenrod)
Nurse plant: *Avena sativa* L. (Common oat)

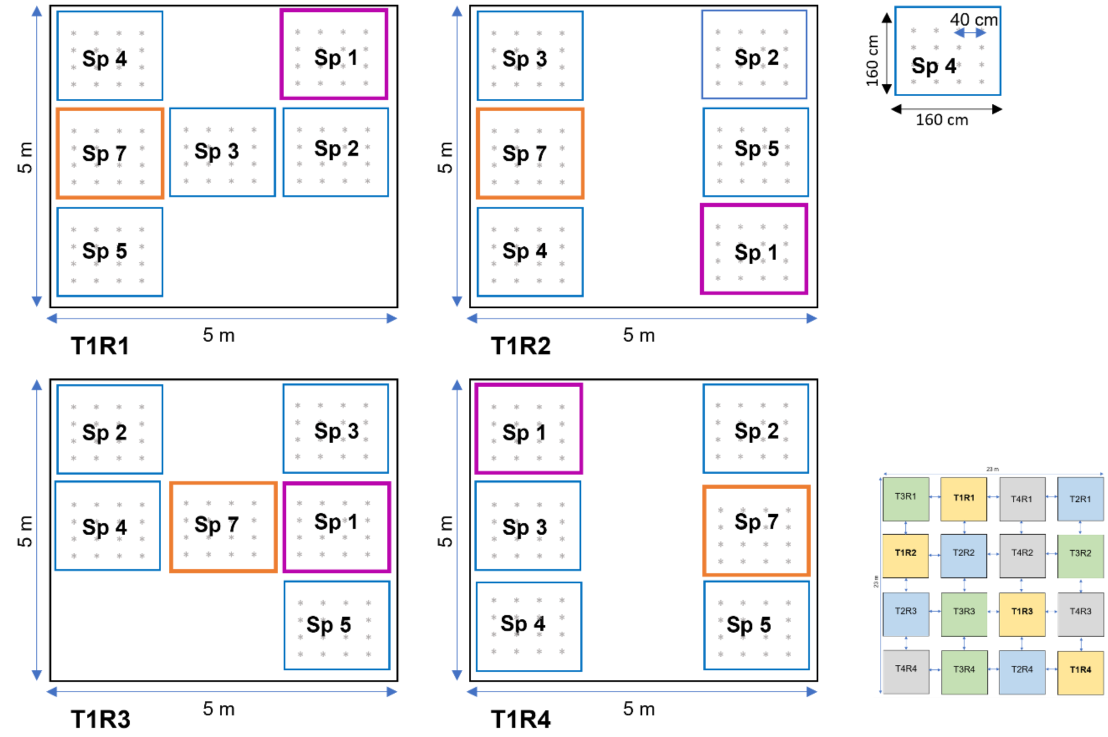
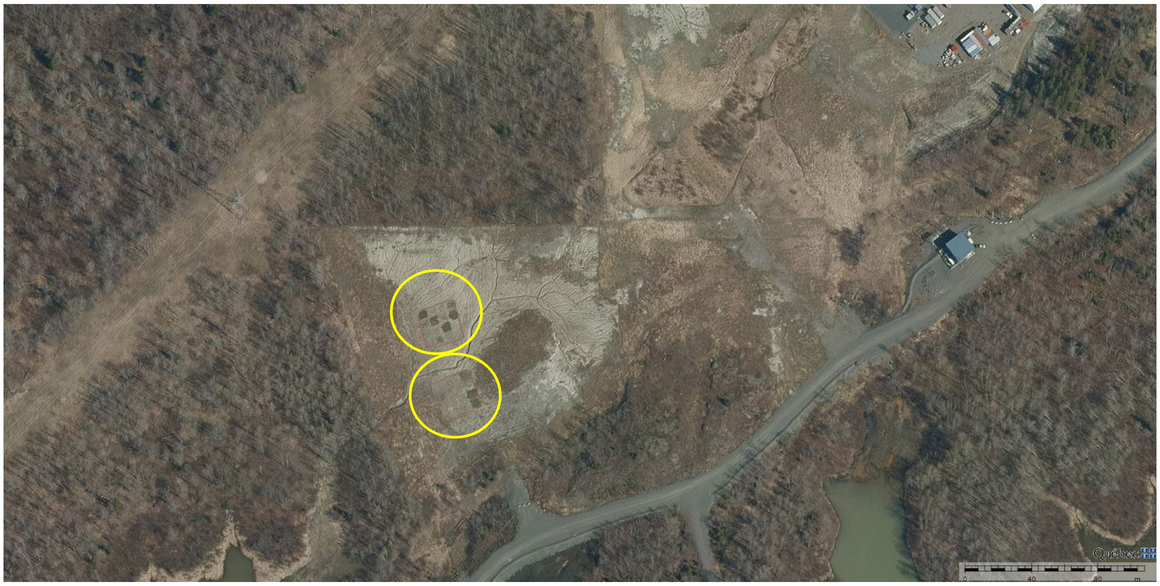


Figure 4
Example of random dispersal of plantings by species in T1 on experimental revegetated mine sites
The 5 m by 5 m treatments on revegetated mine sites are divided into smaller 160 cm by 160 cm plots with up to seven vegetation species. Figure 3 is reprised to demonstrate an example of the random dispersal of T1 repetitions within the experimental mine setting. T1 received 16 seedlings of each species with 40 cm of space between seedlings. Sp 1 is *A. syriaca*, represented in magenta. Sp 7 is *S. canadensis*, represented in orange.

The specific mine site experimental locations with their surrounding areas are shown via satellite imagery in Figures 5a through 5h.

(a) Horne Smelter

(b) Noranda 5-1 and Noranda 5-2



(c) Goldex

(d) Mine Canadian Malartic

(e) LaRonde

(f) Lamaque Eldorado Gold

(g) Manitou

(h) Lapa

Figure 5
Satellite images of mining experimental sites

Source: Ministère des Ressources naturelles et des Forêts (Ministère des Ressources naturelles et Forêts, 2022). Satellite images from Mosaïque Sentinel-2 of experimental revegetated mine settings taken in 2022. Experimental plots circled in yellow include 4 replicates each of the revegetation protocol, except for the Goldex mine site which has 2 replicates. Mine sites were revegetated with 7 agronomic plants including *A. syriaca* and *S. canadensis*. Mines are identified: (a) Horne Smelter, (b) Noranda 5-1 and Noranda 5-2, (c) Goldex, (d) Mine Canadian Malartic, (e) LaRonde, (f) Lamaque Eldorado Gold, (g) Manitou, (h) Lapa.

4.2.3 Experimental setting: Control sites

Control sites received no treatment protocol and were naturally occurring populations of *A. syriaca* and *S. canadensis*, respectively, selected for an existing plant density that approximated the conditions of the T1 repetitions of *A. syriaca* and *S. canadensis*. Control sites (Table 3) for *A. syriaca* were selected in Rouyn-Noranda and Val-d'Or, while the control sites for *S. canadensis* were all located in Rouyn-Noranda.

All plots of control sites for both *A. syriaca* and *S. canadensis* measured 1.2 m by 1.2 m. Plot plant density was one of the variables measured for statistical analysis. Sites in Val-d'Or were omitted from the *S. canadensis* portion of the research due to geographical and labour constraints.

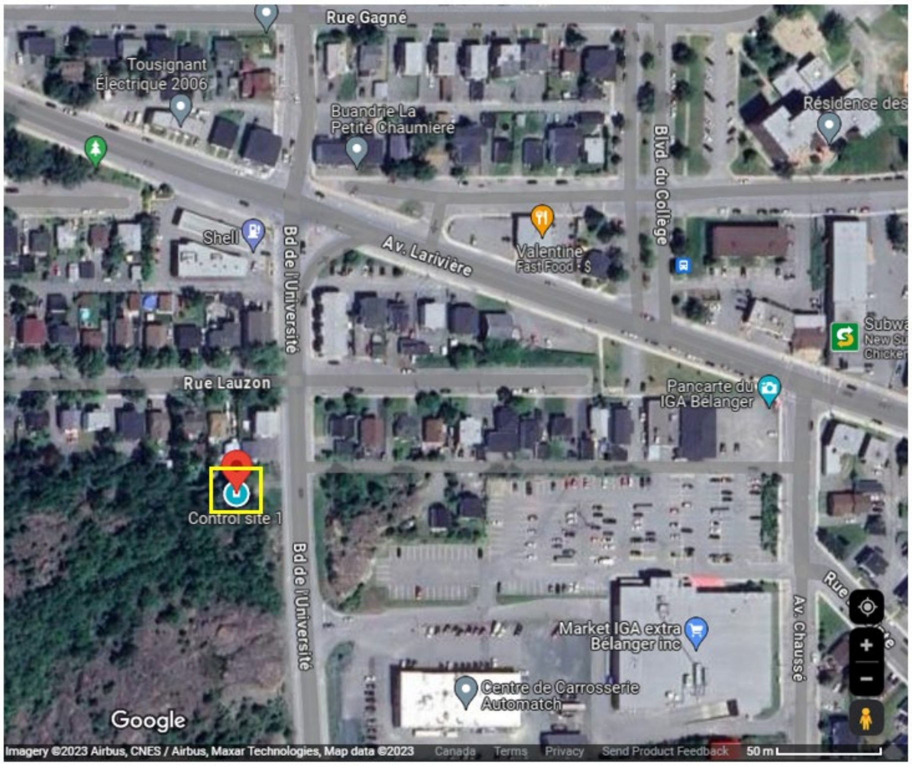
4.2.4 Control sites: *Asclepias syriaca*

Asclepias syriaca is quick to emerge after the snow recedes and the empty seed pods (Borkin, 1982) facilitate identification of potential control sites. The *A. syriaca* control sites (Figures 6a through 6d) were adjacent to roads that had two to four lanes of traffic total, and the *A. syriaca* control spot on Hwy 117 (Control 2) had two lanes but higher volume of traffic. These sites matched characteristics measured in several previous studies on roadside *A. syriaca* presence and *D. plexippus* mortality (Cariveau, Anderson, et al., 2019; Cariveau, Holt, et al., 2019; Lalonde et al., 2022). Roadside sites were selected as a control group for several reasons. Roadside sites are potentially contaminated sites (Kabata-Pendias, 2011; Cariveau, Anderson, et al., 2019; Mitchell et al., 2020; Shephard et al., 2022), the Rouyn-Noranda and Val d'Or zones of Abitibi-Témiscamingue lack actively used farmland that could be easily accessed for control sites, and these agricultural sites may get mowed during the study.

Three initial control sites for *A. syriaca* (Control 1: boul. De l'Université, Control 2: Pont Kinojévis and Control 3: boul. Barrette) were selected and installed during the summer of 2021. These sites represented an urban site with one plot, a rural site with three plots and a rural site with three plots, respectively. A fourth site in an urban setting with two plots was selected during the first week of June 2022: La Maison de l'Envol (Control 4) in Rouyn-Noranda. During the 2021 and 2022 seasons, we advised the administrators for the municipalities of Rouyn-Noranda and Val-d'Or, as well as the Ministère de Transports et de la Mobilité durable of our control site locations of *A. syriaca* and requested that mowing regimes be suspended for the duration of our study as there were no plans to mow the *A. syriaca* plants on the mine sites; the municipalities complied with our request.

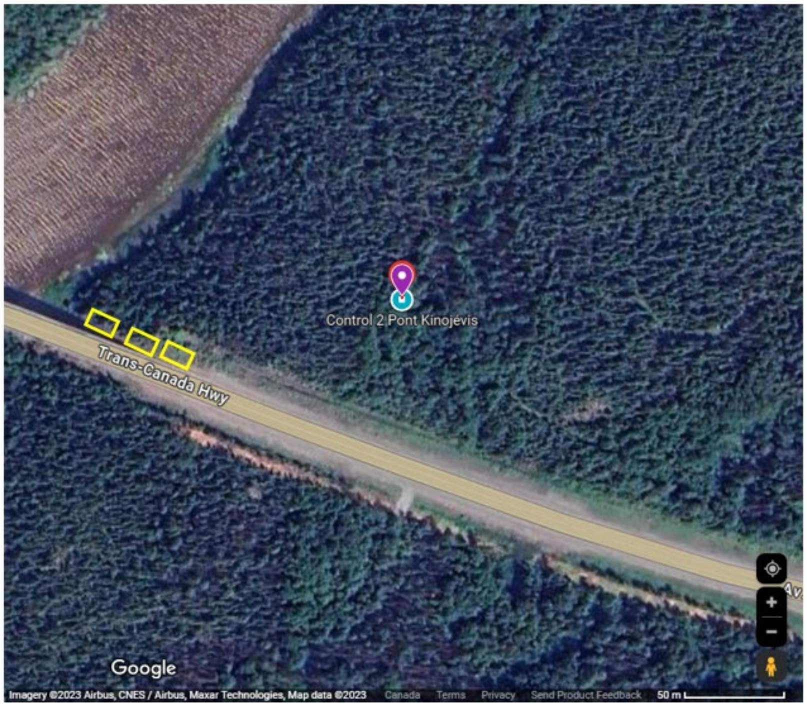
(a) *A. syriaca* control site 1

Boul. de l'Université, Rouyn-Noranda, 48.22608, -79.01019. One plot.



(b) *A. syriaca* control site 2

Pont Kinojévis, Rouyn-Noranda, 48.21576, -78.85018. Three plots.



(c) *A. syriaca* control site 3

Boul. Barrette, Val-d'Or, 48.07853, -77.80834. Three plots.

(d) *A syriaca* control site 4
Maison de l'Envol, Rouyn-Noranda, 48.23822, -78.98725. Two plots.

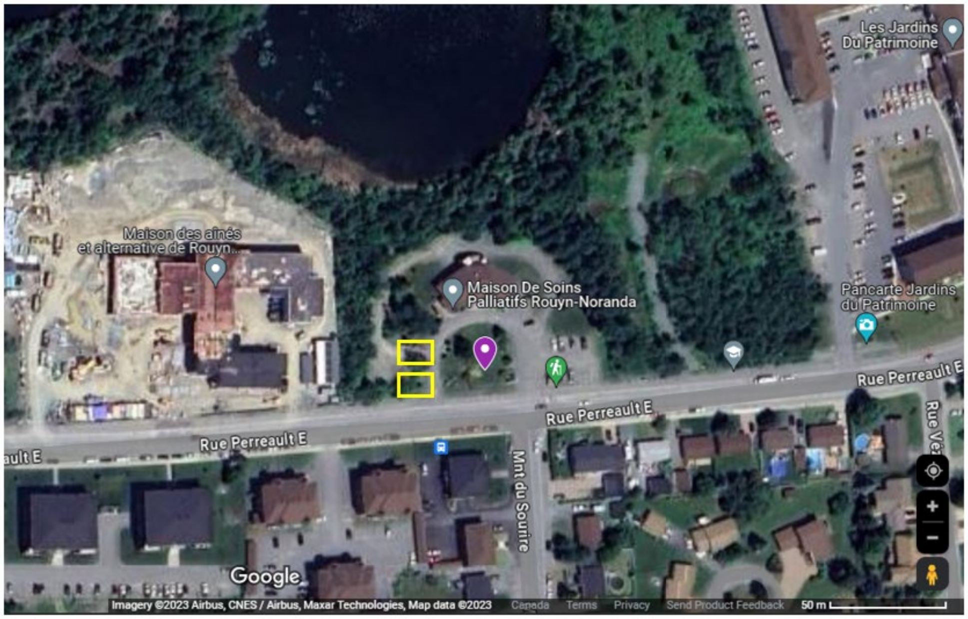


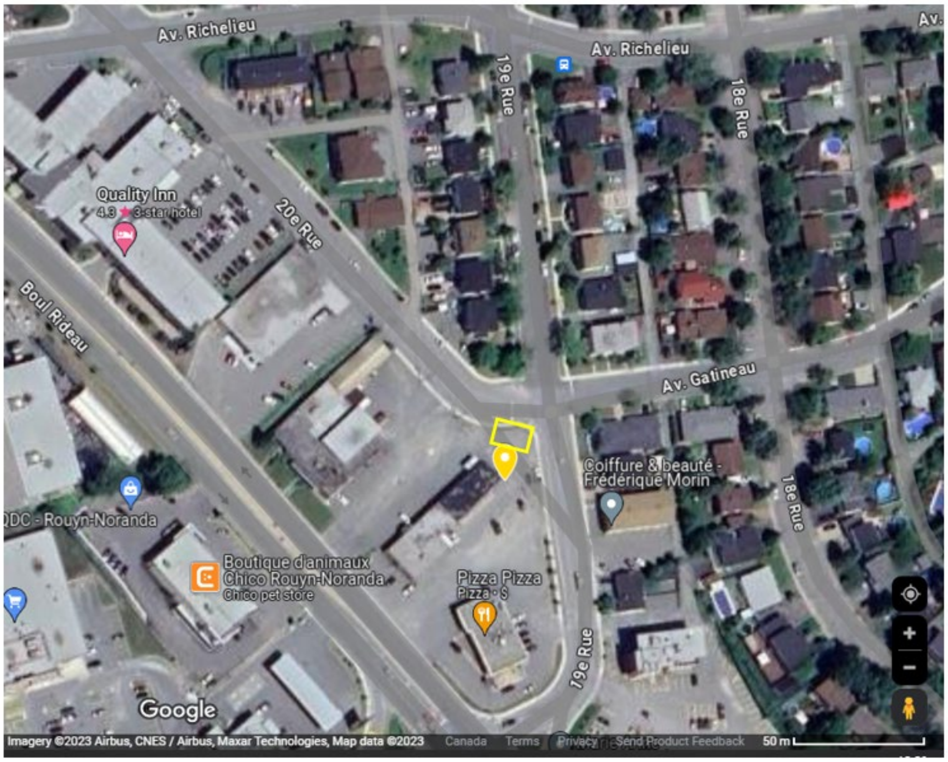
Figure 6
Aerial images of *Asclepias syriaca* roadside control sites
Source : GoogleMaps, December 2023. (a) Boul de l'Université, Rouyn-Noranda (b) Pont Kinojévis, Rouyn-Noranda (c) Boul. Barrette, Val-d'Or, (d) Maison de l'Envol, Rouyn-Noranda. Yellow boxes (not to scale) represent the approximate placement of A. syriaca roadside control sites. All control sites for A. syriaca had existing plants growing in situ of unknown age.

4.2.5 Control sites: *Solidago canadensis*

The four *S. canadensis* (Table 3) control sites (Figures 7a through 7d) had one plot per site and were selected in the region of Rouyn-Noranda. Site selection was finalized during the week of August 1, 2022, and the experimental protocol for *S. canadensis* began on August 8, 2022, once the plants were in full inflorescence.

(a) *S. canadensis* control site 1
Rang Kinojévis, Rouyn-Noranda, 48.20635, -78.85683. One plot.

(b) *S. canadensis* control site 2
Pizza Pizza boul. Rideau, Rouyn-Noranda, 48.24236, -79.03747. One plot.



(c) *S. canadensis* control site 3
UQAT parking lot #3, Rouyn-Noranda, 48.23005, -79.00655. 1 plot.

(d) *S. canadensis* control site 4
Hwy 117 Évain, Rouyn-Noranda, 48.22918, -78.28365. 1 plot.

Figure 7
Aerial images of *Solidago canadensis* roadside control sites
Source : GoogleMaps, December 2023. (a) Rang Kinojévis, Rouyn-Noranda (b) Boul. Rideau, Rouyn-Noranda (c) UQAT parking lot, Rouyn-Noranda, (d) Hwy 117 Évain, Rouyn-Noranda. Yellow boxes (not to scale) represent the approximate placement of *Solidago canadensis* control sites. All control sites for *S. canadensis* had existing plants growing in situ of unknown age.

4.3 Soil characterization in experimental settings

Soil samples for chemical analysis were collected from the mining sites in 2019 during the installation of the treatments. A total of four samples were collected from each mining experimental setting: one from each corner. The soils of the control sites for both *A. syriaca* (Controls 1 through 4) and *S. canadensis* (Controls 5 through 8) were sampled in 2022 using the following sampling protocol: five samples were collected in each plot, one from each corner and one from the centre of the plot. Control sites with multiple repetitions (Controls 2, 3 and 4) had soil samples collected from each plot. The five soil samples of each plot were blended to create one composite sample from each plot to obtain a minimum of 500 grams for analyses. In all sites, the top layer of vegetation litter was omitted, and the soil was sampled to a maximum depth of 10 cm.

All soil samples were dehydrated for a minimum of 48 hours in a 60°C drying oven. Dehydrated soil samples were sifted through a 2 mm mesh sieve then sent for analysis. Soil samples were manipulated under a ventilation hut while wearing a filtering mask hut to avoid inhaling fine particles of soil. Samples were sent for external analysis at SGS Canada Inc. Laboratory.

Parameters examined included a full scan of trace metals including phosphorous via microwave assisted acid digestion followed by metal determination using inductively coupled plasma atomic emission spectroscopy. Analysis also characterized the soil pH, electrical conductivity, organic matter and total nitrogen concentrations of the soil samples. The organic matter analyses used the Walkley-Black method for organic matter testing by oxidizing the material then measuring it in a colourimeter. Characterization of soil pH and electrical conductivity used a 1:2 saturated paste.

A multi-element determination of total metal concentrations in the soil samples was done by aqua regia digestion preparation technique and ICP/MS. The method is derived from EPA 3050B, MA.200-Met. 1.2, EPA SW-846 6020A and O. Reg 153 protocol.

Determining anions of nitrite and nitrate in aqueous, soil and sludge samples using ion chromatography (IC). This method is derived from EPA method 300.1 as well as the Québec Ministry of Environment method MA.303-Ions 3.1.

Nitrogen concentrations in soil were obtained using Kjeldahl Digestion by SFA then colorimetric segmented flow analysis to obtain total Kjeldahl N, expressed as TKN in % of dry soil mass. The procedure is derived from the Standard Methods for the Examination of Water and Wastewater, methods 4500-$N_{org\ D}$.

4.4 Methodology for Danaus plexippus and Asclepias syriaca portion of fieldwork

4.4.1 Methodology for *Danaus plexippus* monitoring 2021

Sites were visited on a once per week basis between June 23 and August 16, 2021, to check for the presence of *D. plexippus*. A total of seven visits were conducted over this nine-week period to count the eggs, caterpillars of each stage of *D. plexippus*' five instar stages (Oberhauser & Kuda, 1997) on each individual stem of *A. syriaca* in each T1 (mine) and control (roadside) plot, and record the presence of chrysalids. If no *D. plexippus* eggs or instars were detected one week, the site was omitted from data collection during the following week, then revisited the following week (two weeks of interval for data collection). All of the mine sites were monitored during the 2021 season, and the numbers one, two and three *A. syriaca* control sites (Figure 8) were included in the 2021 season.

4.4.2 Methodology for *Danaus plexippus* monitoring 2022

Monitoring for the presence of *D. plexippus* eggs and caterpillars on *A. syriaca* began the week of May 30, 2022, and was completed the week of August 1, 2022. No data were collected the week of June 6, and not all sites were surveyed the week of June 27 due to external factors beyond the research team's control. The same variables from 2021 were measured again in 2022. All data was recorded in situ directly into the application KoboCollect in 2022, an offline Android OS mobile application that connects to KoboToolbox. Data was uploaded once daily to the secure KoboToolbox server from a secure Android tablet.

The presence of other insect herbivores on *A. syriaca* was recorded as qualitative data during fieldwork, including *Lygaeus kalmii* Stål, 1874 (common milkweed bug or small milkweed bug), *Labidomera clivicollis* Kirby, 1837 (milkweed leaf beetle), *Aphis nerii* Fonscolombe, 1841 (milkweed aphid or oleander aphid), and *Rhyssomatus lineaticollis* Say, 1824 (milkweed stem pure weevil).

An earlier start date was selected in 2022 based on the results of the 2021 survey as an attempt to observe the presence of the first cohort of *D. plexippus*. Year-over-year variability is correlated with regional weather data and global climate oscillation data (Semmens et al., 2016; Flockhart et al., 2017; Thogmartin, Wiederholt, et al., 2017).

Site 8 (Manitou) was omitted from data collection in 2022 from week 5 onwards due to aggressive vegetation competition in the *A. syriaca* plots. Presence of *A. syriaca* was observed during the initial 4 weeks of the study, however, the plants' development was severely stunted, with stems reaching no more than 1-3 cm in height by the end of June. Competing vegetation was found growing above the *A. syriaca* stems and crowding out the stems; presence could only be observed by moving the competing vegetation aside. These conditions were not favourable for *D. plexippus* ovipositing; thus, the site was removed from the study.

Site 9 (Lapa) was omitted from data collection in 2022 after the first week of observations due to ongoing restoration work with heavy machinery; Agnico Eagle Mines Ltd. had safety concerns for the research team and revoked access to the site. The omission of this site may have negatively impacted the 2022 findings as Lapa had the highest number of stage 5 instars in the 2021 dataset.

Equations 2 and 3 were used to measure two survival rates of the *D. plexippus* caterpillars in our experiment in 2021 and 2022. Equation 2 (Eq. 2), adapted from Nail et al. (2015), concerns the overall survival of *D. plexippus* from the egg to the last mobile larval stages and was used to measure the survival rate of the *D. plexippus* caterpillars in our experiment:

$$\frac{\boldsymbol{Total\ \#\ of\ stage\ 4+5\ caterpillars\ per\ site\ x\ during\ season}}{\boldsymbol{Total\ \#\ of\ eggs\ from\ site\ x\ during\ season}} * \mathbf{100}$$

Equation 2 Stages 4+5 survival

A similar equation (Eq. 3), measuring the survival rate of *D. plexippus* from egg to stage 3 instar larvae was also developed to check if most of the mortality occurred on the immobile stages (stages 1-3) before the onset of the mobile stages of caterpillar development. The survival rate of pre-mobile stage 3 instar larvae was calculated separately from the combined survival rate of stages 4 and 5 instar larvae. This equation is as follows:

$$\frac{\boldsymbol{Total\ \#\ of\ stage\ 3\ caterpillars\ per\ site\ x\ during\ season}}{\boldsymbol{Total\ \#\ of\ eggs\ from\ site\ x\ during\ season}} * \mathbf{100}$$

Equation 3 Stage 3 survival

Survival rates were not measured by cohort as the cohorts overlapped with no clear delineation between the start of one cohort and the end of the previous one.

4.4.3 *Asclepias syriaca* data collection

The following plant-level variables of *A. syriaca* were collected during each *D. plexippus* monitoring visit during the 2021 season and on weekly follow-ups in 2021 and basis during the 2022: season. We counted the number of *A. syriaca* stems per plot, total height (rounded to the closest half per stem in cm), and number of leaves (Pocius et al., 2018) and ramifications, percentage of herbivory, presence or absence of inflorescences for each *A. syriaca* stem were counted. To check for the presence of *D. plexippus*, the tops and bottoms of all *A. syriaca* leaves were examined. On inflorescences, we checked on the tops and bottoms of flower buds, as well as in between the flower buds, which required the field team to investigate each stem on their hands and knees to avoid missing any eggs. All suspected eggs were further examined with 21 mm triplet 10x magnifying glass to confirm that they were *D. plexippus* eggs. Caterpillars were compared to Oberhauser & Kuda's (1997) field guide to confirm the larval instar stage. Time spent per *A. syriaca* stem varied based on the height of the plant, the number of leaves and inflorescences, and how close adjacent *A. syriaca* stems were. Each plot was examined by one individual for the duration of the visit to ensure that the same protocol was employed per plot.

One leaf from each *A. syriaca* individual on each plot was collected during the week of July 11, 2022. The oldest leaves (bottom leaves) and the youngest leaves (top leaves) were omitted from sampling, as were any visibly damaged leaves. *Asclepias syriaca* leaves grow in pairs on opposite sides of the stem: to avoid sampling the oldest leaves, the first 3-4 pairs were left intact. Plants that had fewer than eight leaves (four leaf pairs) were not sampled. Due to a lack of biological material caused by insufficient plant tissue development, only one composite sample per mining and control site (all plots pooled) was analyzed. Samples required 5 grams of plant tissue ground to 1 mm mesh for analysis. All samples of *A. syriaca* were dehydrated for a minimum of 48 hours in a 60°C drying oven.

The dehydrated samples of *A. syriaca* leaves were sent to Lakehead University Environmental Laboratory in Thunder Bay for chemical analysis. Parameters examined included a full scan of trace metals including phosphorus via microwave assisted acid digestion followed by metal determination using inductively coupled plasma atomic emission spectroscopy, and total nitrogen content via combustion then nitrogen analyzer.

4.5 Solidago canadensis portion of fieldwork

Monitoring of insect pollinators was performed on three mine sites (LaRonde, Noranda 5-1 and Noranda 5-2) and the four control sites in 2022, to compare the biodiversity and abundance of pollinator insect species on mining sites with control sites in the region. Solidago canadensis inflorescences began emerging the week of August 1, 2022, however, not all sites were flowering at the same time as the length of its inflorescence season varies from July through September and there is variation within the individual plant phenology (Gross & Werner, 1983; Fenesi et al., 2015). The same soil sampling and analysis methodology described for *A. syriaca* were used on *S. canadensis* experimental mine sites and control sites.

The *S. canadensis* sites were visited 6 times total between August 8, 2022, and August 22, 2022. Each site was visited on two consecutive days in the same week; the first visit each week was to install pan traps, and the second visit was to collect specimens from the pan traps. All data was recorded in situ directly into the application KoboCollect and uploaded once daily to the KoboToolbox server from a secure Android tablet.

All field data was collected using the Android application KoboToolbox.

4.6 Pollinator insect collection

Two methods of quantitative data collection of pollinator insect presence on *S. canadensis* were used: sweep netting and yellow pan traps. Visual observations were also recorded to provide complementary information for the discussion section.

4.6.1 Insect collection on *Solidago canadensis*: Sweep netting

The first technique employed is one of the most common and widely known techniques for collecting flying insects: an active technique which consists of net sweeps (Marshall et al., 1994), the act of sweeping immediately above the plants with an insect net. Each site was visited on two consecutive days in the same week and the net sweep technique was used during each visit during the weeks of August 8, August 15, and August 22, 2022, for a total of 82 samples. Net sweeps were conducted prior to placing pan traps

(Section 4.6.2) during the first visit and repeated prior to collecting pan traps during the second visit.

Ten sweeps of the net were conducted on each plot repetition of *S. canadensis* during each visit in August. Swipes were conducted while actively walking around 2 adjacent sides of the 1.2 m x 1.2 m plot to attempt to sample the largest possible area. After a series of 10 sweeps, the portion of the net containing insects was placed inside a glass jar lined with plaster and dosed with acetone to euthanize specimens quickly and humanely. After insect activity ceased within the jar, the contents were transferred to a Ziplock bag and stored in a cooler for transportation back to the laboratory at UQAT, where specimens were transferred to labelled 30 ml vials with 70% ethanol for storage.

4.6.2 Insect collection on *Solidago canadensis*: yellow pan trap

The second technique of pollinator insect collection involved a passive capture of insects using pan traps (Marshall et al., 1994), also known as bowl traps. Yellow pan traps were selected for this methodology as the inflorescence colour of *S. canadensis* is bright yellow. The pan trap collection method was used during the weeks of August 8, August 15, and August 22, 2022. Pan traps were placed at ground level and filled with tap water containing a few drops of dish soap. Traps were filled to between ½ and ¾ of the plastic dish to avoid losing specimens due to overflowing. Dish soap is essential to a pan trap to break the surface tension in the bowl allowing the insects to sink, otherwise visiting insects may fly away after landing on the surface of the trap.

Five traps were used per plot per week, for a total of 210 pan traps. Traps were placed in each corner of the *S. canadensis* portion of the plot, and a fifth trap was placed at the centre, forming an X (Figure 8). Traps were collected 24 hours later, always starting with trap 1 in the northwest corner and ending with trap 5 in the southeast corner. The contents of each pan trap were stored in a cooler in individually identified Ziplock bags labelled with the date of collection, site ID and repetition information, and bowls 1-5. In the lab, the insect contents were subsequently transferred for storage in 70% ethanol in 15 ml test tubes. Bycatch from this method include ground dwelling and flying non-pollinator insects and arthropods. Bycatch was not identified during the scope of this study.

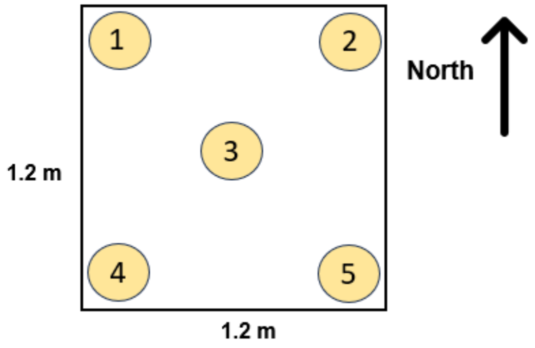


Figure 8
Pan trap configuration
Source : Five yellow pan traps were laid out, with one trap in each corner of the plot and one in the centre as part of an experiment to capture which pollinator insect families forage on *S. canadensis* on revegetated mine sites. Yellow pans were selected for the colour proximity to *S. canadensis* inflorescences. Pans were collected after 24 hours.

The Noranda 5-1 and LaRonde mine sites had four plots each of *S. canadensis* stems as illustrated in Figure 4. The Noranda 5-2 site had two plots of *S. canadensis* stems that were sampled in this experiment. The *S. canadensis* stems in the additional two plots at the Noranda 5-2 site had no inflorescences and the plots were thus omitted.

The four *S. canadensis* control sites illustrated in Figures 7a through 7d had one plot each based on the limitations of finding accessible control sites with the same *Solidago* species used on the mine sites, sufficient density and comparable mean heights as the mining sites. There are multiple species of *Solidago* that grows in Abitibi-Témiscamingue.

4.7 Insect identification

Between September 2022 and October 2023, 341 vials of insect specimens were mounted. The specimens were then identified to the family level of taxonomy using a binocular (Olympus SZ61 and Olympus SZX16 with digital photography capabilities) and various keys (Marshall, 2012; Williams et al., 2014; Marshall, 2017; Carril & Wilson, 2021; Marshall, 2023). Several taxonomic keys were used to differentiate specimens of Halictidae from Colletidae and Andrenidae.

The Shannon Diversity Index, the Simpson Diversity Index and the Bray-Curtis Index of Dissimilarity were used to measure the diversity of the identified families.

4.7.1 Shannon diversity index

The Shannon diversity index, or Shannon-Weiner index, measures total species richness per plot, or a measurement of α diversity. The Shannon index is more sensitive to the presence of rare species or families (MacInnis et al., 2023). Shannon's index (Equation 4) (Magurran, 2004):

$$\boldsymbol{H' = \sum p_i \, ln p_i}$$

Equation 4 Shannon Index

Where $p_i = n_i/N$; n_i = the abundance of the *i*th species; and N = the total abundance (Magurran, 2004). The Shannon index can be calculated in the *R* software (R Core Team, 2024) using the vegan package (Oksanen et al., 2022).

4.7.2 Simpson's diversity index

The Simpson diversity index is able to rank communities of species with sample sizes as small as 80 individuals, as the estimator is independent of sample size (Magurran, 2004), or a measurement of α diversity. Simpson's diversity index makes no assumption about the underlying species abundance distribution, and thus it is a nonparametric diversity index (Magurran, 2004). The Simpson's index is more weighted towards common species or families (MacInnis et al., 2023). Simpson's index (Equation 5) (Magurran, 2004):

$$\boldsymbol{D} = \sum \boldsymbol{p}_{\boldsymbol{i}}^{\boldsymbol{2}}$$

Equation 5 Simpson's Index

Where p_i = the proportion of individuals in the *i*th species (Magurran, 2004). The Simpson index can be calculated in the *R* software (R Core Team, 2024) using the vegan package (Oksanen et al., 2022).

4.7.3 Bray-Curtis dissimilarity calculator

A measurement of dissimilarity also called the Sorensen dissimilarity index or matrix, or Sorenson distance. This calculates the dissimilarity between family or genera (Bueno et al., 2023), or a measurement of β diversity. The Bray-Curtis distance matrix is useful to assess the significance of differences among sites for overall family assemblages (Mlynarek et al., 2018). It is a form of non-metric Multidimensional Scaling (NMS) ordination for family occurrence data of treatment quarters (Ingerpuu et al., 2019). The Bray-Curtis dissimilarity distance (Equation 6) (Magurran, 2004):

$$\boldsymbol{BC_{ij}} = \mathbf{1} - \frac{\mathbf{2}\boldsymbol{C_{ij}}}{\boldsymbol{S_i} + \boldsymbol{S_j}}$$

Equation 6 Bray-Curtis distance

Where i & j are the two sites; S_i is the total number of specimens counted on site i; S_j is the total number of specimens counted on site j; and C_{ij} is the sum of only the lesser counts for each species found in both sites (Magurran, 2004).

4.8 Measurements on Solidago canadensis plants

At each week of visit, the height in cm (rounded to the closest half cm) of 3 randomly chosen *S. canadensis* stems was measured, and the stage of inflorescence was noted. The heights from the week of August 8-12, 2022 were used in the statistical analyses; the net sweeps negatively impacted the total height of the stems by knocking inflorescences off the stems. The number of stems in each plot was counted during the week of August 15-19, 2022. For the two Noranda sites and all the control sites, we counted the number of individual stems in the plot. This was not possible at the

LaRonde mine site as the *S. canadensis* plots had a large number of stems growing extremely close to one another in each plot. For the LaRonde plots, we counted the number of stems on two adjacent sides of the plot to form a 90-degree angle and multiplied these numbers to calculate the total number of stems in the plot. Accessing the *S. canadensis* stems at the centre of the plot would have been detrimental to the plot and the experiment.

5. STATISTICAL ANALYSIS

All statistical analyses were conducted in R (R Core Team, 2024) using the RStudio platform version 2023.09.1+494 or RStudio platform version 2024.04.0+735. For all analyses the experimental unit is the individual plot (4 plot repetitions by mine site, 1-3 repetition by control site), and the statistical level of significance was fixed at 0.05. The R packages ggplot2, ggpmisc, ggpubr, dplyr, tydiverse, hrbrthemes, viridis, hablar, rstatix, dunn.test, devtools, ggvegan, vegan, and FactoMineR were used.

5.1 Statistical analysis for Asclepias syriaca and Danaus plexippus portion

ANOVA tests with the site as a fixed factor were used to compare the pooled number of leaves, pooled number of stems and pooled total height of *A. syriaca* stems by plot, followed by a post-hoc TukeyHSD test to determine if there were significant differences between specific sites.

Variables at the plant level of *A. syriaca* were then analyzed as predictors of the larval numbers of *D. plexippus*. We used the pooled total height in cm of *A. syriaca* at the plot level, the pooled total number of leaves per plot, and the total number of stems per plot (Table 4) at the peak of *D. plexippus* larval activity and compared these values with the pooled larval numbers for the entirety of the 2021 season using linear regression models. Variables at the soil level were also related to larval numbers of *D. plexippus* in all sites with linear regression models.

Next, ANOVA tests were used to compare the pooled number of eggs and larvae per plot among sites for each week separately in 2021 and 2022. Where there was significance, a post-hoc TukeyHSD test was performed to determine the significance of the differences between specific sites.

When the ANOVA assumptions were not met as the data distribution is not normal, the nonparametric Kruskal Wallis rank sum test was used to determine if differences were statistically significant between mining sites and control sites regarding the survival of

D. plexippus eggs to stage 3 instar larvae, and to stages 4-5 instar larvae. This was followed by a post-hoc Dunn test for pairwise comparisons. The Dunn test was run both with and without the Bonferroni correction.

5.2 Statistical analysis for Solidago canadensis portion

ANOVA with site as fixed factor tests followed a TukeyHSD test were performed to test for statistical differences between mining sites and control sites for the number of individuals (combined for all three dates) of a pollinator family per plot.

A linear model was used to compare the number of *S. canadensis* stems to the total number of pollinator insects for the three follow-up dates combined.

Next, family diversity analyses were conducted using the Shannon Diversity Index and the Simpson Diversity Index, and the community diversity was investigated using the Bray-Curtis Index of Dissimilarity.

5.3 Soil chemical analysis portion

Soil element concentrations were analyzed using ANOVA tests followed by a TukeyHSD test to determine differences in concentration of trace metals and physicochemical properties using site as fixed factor. Soil element concentration analyses included all *A. syriaca* and all *S. canadensis* control sites.

No statistical analyses were run on the foliar elemental concentrations of *A. syriaca* as there was only one analyzed sample by site (no repetitions). A table of concentrations per site is situated in the results section (Table 9).

No trace metal analysis was conducted on *S. canadensis* plant material as it is not the sole source of nectar nutrition for any of the pollinator insects collected.

6. RESULTS

6.1 Asclepias syriaca and Danaus plexippus results

6.1.1 *Asclepias syriaca* observations

During the 2021 season, a total of $n = 619$ *A. syriaca* stems were counted on all investigated sites during the week of July 12-16 (except for the Horne Smelter mine site and the two Noranda-5 sites which were counted during the week of July 19-23). For the 2022 season, a total of $n = 610$ *A. syriaca* stems were counted during the week of July 11-15. The number of stems presented in Table 4 is based on the total pooled number of stems present on each site during the peak presence of *D. plexippus* eggs and larvae. Data presented in section 6.1.1 is based on the measurements in Table 4.

The number of *A. syriaca* stems, the mean height, and the mean number of leaves for both the mining sites and the control sites are presented in Table 4. In general, mine sites had a greater number of stems than in control plots, with a maximum found at Noranda 5-1. The Noranda 5-1 mine site had the greatest number of *A. syriaca* stems out of both the mining and control sites during both years, while the mean height and mean number of leaves per stem at this site closely resemble the other mining sites in Table 4. Only the number of *A. syriaca* stems from the LaRonde mining site resembles the data from the control sites during both years, however, the control sites had a lower number of plots. *Asclepias syriaca* on control sites tended to have mean taller stems, and more leaves compared to the mining sites.

In 2021, the total number of *A. syriaca* stems on mining sites ranged from 18 to 146 stems, with a mean number of 61 stems per site. On the control sites, the total number of stems ranged between 11 and 29, with a mean number of 23 stems per site.

In 2022, the total number of *A. syriaca* stems on mining sites ranged from 18 to 149 stems, with a mean number of 64 stems per site. On the control sites, the total number of stems ranged between 12 and 59, with a mean number of 40 stems per site.

The mean height of *A. syriaca* stems in 2021 ranged from 1.99 cm (Lapa) to 52.59 cm (LaRonde) on the mine sites, with a mean height overall of 9.81 cm. On the control sites, the mean height ranged from 47 cm to 60.98 cm, with a mean overall height of 51.76 cm.

During the 2022 season, the mean height of *A. syriaca* stems ranged from 4.06 cm (Mine Canadian Malartic) to 73.25 cm (LaRonde) on the mine sites, with a mean height of 16.62 cm. On the control sites, the mean height ranges from 46.67 cm to 78.03 cm, with a mean overall height of 61.62 cm.

The differences between the mining sites and the control sites continue at the leaf-level in Table 4. During the 2021 season, the mining sites ranged from 0.56 leaves (Goldex) to 17.94 leaves (LaRonde), with a mean of 6.2 leaves per stem.

Note that the Goldex site had clear evidence of *D. plexippus* herbivory and the presence of frass in T1 during the week of July 12-16, 2021, with numerous leaves completely consumed by a missed cohort of earlier *D. plexippus* larvae. As for the control sites, the mean number of leaves per *A. syriaca* stem ranged from 12 to 16.39 leaves, with an overall mean number of leaves of 14.38 leaves per stem.

Finally, during the 2022 season, the mining sites ranged from 0.86 leaves (Notre-Dame Neighbourhood) to 20.61 leaves (LaRonde), with a mean of 7.89 leaves per stem. *Danaus plexippus h*erbivory and frass were observed at both LaRonde and Goldex during this visit. The control sites ranged from 13.41 to 18.98 leaves, with a mean of 16.86 leaves per stem. There was evidence of *D. plexippus* herbivory and frass at Controls 3 and 4.

6.1.2 *Danaus plexippus* observations

There were a total of 195 combined *D. plexippus* eggs and instar larvae observed in 2021 and 18 total combined *D. plexippus* egg and instar larvae observed in 2022 (Table 5). The total number of eggs observed on all sites combined was 60 in 2021, while it was only 11 in 2022 (Table 5). Accordingly, between 16 and 33 instar larvae of each stage were observed in 2021, while the number of instar larvae observations varied from 0 to 4 in 2022. During both years, eggs and instar larvae were observed at the LaRonde mine site as well as Controls 2-3 (Table 5). In 2021, no *D. plexippus* were observed on the two following mining sites: Manitou and Lamaque. For the Goldex mine site, no *D. plexippus* were detected on T1 plots during either year of monitoring, although T1 *A. syriaca* had clear evidence of *D. plexippus* specific herbivory and frass at the time of observations, and *D. plexippus* stages 4 and 5 instar larvae were observed on T4 plots.

6.1.3 Phenological activity of *Danaus plexippus*

All stages of instar larvae were detected on some sites during the first week of observation beginning on June 23, 2021 (Table 6), indicating that the first cohort was oviposited 7-20 days prior and that the arrival of the first cohort was missed. More precisely, the Noranda 5-1 and Noranda 5-2 sites had various stages of *D. plexippus* instar larval development during the first week of data collection, including stage 5 instar larvae, and Canadian Malartic mine site showed stage 1 instar larvae. The presence of stages 4 and 5 instar larvae at the Noranda 5 sites during the week of June 23 indicate an early June arrival for the first cohort.

Table 4

Number of *Asclepias syriaca* stems & leaves with mean stem height

Total number of *A. syriaca* stems and leaves, along with the mean stem height measured during the peak weeks of *D. plexippus* presence (week of July 12-16, 2021 and week of July 11-15, 2022) at 8 revegetated mine sites and 4 roadside control sites. Mine sites received treatment of 16 seedlings per plot in 2019, controls received none. Table represents the total numbers per plot per year.

Site	**Total # *A. syriaca* stems per site '21**	**Total height all stems cm '21**	**Mean height cm '21**	**Total # leaves '21**	**Mean # leaves per stem '21**	**Total # *A. syriaca* stems per site '22**	**Total height all stems cm '22**	**Mean height cm '22**	**Total # of leaves '22**	**Mean # leaves per stem '22**
Horne Smelter (4 plots)	53	302.5	**5.71**	336	**6.34**	37	155.0	**4.19**	216	**0.86**
Noranda 5-1 (4 plots)	146	756.2	**5.18**	508	**3.48**	149	971.0	**6.52**	1083	**7.27**
Noranda 5-2 (4 plots)	41	131.8	**3.21**	141	**3.43**	39	153.5	**17.06**	242	**6.21**
Goldex (2 plots)	48	162.8	**3.39**	27	**0.56**	54	315.0	**5.83**	247	**4.57**
Mine Canadian Malartic (4 plots)	48	229.4	**4.78**	341	**7.10**	67	272.0	**4.06**	507	**7.57**
LaRonde (4 plots)	18	946.7	**52.59**	323	**17.94**	18	1318.5	**73.25**	371	**20.61**
Lamaque (4 plots)	87	593.2	**6.82**	785	**9.02**	87	474.5	**5.45**	710	**8.16**
Manitou (4 plots)	39	179.1	**4.59**	224	**5.74**	NA	NA	**NA**	NA	**NA**
Lapa (4 plots)	71	141.9	**1.99**	158	**2.23**	NA	NA	**NA**	NA	**NA**
Control 1 (1 plot)	11	517.0	**47.0**	132	**12.0**	12	560.0	**46.67**	161	**13.41**
Control 2 (3 plots)	28	1707.3	**60.98**	459	**16.39**	36	2089.5	**58.04**	641	**17.81**
Control 3 (3 plots)	29	1370.5	**47.26**	428	**14.76**	52	3314.5	**63.73**	914	**17.25**
Control 4 (2 plots)	NA	NA	**NA**	NA	**NA**	59	4604.0	**78.03**	1120	**18.98**

Table 5

Number of observations of the different life stages of *Danaus plexippus* for each sampling year at each site

Total number of *D. plexippus* eggs and larval instar stages found on a mining revegetation experiment, broken down per site, per stage of development, and per year. Total figures represent the combined total of eggs and larval instar stages.

	Larval stage	**HRN**	**NOR 5-1**	**NOR 5-2**	**GOL**	**MCM**	**LAR**	**LAM**	**MAN**	**LAP**	**CTR 1**	**CTR 2**	**CTR 3**	**CTR 4**	**ALL**
2021	Egg	4	6	3	0	3	11	0	0	0	18	4	11	NA	***60***
	1st instar	0	6	1	0	0	3	0	0	1	0	1	4	NA	***16***
	2nd instar	0	12	3	0	5	2	0	0	0	2	2	4	NA	***30***
	3rd instar	0	8	0	0	8	6	0	0	1	1	4	3	NA	***31***
	4th instar	0	1	1	0	4	4	0	0	6	1	5	3	NA	***25***
	5th instar	0	6	0	0	7	0	0	0	9	2	6	3	NA	***33***
2021	**Total eggs and instar larvae**	**4**	**39**	**8**	**0**	**27**	**26**	**0**	**0**	**17**	**24**	**22**	**28**	**-**	***195***
2021	Egg	0	0	0	0	0	1	0	NA	NA	0	2	2	6	***11***
	1st instar	0	0	0	0	0	0	0	NA	NA	0	0	0	0	***0***
	2nd instar	0	0	0	0	0	1	0	NA	NA	0	0	0	1	***2***
	3rd instar	0	0	0	0	0	0	0	NA	NA	0	0	1	3	***4***
	4th instar	0	0	0	0	0	0	0	NA	NA	0	0	0	0	***0***
	5th instar	0	0	0	0	0	1	0	NA	NA	0	0	0	0	***1***
2022	**Total eggs and instar larvae**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**-**	**-**	**0**	**2**	**3**	**10**	***18***

A second distinct cohort of *D. plexippus* eggs in 2021 can be identified during the weeks of July 5 through July 19 in Figure 9. This second cohort was observed on different sites than the first cohort: LaRonde, Mine Canadian Malartic, and all of the control sites hosted the second cohort, while Lapa, Horne Smelter, and the two Noranda 5 sites were largely excluded from this second cohort.

The peak of *D. plexippus* eggs and larval presence occurred during the weeks beginning July 5 and July 12 in 2021 (Figure 9), and the weeks beginning July 4 and July 11 in 2022. Data from 2022 is not represented in Figure 9 due to too low number of observations. Some sites were not visited during certain weeks of 2021: the two Noranda-5 sites had *D. plexippus* larval presence during the week of June 21, 2021, but were not visited the following week. Lapa had *D. plexippus* presence during the week of June 28, 2021, but was not visited the previous week.

Table 6

Mine sites with *Danaus plexippus* detected week of June 23, 2021

Table with the number of D. plexippus observed during the first week of a mine site revegetation experiment in 2021. Results in the instar larval stage column indicate that the arrival of a first generation of D. plexippus was missed on the two Noranda-5 sites and the Mine Canadian Malartic site in 2021.

Site	Eggs (Pooled)	Instar larval stages (Pooled)
Horne Smelter	0	0
Noranda 5-1	0	6 x stage 1 10 x stage 2 3 x stage 3 2 x stage 5
Noranda 5-2	2	1 x stage 1 3x stage 2 1x stage 4
Goldex	NA	NA
Mine Canadian Malartic	0	5 x stage 1
LaRonde	NA	NA
Lamaque Eldorado Gold	0	0
Manitou	0	0
Lapa	NA	NA

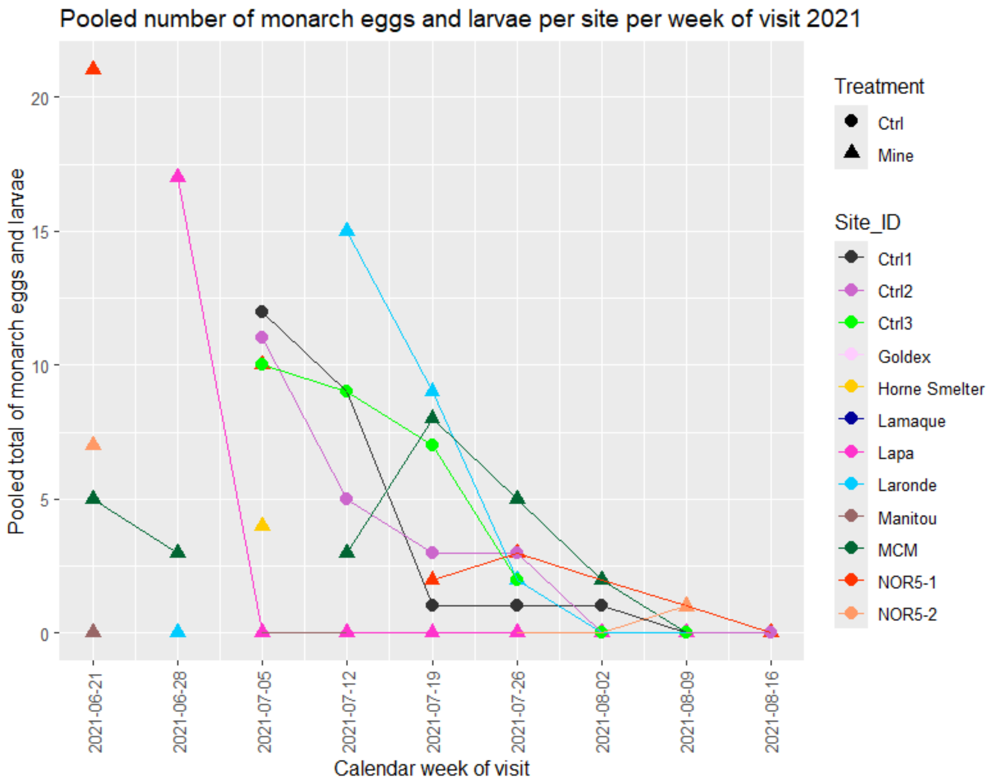


Figure 9
Pooled number of *Danaus plexippus* eggs and larvae per site per week of visit in 2021
Descriptive illustration of monarch eggs and larval instar presence during 2021. Monarch presence was observed during the week of June 21, 2021, with a second cohort of monarchs observed peaking between the weeks of July 5 through July 19, 2021. Mine sites are represented by triangles, control sites by circles. No statistical analysis was conducted for this illustration.

6.1.4 *Danaus plexippus* survival

There was no marked difference in the number of *D. plexippus* larvae and their survival between some mine sites and control sites. There was a lot of variability in the results among mine sites, including several mine sites without any presence of *D. plexippus* (Lamaque Eldorado Gold, Manitou).

Sites were visited on a once per week basis between June 23 and August 16, 2021. A total of 7 visits to the sites were conducted over this 9-week period. If no *D. plexippus* ova or instars were detected one week, the site was omitted from data collection the following week, then revisited the week after (2 weeks of interval for data collection). Data collection ceased once no *D. plexippus* larval presence was detected at all mining sites and control, suggesting that the reproductive cohort had matured, and adult *D. plexippus* were not the focus of this study.

Table 7 compiles the survival rates from eggs to mobile instar larvae (stages 4-5), and from eggs to immobile instar larvae (stage 3) in 2021, pooled per site, applying the omissions mentioned above if the site was not visited one week but a stage 3 instar larvae or stages 4-5 instar larvae were observed the following week. Statistical tests were not run on the 2022 *D. plexippus* survival data due to the small number of eggs and larvae observed. A Kruskal-Wallis rank sum test ($Chi^2 = 13.554$, df = 7, p = 0.0597) on the 2021 data followed by a post-hoc Dunn test without the Bonferroni correction indicated that the site did have a significant impact on predicting the percentage of *D. plexippus* eggs surviving to stage 3 instar larvae (Table 7).

For stages 4-5 instar larvae, a Kruskal-Wallis rank sum test ($Chi^2 = 21.359$, df = 11, p = 0.03) followed by a post-hoc Dunn test without the Bonferroni correction showed that the site had a more significant impact in 2021 on predicting the percentage of *D. plexippus* eggs surviving to stages 4-5 instar larvae (Table 7).

Table 7

Survival rate for *Danaus plexippus* from egg to instar larvae 3, and from egg to instar larvae stages 4-5 for 2021

NA if 0 eggs detected but instar larvae later detected, 0% if eggs detected but no instar larvae detected, and – if no eggs or instar larvae detected. There was a statistical difference between sites for stage 3 survival, as well as stages 4-5 survival. (Numbers in parentheses indicate the number of eggs / number of stages 3 found, or number of combined stages 4-5.)

Site	Eggs	Stage 3 individuals	Stage 3 survival %	Stages 4-5 individuals	Stages 4-5 survival %
Horne Smelter	4	0	0% (*d*)	0	0% (*d*)
Noranda 5-1	6	1	17% (*c*)	4	67% (*b*)
Noranda 5-2	3	1	33% (*b*)	0	0% (*d*)
Goldex	0	0	-	0	-
Mine Canadian Malartic	3	4	133% (*a*)	7	233% (*a*)
LaRonde	11	4	36% (*b*)	*0*	0% (*d*)
Eldorado Gold Lamaque	0	0	-	0	-
Manitou	0	0	-	0	-
Lapa	0	6	NA	9	NA
Ctrl 1 (Rouyn-Noranda)	18	1	6% (*d*)	2	11% (*c*)
Ctrl 2 (Rouyn-Noranda)	4	5	125% (*a*)	4	100% (*a*)
Ctrl 3 (Val-d'Or)	11	1	9% (*d*)	2	18% (*c*)

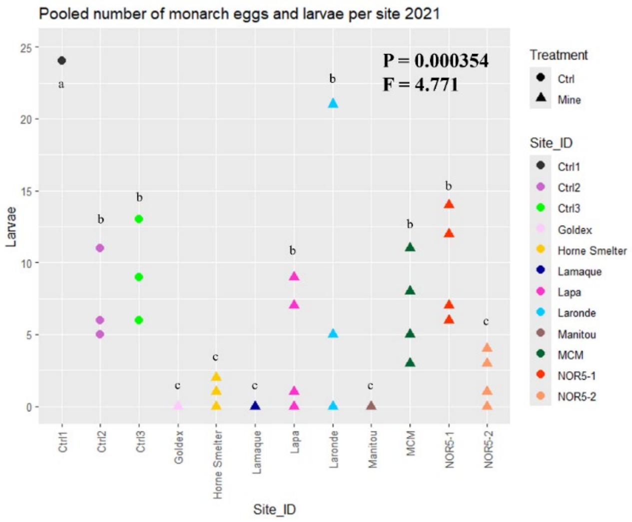
6.1.6 Sites as predictors of *Danaus plexippus* presence

Figure 10 represents the pooled totals per site and per plot, with each coloured dot representing a different plot. An ANOVA test showed that the site was an important predicting factor for observing the presence of *D. plexippus* eggs or larvae (2021 had F = 4.771 and p = 0.000354, while 2022 had F = 4.672 and p = 0.000952). The results of the TukeyHSD test are labelled on Figure 10 to show which sites resemble each other. The mining sites with the most *D. plexippus* eggs and larvae in 2021 were Noranda 5-1 (39), Mine Canadian Malartic (27), LaRonde (26) and Lapa (17) but their numbers resembled controls 2 and 3. The other mine sites (Manitou, Horne Smelter, Lamaque, Noranda 5-2 and Goldex) had significantly lower numbers of eggs and larvae. The control sites each had between 22 and 28 eggs and larvae.

There were so few eggs and larvae observed in 2022 (Figure 10) that the TukeyHSD test failed to detect significant differences between sites with 0 observations (the mine sites of Goldex, Lamaque Eldorado Gold, Mine Canadian Malartic, both Noranda 5 sites, Horne Smelter, and Control 1) and sites with small numbers of observations (Controls 2 and 3 resemble each other, LaRonde and Control 3 resemble each other). Only Control 4 was truly distinct in 2022 according to the TukeyHSD test. During the 2022 season, Control 1 had no detected presence of *D. plexippus*, whereas at the other 3 control sites, we detected few eggs and larvae. This is a stark difference, as during the 2021 season, Control 1 had the most *D. plexippus* larvae.

Figure 10 illustrates the presence of *D. plexippus* eggs and larvae on the mine and control sites each week during the 2021 season. An ANOVA followed by a post-hoc TukeyHSD test were run for each week of data to determine if there were differences between the mining sites and control sites on a weekly basis. The p-value for each week is shown in Figure 10, with the results of the post-hoc TukeyHSD test indicated with letters to differentiate between sites.

a) 2021



b) 2022

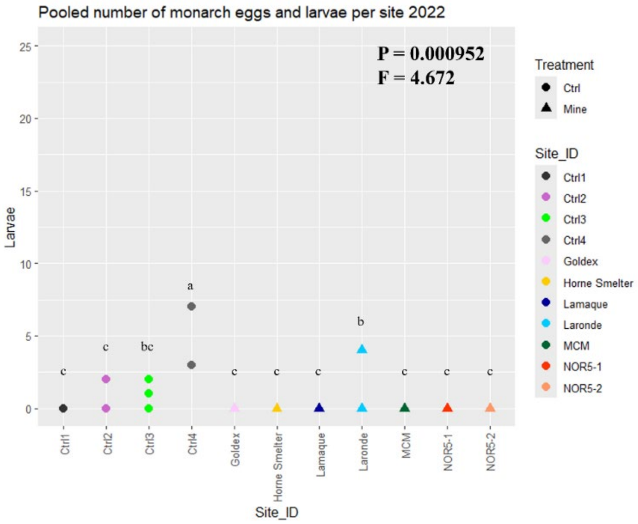


Figure 10

Pooled number of *Danaus plexippus* eggs and larvae in T1 plots per site for (a) 2021 and (b) 2022

Illustration of the number of *D. plexippus* eggs and total number of larvae plots pooled together, for mine sites revegetated with *A. syriaca* and roadside control sites. ANOVA test with p-values and F-statistic for each year. Results of post-hoc TukeyHSD test identified from highest value to lowest value (a, b, c, etc.) to identify similar sites.

The results illustrated in Figure 11 show that we detected the presence of *D. plexippus* larvae on different mine and control sites each week during the 2021 season. Once the control sites were introduced during week 3 of monitoring (week of July 5, 2021), we detected the presence of *D. plexippus* larvae consistently on at least one of these sites each week until week 7 (week of August 2, 2021). Comparing the differences between sites week over week, there were significant p-values for weeks 1, 3, 4, and 7. The graphs in Figure 11 show that even during the weeks when the p-value was $p > 0.05$, there were still sites with important differences compared to the other sites.

During week 1 (Figure 11), Noranda 5-1 was distinct from all other sites as *D. plexippus* eggs or larvae were observed on 3 of the 4 plots. Mine Canadian Malartic and Noranda 5-2 resembled each other as they both had *D. plexippus* on two plots, and these two sites had comparable numbers of *D. plexippus*. All other sites lacked observations of *D. plexippus* during week 1.

The differences between sites continued to be significant during week 2. The Lapa mine site was distinct from all other sites in week 2 (Figure 11), as *D. plexippus* eggs or larvae were present and abundant on 2 plots. Mine Canadian Malartic was also distinct from all other sites during week 2, as *D. plexippus* eggs or larvae were observed albeit in significantly lower numbers than at Lapa. All other sites lacked *D. plexippus* eggs or larvae observations during week 2.

Control sites were included in the dataset as of week 3, and all the control sites had *D. plexippus* eggs or larvae observations during this week. Control 1 was distinct from all the control sites as well as all the mining sites during week 3, with a greater number of *D. plexippus* individuals. Controls 2 and 3 resembled the Noranda 5-1 site, with *D. plexippus* observations on two plots at each site. The Horne Smelter also had *D. plexippus* observations during week 3, but at a lower abundance than all other sites with observations. The remaining mining sites had no observations during week 3.

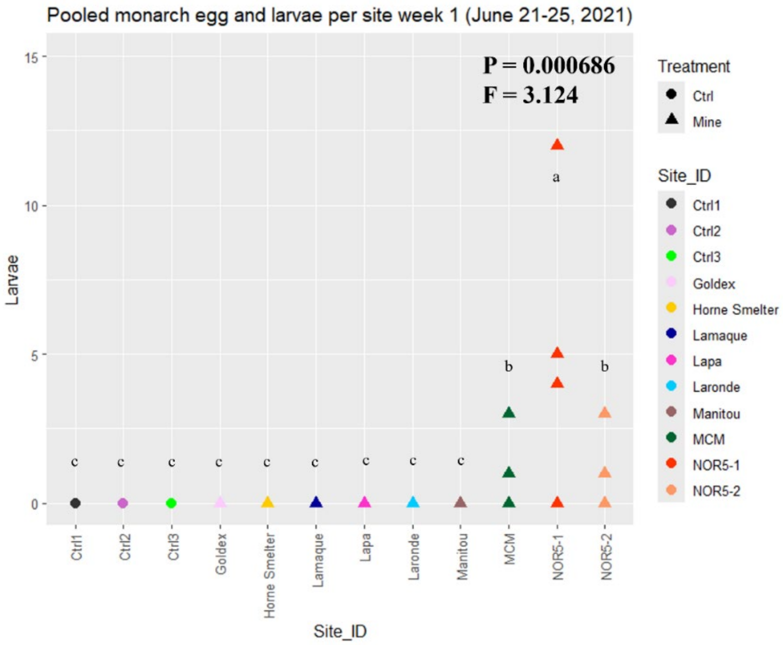
During week 4, LaRonde mine is significantly different from all other sites with greater numbers of eggs and larvae. In second, Control 1 has the greater number of *D. plexippus* eggs and larvae observations during this week, compared to all other sites except LaRonde. Next, we see that all the control sites have *D. plexippus* observations, however, they are all distinct from one another. Control 2 has a small number of *D. plexippus* eggs and larvae and it resembles Mine Canadian Malartic this week. Control 3 is distinct from all other sites this week; it has more *D. plexippus* eggs and larvae than Control 2, but significantly fewer than Control 1. Finally, all the other mine sites (except for LaRonde and Mine Canadian Malartic) have no *D. plexippus* eggs or larvae and are indistinguishable between themselves.

The p-values for weeks 5 and 6 were $p > 0.05$, thus the statistical differences between sites during weeks 5 and 6 were not significant.

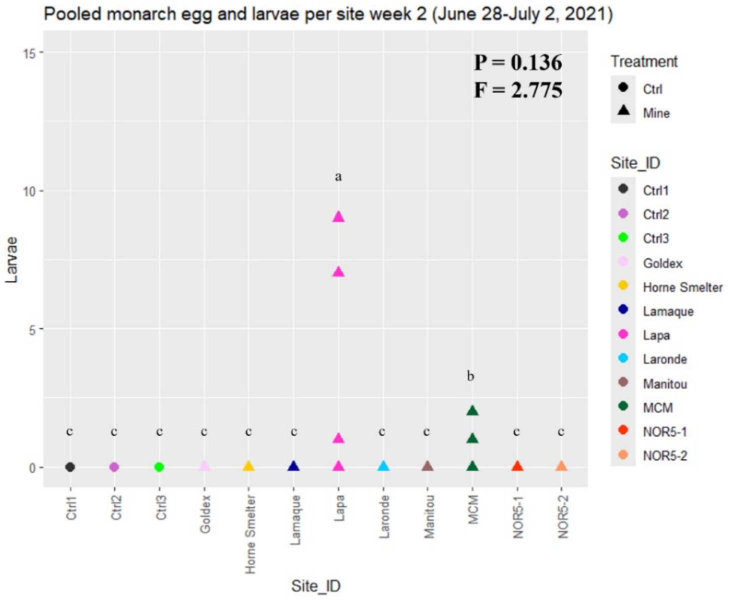
Week 7 is the last time that the differences between sites are significant ($p = 0.0065$). The distinct sites were Mine Canadian Malartic, Noranda 5-1 and Control 1 for having observations of *D. plexippus* eggs or larvae (Figure 11) while all other sites lacked observations.

The p-values for weeks 8 and 9 indicate that there were no significant differences between the various sites during the end of the season. Monitoring ended after all sites had zero observations of *D. plexippus* during week 9, indicating the end of the cohort.

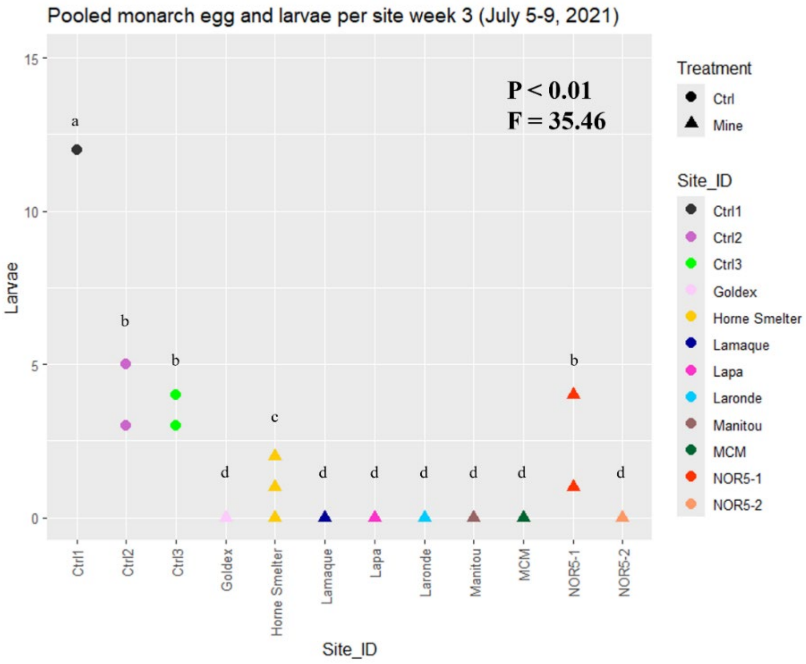
a) Week 1 (June 21-25, 2021)



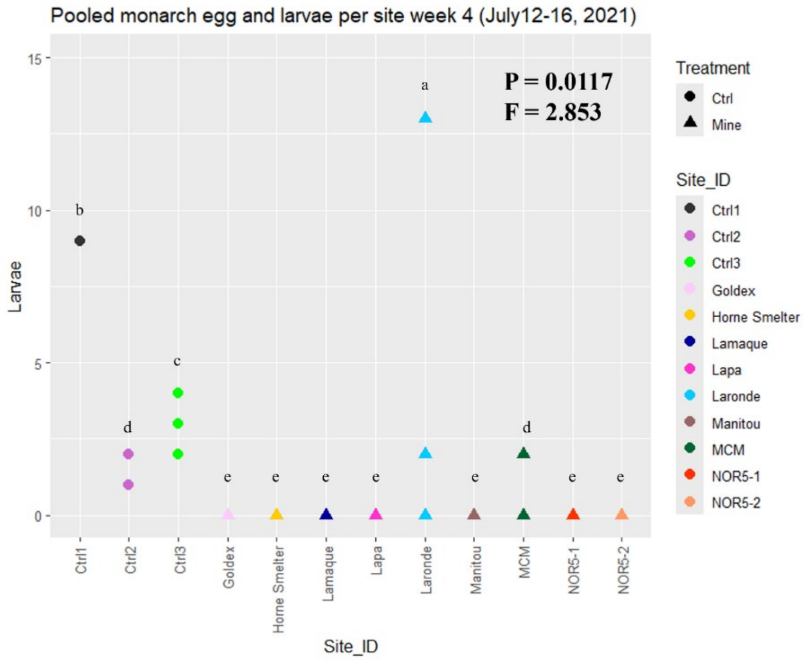
b) Week 2 (June 28-July 2, 2021)



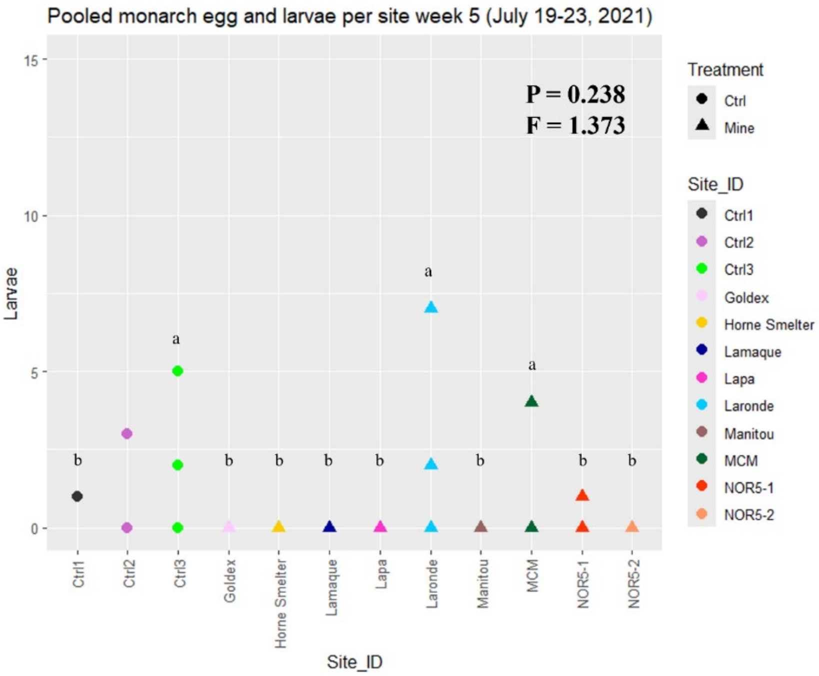
c) Week 3 (July 5-9, 2021)



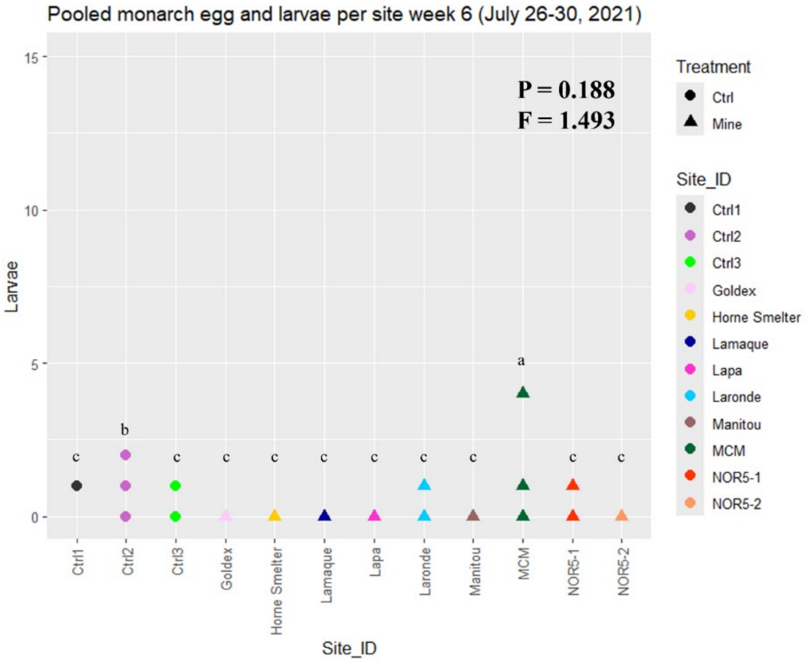
d) Week 4 (July 12-16, 2021)



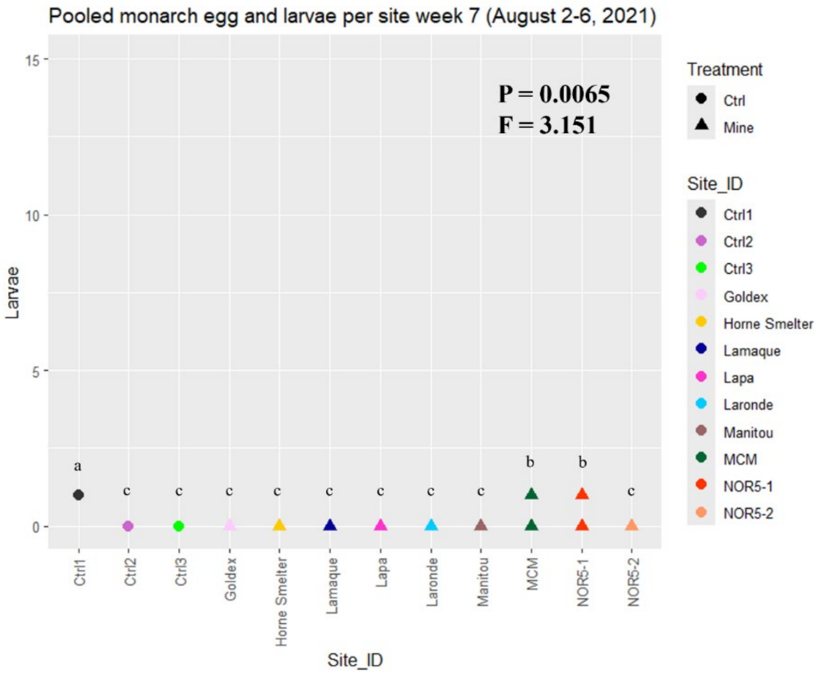
e) Week 5 (July 19-23, 2021)



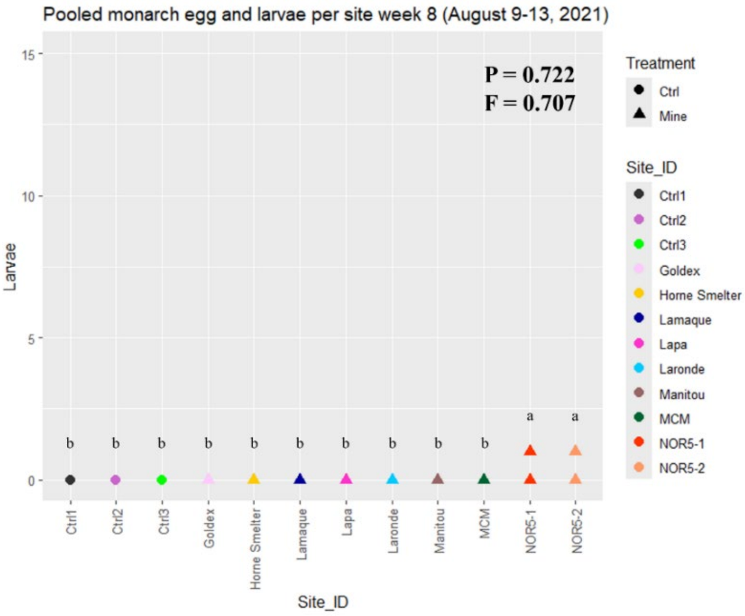
f) Week 6 (July 26-30, 2021)



g) Week 7 (August 2-6, 2021)



h) Week 8 (August 9-13, 2021)



i) Week 9 (August 16-20, 2021)

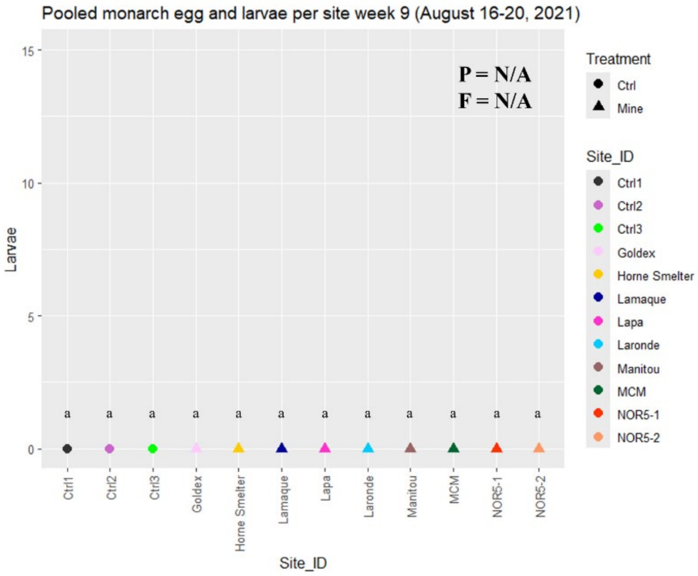


Figure 11

Pooled number of *Danaus plexippus* eggs and larvae per site on a weekly basis for 9 weeks (Figures 11a through 11i) for 2021

Nine figures illustrating the total pooled number of monarch eggs and caterpillars per week over the 9 weeks of observations in 2021 on revegetated mine sites and roadside control sites. ANOVA test with p-values and F-statistic for each week included in the graphs. Results of post-hoc TukeyHSD test identified from highest value to lowest value (a, b, c, etc.) to identify similar sites.

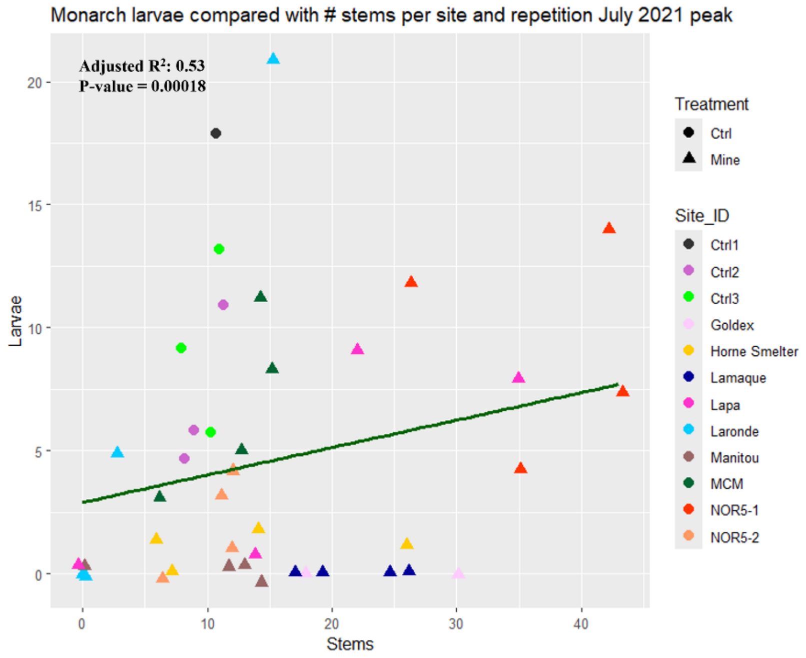
6.1.7 *Asclepias syriaca* characteristics as predictors of *Danaus plexippus* presence

The three *A. syriaca* variables each have a positive linear relation with the total number of *D. plexippus* observed (Figure 12). The variable with the most significant positive relationship to *D. plexippus* larval presence was the total pooled height of the stems in a plot during the peak of *D. plexippus* larval development.

a)


Monarch larvae compared with # leaves per site and repetition, July 2021 peak
Adjusted R²: 0.28
P = 0.03
Pooled larvae 2021 season
0
5
10
15
20
0
100
200
Pooled # leaves per repetition mid July
Treatment
Ctrl
Mine
Site_ID
Ctrl1
Ctrl2
Ctrl3
Goldex
Horne Smelter
Lamaque
Lapa
Laronde
Manitou
MCM
NOR5-1
NOR5-2

b)


Monarch larvae compared with # stems per site and repetition July 2021 peak
Adjusted R²: 0.53
P-value = 0.00018
Larvae
0
5
10
15
20
0
10
20
30
40
Stems
Treatment
Ctrl
Mine
Site_ID
Ctrl1
Ctrl2
Ctrl3
Goldex
Horne Smelter
Lamaque
Lapa
Laronde
Manitou
MCM
NOR5-1
NOR5-2

c)

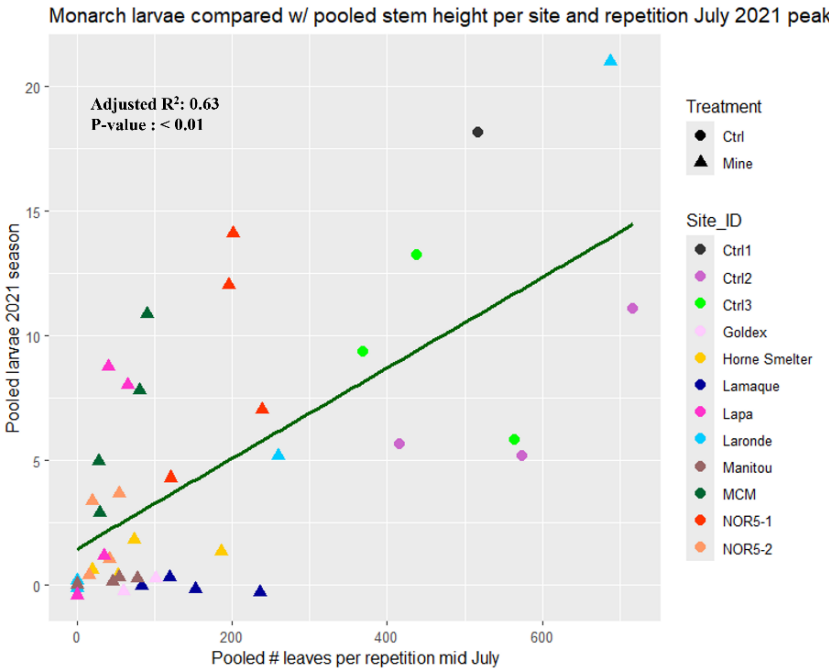


Figure 12

Total number of *Danaus plexippus* larvae by repetition compared with pooled number of (a) *A. syriaca* leaves, (b) *A. syriaca* stems, (c) cumulative *A. syriaca* height during the second week of July 2021

Three linear models comparing the number of *D. plexippus* eggs and caterpillars against the number of *A. syriaca* leaves, number of *A. syriaca* stems, and the total height of *A. syriaca* stems per plot. All *A. syriaca* level variables had p-values that indicate a significant impact on the quantity of *D. plexippus* observed, with the most significant variable being the total height of the available *A. syriaca* stems on a given plot

6.2 Asclepias syriaca foliar chemical analysis.

No statistical analysis was conducted on the *Asclepias syriaca* foliar chemical analysis as only one mixed sample per site was available due to a lack of biological material. Table 8 illustrates the pooled sample concentrations in total trace elements in µg/g for the four *Asclepias syriaca* control sites and the 7 mining sites sampled in 2022. Values for total nitrogen concentrations are represented as a percentage of dry mass.

Table 8
Asclepias syriaca foliar chemical analysis for 2022

Site	N %	Al µg/g	As µg/g	Bo µg/g	Ba µg/g	Be µg/g	Ca µg/g
AS_Ctrl1	3.16	53.3	11.0	10.2	5.3	< 0.05	8148
AS_Ctrl2	2.36	51.4	11.1	16.7	20.6	< 0.05	13614
AS_Ctrl3	2.88	173.5	23.4	11.7	38.9	< 0.05	13148
AS_Ctrl4	3.52	103.8	2.8	21.1	6.4	< 0.05	13731
Horne	2.43	411.6	31.2	22.4	29.6	< 0.05	12041
Noranda 5-1	1.96	12913.9	31.6	22.5	80.5	0.37	20566
Noranda 5-2	1.73	10410.2	1.7	12.7	79.5	0.25	11650
Goldex	1.84	121.7	20.9	7.2	11.0	< 0.05	11010
MCM	2.18	2344.4	20.1	8.2	19.1	< 0.05	12506
LaRonde	2.74	281.7	13.9	16.4	5.6	< 0.05	13229
Lamaque	2.96	601.3	13.9	18.5	1.5	< 0.05	13211

Site	Cd µg/g	Co µg/g	Cr µg/g	Cu µg/g	Fe µg/g	K µg/g	Mb µg/g
AS_Ctrl1	< 0.07	0.16	1.25	19.3	127	34414	< 2.5
AS_Ctrl2	< 0.07	0.12	1.02	22.5	155	34430	< 2.5
AS_Ctrl3	< 0.07	0.22	2.36	13.2	299	25846	< 2.5
AS_Ctrl4	0.12	0.16	2.15	35.9	225	28820	< 2.5
Horne	1.19	1.24	2.98	312	813	14662	< 2.5
Noranda 5-1	< 0.07	6.28	32.88	31.2	13452	19345	< 2.5
Noranda 5-2	0.21	5.44	28.09	21.0	11255	18868	< 2.5
Goldex	< 0.07	0.24	1.46	11.5	237	25799	< 2.5
MCM	< 0.07	1.67	12.76	11.5	3233	19827	< 2.5
LaRonde	< 0.07	0.21	1.56	7.8	379	30378	< 2.5
Lamaque	< 0.07	0.45	1.67	10.6	1091	22504	< 2.5

Site	Mg µg/g	Mn µg/g	Na µg/g	Ni µg/g	P µg/g	Pb µg/g	S µg/g	Sb µg/g
AS_Ctrl1	2650	36.7	66.2	3.39	3609	3.68	3713	< 1.50
AS_Ctrl2	2693	50.3	18.4	2.53	1630	1.83	2884	< 1.50
AS_Ctrl3	2053	56.7	113.1	4.06	1973	< 0.84	2776	< 1.50
AS_Ctrl4	3242	32.3	33.3	1.96	2681	1.68	3019	< 1.50
Horne	4396	76.2	194.3	16.87	1661	15.11	4914	3.5
Noranda 5-1	6952	261.8	353.5	17.11	2054	4.62	1779	< 1.50
Noranda 5-2	7086	233.6	376.3	14.61	2442	3.05	2523	< 1.50
Goldex	1836	53.6	29.1	3.15	1546	< 0.84	2114	< 1.50
MCM	4304	82.8	132.9	7.36	1477	1.07	5030	< 1.50
LaRonde	3107	52.4	26.5	1.07	2107	< 0.84	3011	< 1.50
Lamaque	2819	62.9	27.4	1.09	1241	< 0.84	6473	< 1.50

Site	Se µg/g	Si µg/g	Sn µg/g	Sr µg/g	Ti µg/g	Tl µg/g	V µg/g	Zn µg/g
AS_Ctrl1	1.52	214	< 2.5	37.1	4.9	< 2.5	< 0.50	31.8
AS_Ctrl2	0.85	149	< 2.5	51.8	4.1	< 2.5	< 0.50	42.6
AS_Ctrl3	< 0.80	186	< 2.5	46.2	14	< 2.5	0.51	20.6
AS_Ctrl4	0.91	161	< 2.5	21.8	6.6	< 2.5	< 0.50	34.6
Horne	1.28	582	< 2.5	28.2	28	< 2.5	0.76	184.0
Noranda 5-1	< 0.80	299	< 2.5	36.1	625	< 2.5	26.7	36.0
Noranda 5-2	< 0.80	326	< 2.5	37.0	600	< 2.5	22.4	30.3
Goldex	0.91	198	< 2.5	25.7	7.9	< 2.5	< 0.50	18.8
MCM	< 0.80	248	< 2.5	82.5	192	< 2.5	6.58	30.8
LaRonde	< 0.80	182	< 2.5	17.9	17	< 2.5	0.62	23.8
Lamaque	< 0.80	209	< 2.5	18.6	9.4	< 2.5	1.56	17.2

6.3 Solidago canadensis results

We conducted visual observations at the *S. canadensis* plots before conducting net sweeps. This data was qualitative in nature and does not permit statistical analysis; it was recorded to complement the quantitative data collected. There were more Megachilidae and Syrphidae individuals observed than collected.

6.3.1 *Solidago canadensis* observations

A total of 4458 *S. canadensis* stems were counted during the week of August 22-26, 2022, with a mean height of 99.4 cm (Table 9). All investigated plots had *S. canadensis* with inflorescences except two plots with dwarf sized plants at Noranda 5-2 site (despite being 4 years old), which were omitted from sampling. The *S. canadensis* stems at LaRonde were noticeably taller than at all other sites, with the stems more densely clustered together in the 1.2 m × 1.2 m plots.

Table 9
***Solidago canadensis* observations, week of August 22-26, 2022**

Site	Number of 1.2 m × 1.2 m plots	Pooled number of stems	Mean height in cm, N = 3 stems (± standard deviation)
Noranda 5-1	4	458	100 (±5.29)
Noranda 5-2	2	137	79 (±9.05)
LaRonde	4	3690	138 (±1.16)
SC Control 1	1	35	86 (±7.27)
SC Control 2	1	66	92 (±5.95)
SC Control 3	1	31	100 (±4.40)
SC Control 4	1	41	101 (±1.70)

6.3.2 Abundance of pollinator individuals per site and per family

A total of 203 pollinator insects (*N* = 203) were collected along the three sampling weeks in August 2022, of which 185 were native bees (*N* = 185).

Figure 13 illustrates the total number of insects collected for each site. A total of 23 pollinator insects were collected from the 4 control sites pooled together (4 x 1 repetition). The control sites were pooled as they only had one repetition each due to the natural conditions of the sites.

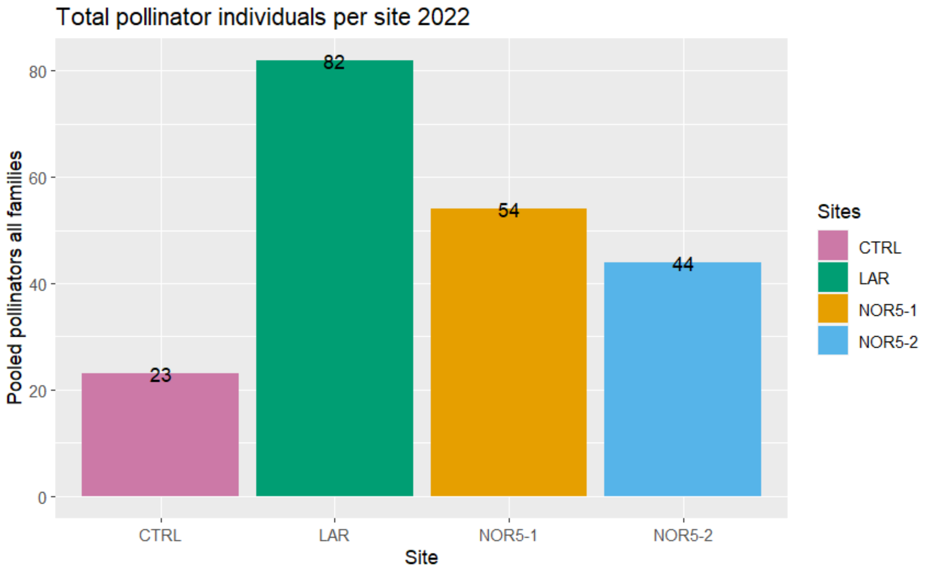


Figure 13
Total number of pollinator individuals per site 2022
Total number of pollinator insects recovered on *S. canadensis* for 3 revegetated mine sites and a pooled total for the roadside control sites. The total number of pollinator insects per site is represented here, with no statistical analysis represented.

The LaRonde mine site had 82 pollinator insects, Noranda 5-1 had 54 pollinator insects, while the Noranda 5-2 mine site had 44 pollinator insects. An ANOVA determined that the site was not significant in predicting the overall total number of pollinator individuals (F = 1.929, p = 0.179).

There were five families of bees (Halictidae, Apidae, Megachilidae, Andrenidae, and Colletidae) and one family of flower flies (Syrphidae) collected from *S. canadensis* on mine sites and roadside control sites. Halictidae was the most abundant bee family (133 individuals), followed by Apidae (39 individuals) and Syrphidae (18 individuals), Megachilidae (7 individuals), Andrenidae (5 individuals) and Colletidae (1 individual) (Figure 14).

Halictidae were most abundant on the three mining sites (Noranda 5-1, Noranda 5-2 and LaRonde) (Figure 15) compared to the other families. At the control sites, we collected Halictidae and Apidae in equal numbers (Figure 15).

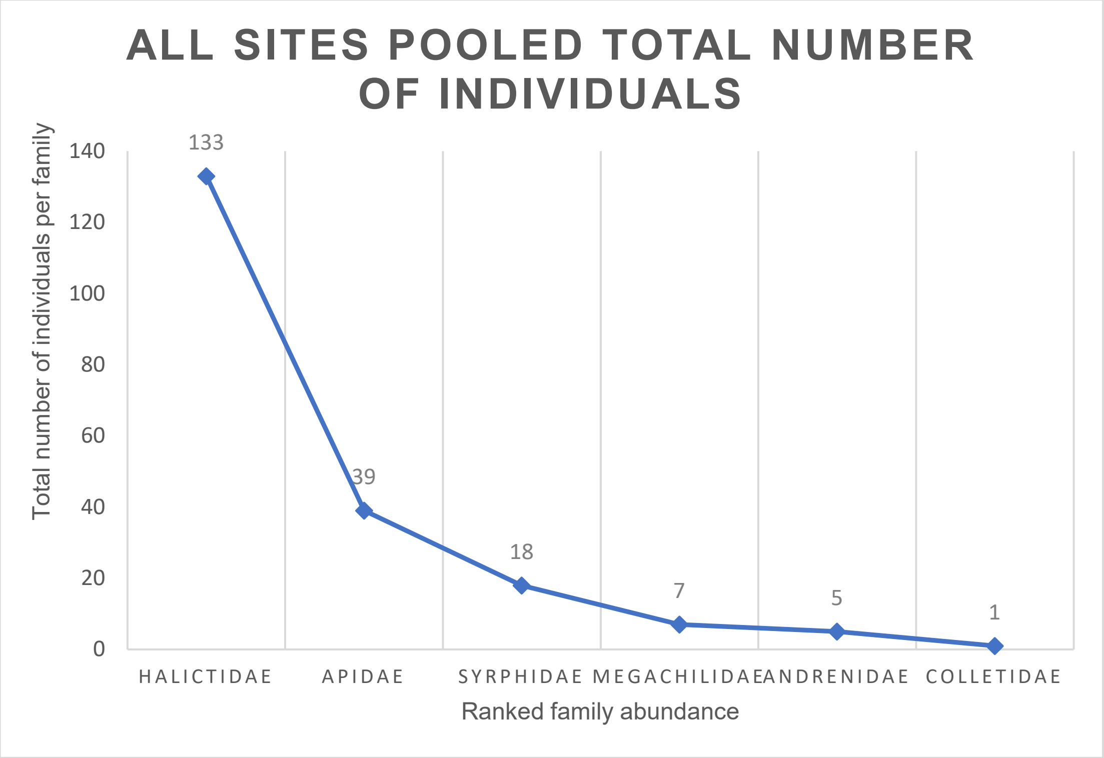


Figure 14
Abundance curve of pollinator individuals per ranked family in 2022
Ranked family abundance data (pooled for all sites) showing the 6 families of pollinator insects collected using yellow pan traps and net sweeps on *S. canadensis* used to revegetate mine sites, as well as roadside control sites.

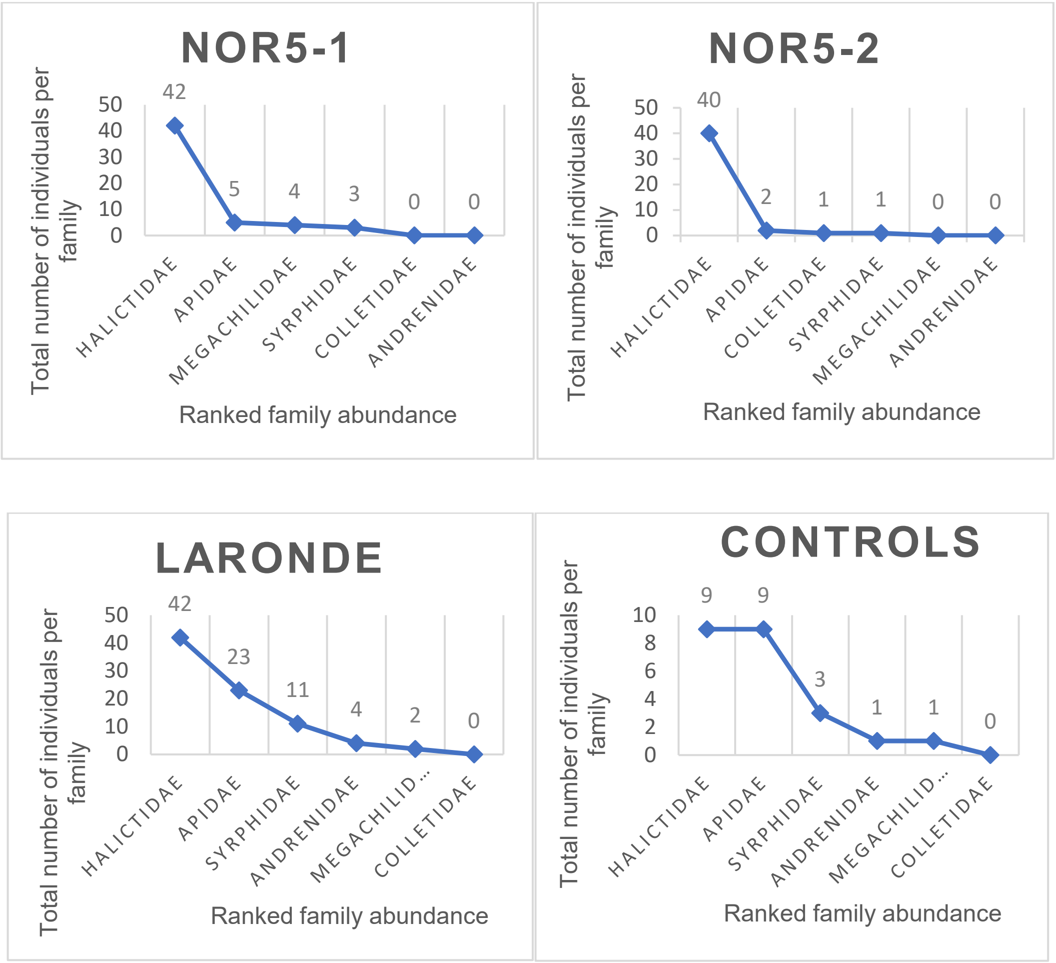


Figure 15
Abundance curve of pollinator insects ranked from most to least abundant families, broken down by site in 2022
Ranked family abundance data for 3 mine sites revegetated with S. canadensis and a pooled graph for all the roadside control sites. There were 6 families of pollinator insects collected using yellow pan traps and net sweeps in this experiment.

6.3.3 Diversity indexes of insect pollinators

The Shannon diversity index (Table 10) ranged from 0.33 at Noranda 5-2 to 1.55 at *S. canadensis* Control 3. *Solidago canadensis* Control 3 had the highest Shannon index of diversity (1.55), with 5 families present but only 7 individual pollinator insects were collected.

Table 10
Shannon and Simpson Diversity indexes of insect pollinators
Table comparing the Shannon and Simpson diversity indexes of collected insect pollinators found at mine sites revegetated with *S. canadensis* and roadside control sites.

Index	NOR5-1	NOR5-2	LAR	CTRL1	CTRL2	CTRL3	CTRL4
Shannon	0.68	0.33	0.98	0.69	0.64	1.55	0.95
Simpson	0.39	0.17	0.56	0.50	0.44	0.78	0.78

According to the Shannon diversity index test, the most diverse mine site was LaRonde (0.98), however, there was a high degree of variability between the different plots at LaRonde, ranging from 2 families to 5 families. *Solidago canadensis* Control 3 was the most diverse site overall (1.55) according to the Shannon diversity index test. Both plots at Noranda 5-2 were the least diverse according to the Shannon diversity index (0.33).

The Simpson Diversity Index (Table 10) had values ranging from 0.17 to 0.78. The Simpson Diversity Index indicated the lowest level of diversity in the two sampled plots at Noranda 5-2 (0.17), and the next most diverse site was the Noranda 5-1 site (0.39). The LaRonde site (0.50) and the control sites (values between 0.44 and 0.78) had comparable levels of diversity between them. Just like the Shannon diversity index test, the Simpson Diversity Index showed the highest level of diversity at *S. canadensis* Control 3 (0.78), a score shared with Control 4.

The minimum Bray-Curtis dissimilarity distance (Table 11) was between Noranda 5-1 and Noranda 5.2 (0.12) and the maximum distance was between Noranda 5-2 and the pooled *S. canadensis* control sites (0.64). The mean distance was 0.40.

Table 11

Bray-Curtis Dissimilarity Distance

Table using the Bray-Curtis Dissimilarity Distance to represent the insect pollinators collected at mine sites revegetated with *S. canadensis* and roadside control sites.

	NOR5-1	**NOR5-2**	**LAR**
NOR5-2	0.12		
LAR	0.24	0.32	
CTRL (pooled)	0.53	0.64	0.56

The two Noranda 5 sites most closely resemble each other with a Bray-Curtis distance of 0.12 total. The control sites show consistently the greatest distance from the mine sites with this dissimilarity analysis, with Bray-Curtis values ranging from 0.53 to 0.64. There is a notable differentiation between the mining sites and control sites.

6.3.4 Halictidae results per site

A total of 133 Halictidae bees were collected (Figure 16), consisting of 65.5% of the pollinator insects collected and 71.9% of the native bees. The Halictidae family was evenly distributed between the three mining sites, with 42 individuals recovered each from the Noranda 5-1 and LaRonde sites, and 40 individuals from the Noranda 5-2 site. The control sites had a pooled total of 9 Halictidae in comparison.

An ANOVA test comparing the number of Halictidae between each site ($F = 1.408$, $p = 0.288$) using the site as fixed factor indicated that the differences between sites was not significant for this family.

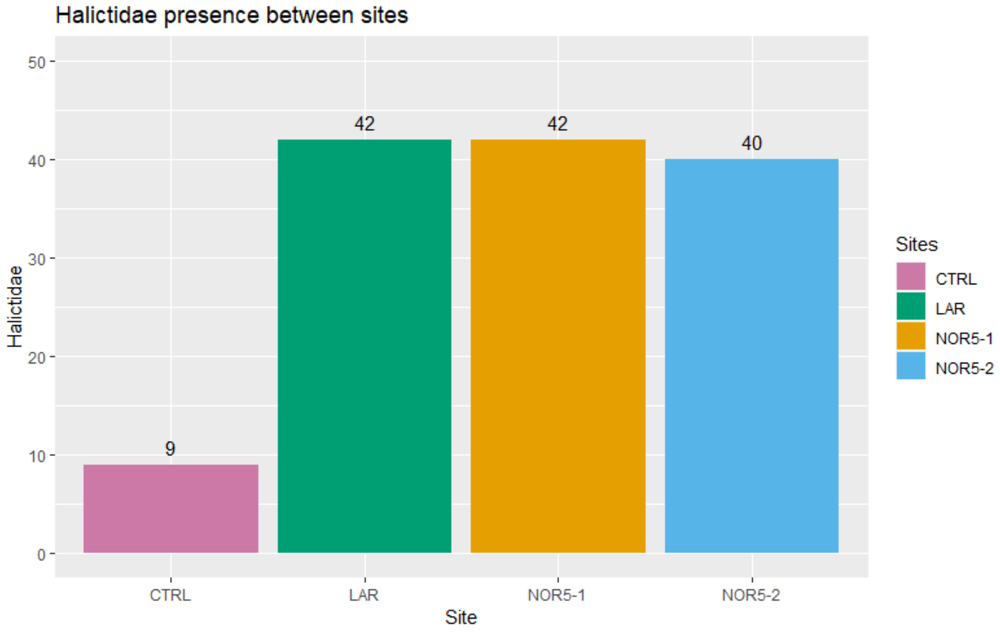


Figure 16
Pooled number of Halictidae per site in 2021
Total number of Halictidae recovered on *S. canadensis* for 3 revegetated mine sites and a pooled total for the roadside control sites. The total number of Halictidae per site is presented here, with no statistical analysis represented.

6.3.5 Apidae results per site

The Apidae family of bees were found at every site (Figure 17), for a total of 39 individuals, representing 19.2% of the pollinator individuals collected and 21.1% of the native bees. The LaRonde mine site had the most Apidae, with 23 individuals. Noranda 5-2 had the least number of Apidae, with 2 individuals.

While Apidae were recovered from all the mine sites, they were not present at every plot. No Apidae were recovered at *S. canadensis* control 1. An ANOVA test comparing the number of Apidae between each site (F = 2.066, p = 0.158) using the site as fixed factor, indicated that the differences between sites were also not significant for this family.

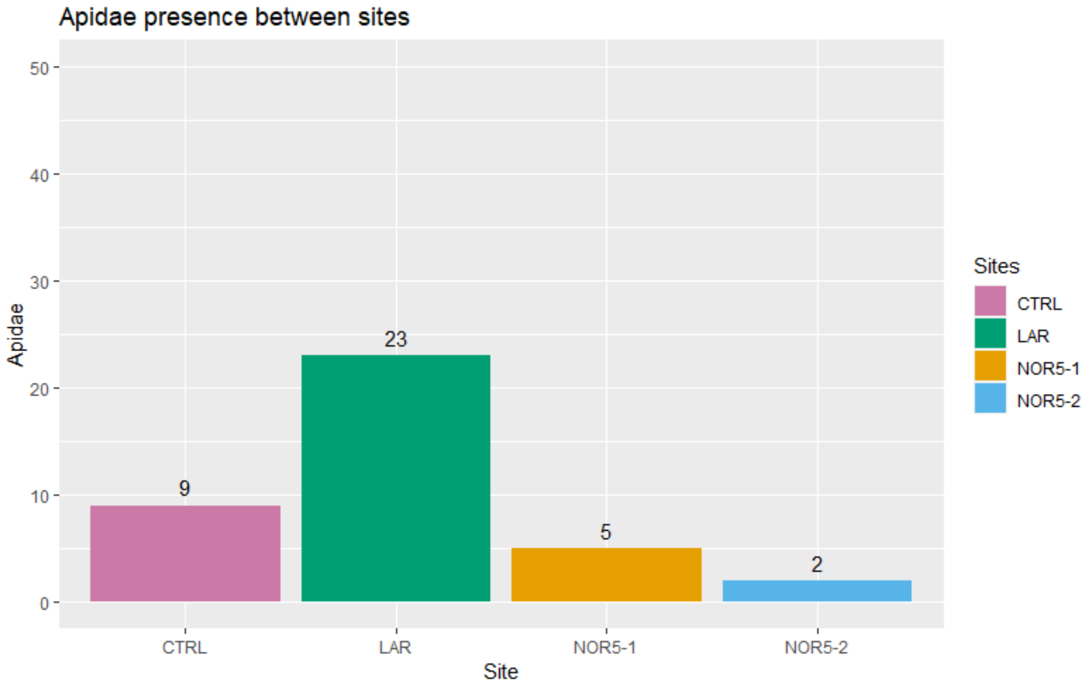


Figure 17

Pooled number of Apidae per site in 2022e

Total number of Apidae recovered on S. canadensis for 3 revegetated mine sites and a pooled total for the roadside control sites. The total number of Apidae per site is presented here, with no statistical analysis represented.

6.3.6 Syrphidae per site

A total of 18 Syrphidae were recovered, consisting of 8.87% of the pollinator insects collected (Figure 18). Syrphidae were present at all the mining sites and three of the four control sites. An ANOVA test comparing Syrphidae presence per site (F = 11.8, p < 0.001) indicated that the differences between sites were significant. The LaRonde site had more Syrphidae (11 individuals) than all the other sites combined. The pooled control sites had the same results as the pooled Noranda 5-1 plots, with 3 Syrphidae each. Noranda 5-2 was distinct from the other sites, with only 1 Syrphidae collected.

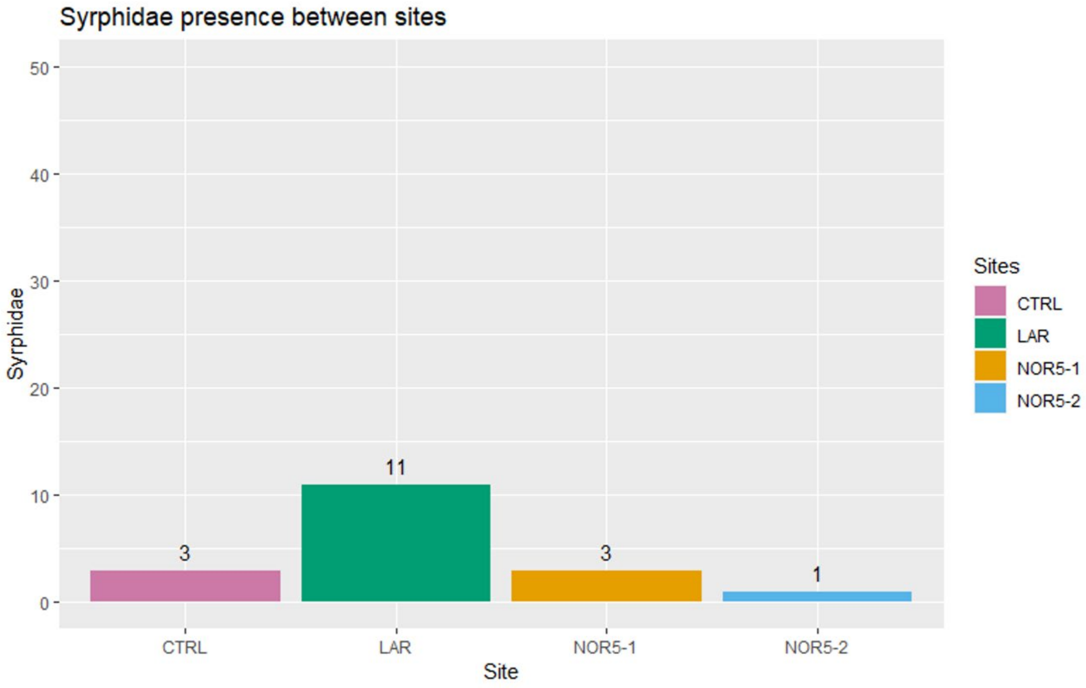


Figure 18
Pooled number of Syrphidae per site in 2022
Total number of Syrphidae recovered on *S. canadensis* for 3 revegetated mine sites and a pooled total for the roadside control sites. The total number of Syrphidae per site is presented here, with no statistical analysis represented.

6.3.7 Colletidae, Andrenidae and Megachilidae results

No statistical analysis was performed on the Colletidae, Andrenidae and Megachilidae families as not enough individuals were collected. Table 12 shows the pooled number of pollinator insects per family per site. A total of one Colletidae bee, five Andrenidae bees, and seven Megachilidae bees were collected, for a total of 13 individuals out of 203 pollinators. These three families represent 6.4% of the total population of pollinator insects found on *S. canadensis* in 2022, or 7% of the native bees.

Table 12
Colletidae, Andrenidae and Megachilidae results
Total number of Colletidae, Andrenidae and Megachilidae insects recovered on *S. canadensis* for 3 revegetated mine sites and a pooled total for the roadside control sites. The total number of individuals per family per site is presented here, with no statistical analysis represented.

Site	Colletidae	Andrenidae	Megachilidae
Noranda5-1	0	0	4
Noranda5-2	1	0	0
LaRonde	0	4	2
Control	0	1	1

6.3.8 Number of *Solidago canadensis* stems impacts on the total number of pollinator individuals

There was a positive correlation between the total number of *S. canadensis* stems and the total number of individual pollinator insects collected ($F_{1,12} = 4.154$, $p = 0.06421$, adj $R^2 = 0.1952$), although the p-value was not within the 95% confidence interval. We can thus reject the hypothesis that the density of *S. canadensis* stems had a positive impact on the number of pollinators present. Figure 19 illustrates that the LaRonde site is clearly driving the significant relationship, as the relationship is heavily impacted by the large number of *S. canadensis* stems present at the LaRonde site. The small sample size was not conducive to a generalized linear model analysis.

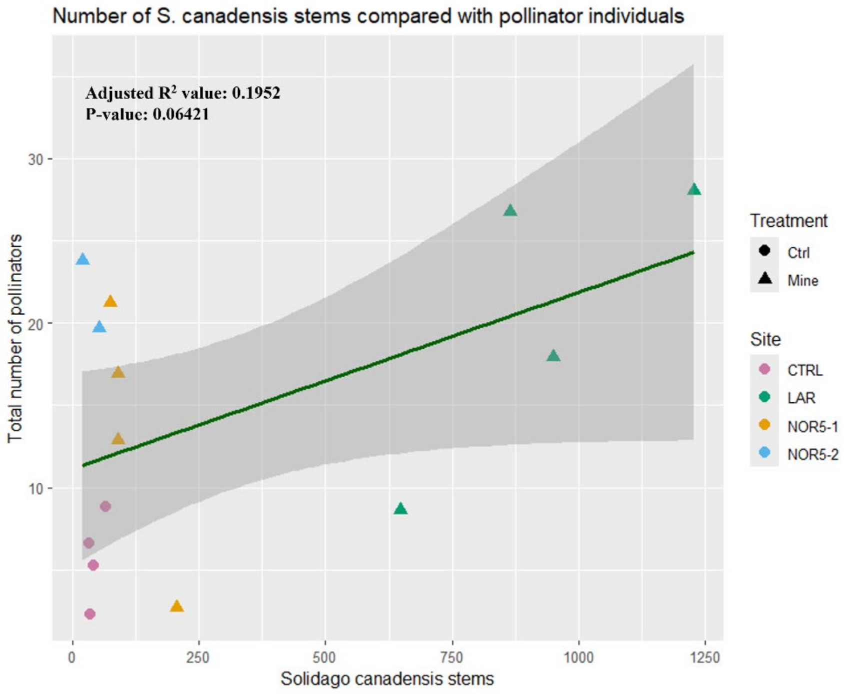


Figure 19

Number of *Solidago canadensis* stems compared with the number of pollinator individuals

Linear regression model of a mining revegetation experiment using S. canadensis, comparing the total number of pollinator individuals per site (mines vs. roadside controls) with the total number of S. canadensis stems per site, with a confidence interval of 95%.

6.3.9 Number of *Solidago canadensis* stems impacts on Apidae presence

There was a significant positive correlation ($F_{1,12} = 9.582$, $p = 0.009267$, adj $R^2 = 0.3977$) between the number of *S. canadensis* stems and Apidae numbers, as illustrated in Figure 19. The LaRonde site is also driving the significant relationship between the number of *S. canadensis* stems and Apidae presence in Figure 20.

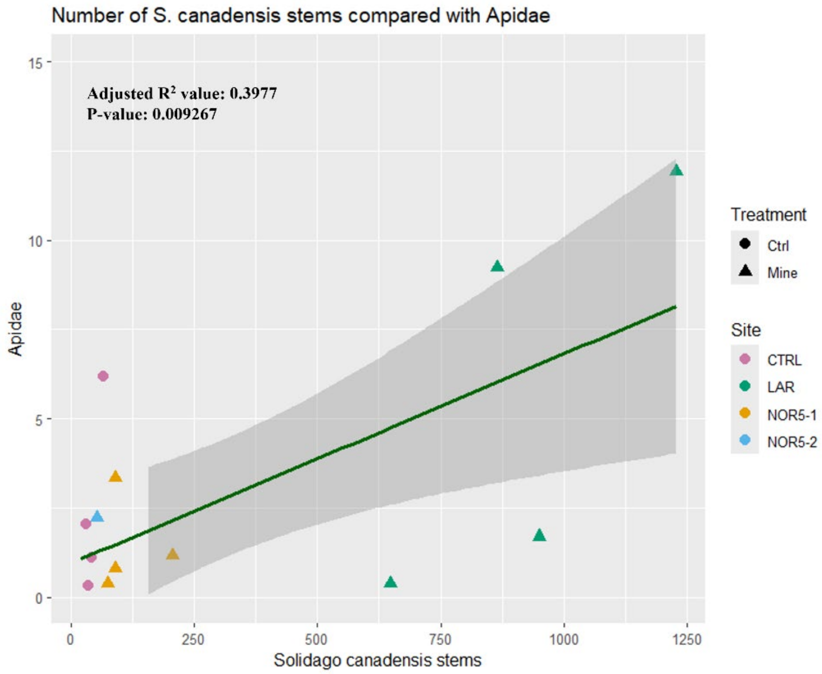


Figure 20
Number of *Solidago canadensis* stems compared with the number of Apidae individuals
Linear regression model of a mining revegetation experiment using *S. canadensis*, comparing the total number of Apidae individuals per site (mines vs. roadside controls) with the total number of *S. canadensis* stems per site, with a confidence interval of 95%.

6.3.10 Number of *Solidago canadensis* stems impacts on Halictidae presence

There was no statistically significant impact of the number of *S. canadensis* stems on the presence of Halictidae bees, as the p-value was 0.44.

6.3.11 Number of *Solidago canadensis* stems impacts on Syrphidae presence

The most significant positive correlation ($F_{1,14} = 25.69$, $p = 0.0001714$ adj $R^2 = 0.6221$) occurred between the number of *S. canadensis* stems and Syrphidae presence, as illustrated in Figure 21. As with Figure 19, which represents all pollinator families, and Figure 20, which represents Apidae, the LaRonde site has a significant impact on the relationship between the number of *S. canadensis* stems and Syrphidae individuals.

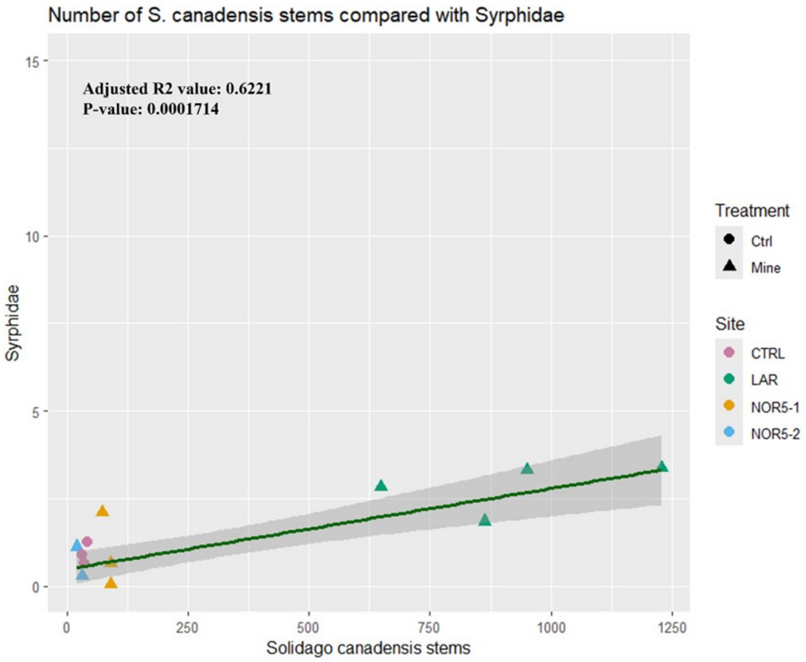


Figure 21

Number of *Solidago canadensis* stems compared with the number of Syrphidae individuals

Linear regression model of a mining revegetation experiment using *S. canadensis*,comparing the total number of Syrphidae individuals per site (mines vs. roadside controls) with the total number of *S. canadensis* stems per site, with a confidence interval of 95%.

6.4 Soil chemistry analysis

The Ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques have guidelines established to prevent soil and groundwater contamination and assure the rehabilitation of the territory (Beaulieu, 2021). The graphs in this section include the thresholds for these element concentration criteria. Where no guidelines are available, no criterion was entered on the graph.

- **Criteria A:** Naturally occurring quantity that one might find in the soil. No restrictions for usage or activities on the soil, illustrated by the hashed green horizontal line in Figures 22 through 24.

- **Criteria B:** Maximum permitted value acceptable for residential use, or maximum permitted amount within the first metre of soil for playgrounds in municipal parks. Approved for residential usage as well as commercial or institutional usage including sensitive usage such as primary and secondary schools, daycare centres (CPE, or centre de la petite enfance), hospitals, long-term care centres (CHSLD, or centre d'hébergement et de soin de longue durée), rehabilitation centres, and detention centres. Illustrated by the hashed orange horizontal line in Figures 22 through 24. When no Criteria B is suggested in the guideline, it will jump from Criteria A to Criteria C directly.
- **Criteria C:** Maximum acceptable limits for industrial, commercial and non-sensitive institutional use, recreational use such as bicycle paths and municipal parks aside from the first metre of soil in playgrounds, road surfaces or sidewalks adjacent to said road surfaces. Illustrated by the hashed red horizontal line in Figures 22 through 24.
- **≥ Criteria:** Soils that surpass Criteria C are only recommended for backfilling and on-site decontamination.

Asclepias syriaca controls are labelled as AS_Ctrl# on the left most portion of the x-axis and *S. canadensis* controls are labelled as SC_Ctrl# on the right most portion of the x-axis of the graph in Figures 22 through 24. The white dot represents the mean concentrations of the trace metal or element for that site.

Federal guidelines (Canadian Council of Ministers of the Environment, 2007) are used for pH and electrical conductivity in [dS/m] in Figure 22 as no Québec provincial guidelines exist. The green hashed line represents the lower guidelines, and the red hashed line represents the upper guidelines. Lower thresholds are indicated for agricultural or residential/parkland usage, while higher thresholds are indicated for commercial or industrial use.

6.4.1 Soil chemical analysis for pH, Sulfur, Sodium, Total salinity, Organic matter and Nitrogen

The federal level guidelines recommend a pH level between 6 and 8 for residential, commercial and industrial use (Canadian Council of Ministers of the Environment, 2007), with a neutral pH (7) represented by a solid blue line in Figure 22. The mining sites all had basic soil with a mean pH higher than 7, except for the Horne Smelter site that had a mean pH below 5 (Figure 22). The Goldex site stood out with the most basic soil with a pH approaching 9, and as the only site exceeding the federal pH recommendation of 6 to 8. The roadside control sites had mostly acidic soil except for *A. syriaca* Control 2, which had basic soil. The Horne Smelter and the *S. canadensis* controls 1 and 4 were below the federal recommendation of pH 6 for acidic soil. Sites were a strong predictor of pH values ($F = 61.51$, $p < 0.05$) with a 95% confidence interval.

There were two sites that stand out as significantly different than all other sites for total sulfur, which were both in excess of Criteria C. These two sites also differed from each other: the Lamaque mine site and the LaRonde mine site (Figure 22). Both sites surpassed Criteria C for total S concentrations, with the LaRonde site having a mean value approximately 6 times higher than Criteria C's value of 2000 µg/g. Sites were a strong predictor of total sulfur values ($F = 57.36$, $p < 0.05$) with a 95% confidence interval.

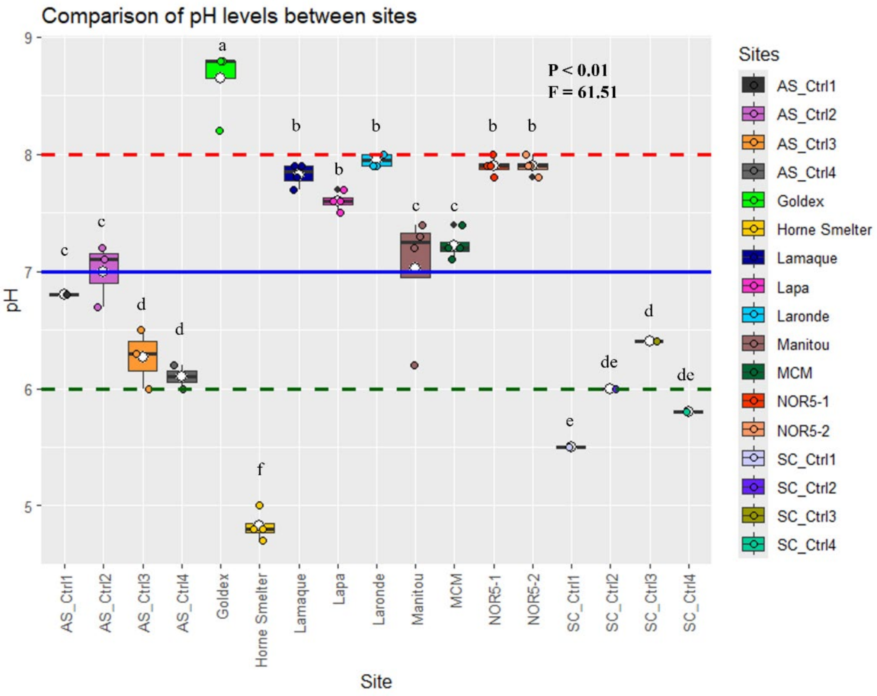
There are no provincial or federal soil Na concentration guidelines; provincially there are recommendations for drinking water in µg/L (Beaulieu, 2021). There were three main groupings for sodium concentration in µg/g that stand out in Figure 22. Three of the *A. syriaca* control sites (Controls 1-3) and two of the *S. canadensis* (Controls 2 and 4) were statistically similar to each other, and different from all of the mine sites. The LaRonde mine site resembled the Goldex mine site and two control sites (*A. syriaca* Control 4 and *S. canadensis* Control 3), while the Goldex mine site only resembled the LaRonde mine site. The remaining mine sites closely resembled each other, with very low concentrations of soil Na, with mean values of > 50 µg/g. The control sites all had much higher mean soil Na concentrations that were < 200 µg/g, with *S. canadensis* Control site 1 having the highest concentration. Sites were a strong predictor of Na concentrations ($F = 35.84$, $p < 0.01$) with a 95% confidence interval.

Electrical conductivity did not differ significantly among the mining sites and control sites (Figure 22), except for the LaRonde mine site that showed a mean concentration more than 8 times greater than in other sites (Figure 22). The CCME guidelines (2007) have a threshold of 2 dS/m for agricultural and residential/parkland soils, and a threshold of 4 for commercial and industrial soils. Only the LaRonde mine site that had some of its values that surpassed the threshold for agricultural and residential/parkland soils, although the mean values for LaRonde were still under the threshold of 2 dS/m. Sites were a strong predictor of electrical conductivity ($F = 11.38$, $p < 0.01$) with a 95% confidence interval.

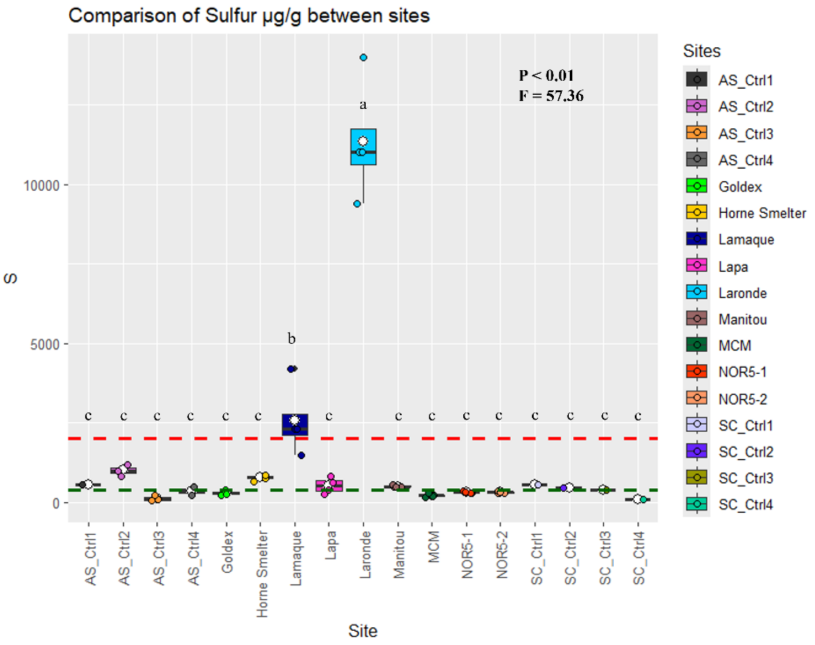
There are no provincial or federal guidelines governing the percentage of organic matter concentrations in residual mineral soils. The mining sites showed lower mean organic matter concentrations in the soils (from < 0.01% to 0.19%) than in the control sites (from 0.04% to 0.13%) (Figure 22), except AS-Ctrl3 and SC-Ctrl1 that had intermediate concentrations (from 0.11% to 0.38%) (Figure 18). The Horne Smelter mine site was different from all other sites as it had significantly more organic matter in the soil than all other sites (range of 7.9% to 9.3%). Sites were an important predictor of the percentage of organic matter in the soil ($F = 42.13$, $p < 0.01$) with a 95% confidence interval.

The total nitrogen elemental concentration had two main groups of results. *Solidago canadensis* control 1 was different from all other sites with the highest concentration of total N in the soil (0.38%). Concentrations of total nitrogen were below the threshold of detection (< 0.01%) in the Goldex, Lapa and LaRonde mining samples and are illustrated as NA on Figure 21. There is one grouping of control sites with minimal differences between them, and a second grouping of control sites that resemble the remaining mining sites. Sites were a strong predictor of total N concentration (F = 17.79, $p < 0.01$) with a 95% confidence interval.

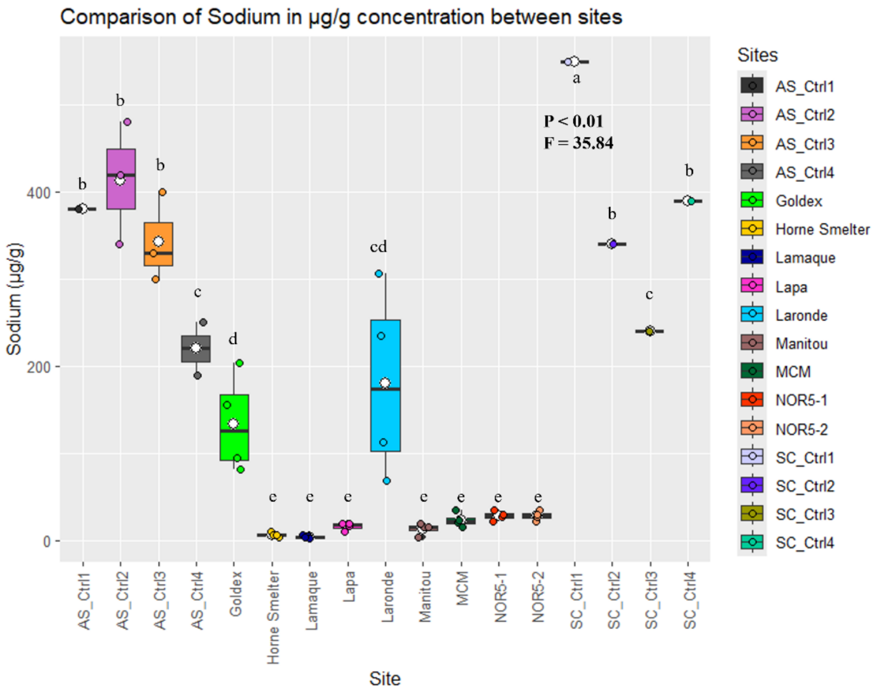
(a) pH



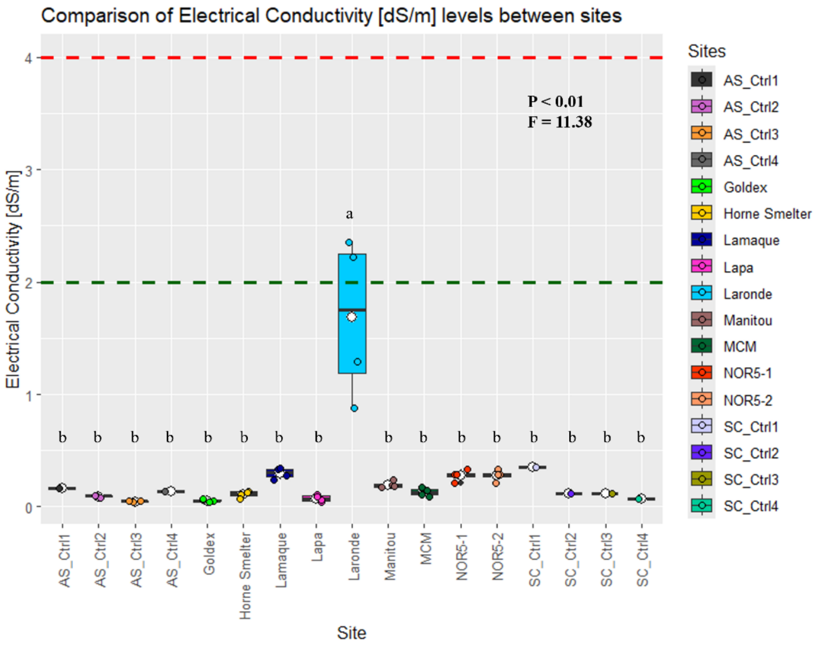
(b) Sulfur



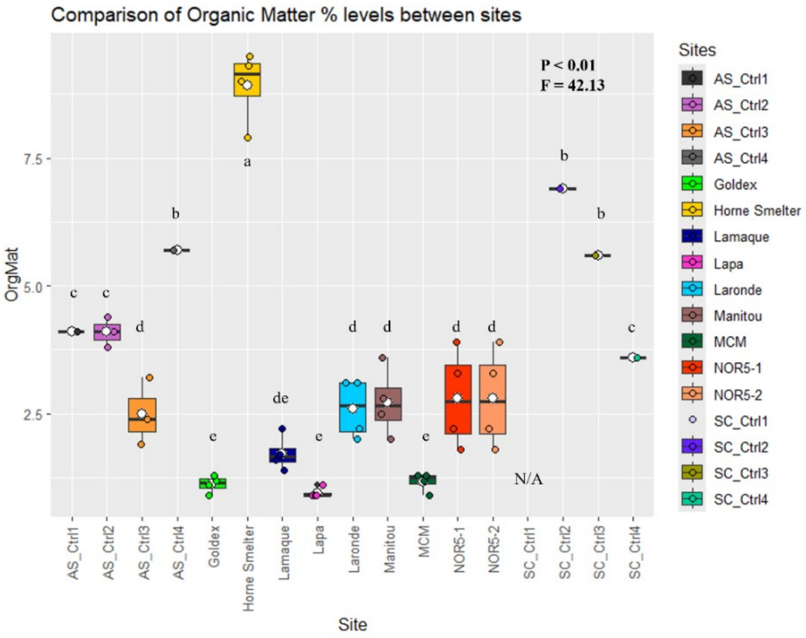
(c) Sodium



(d) Electrical Conductivity [dS/m]



(e) Organic materials %



(f) Nitrogen T/K /N%

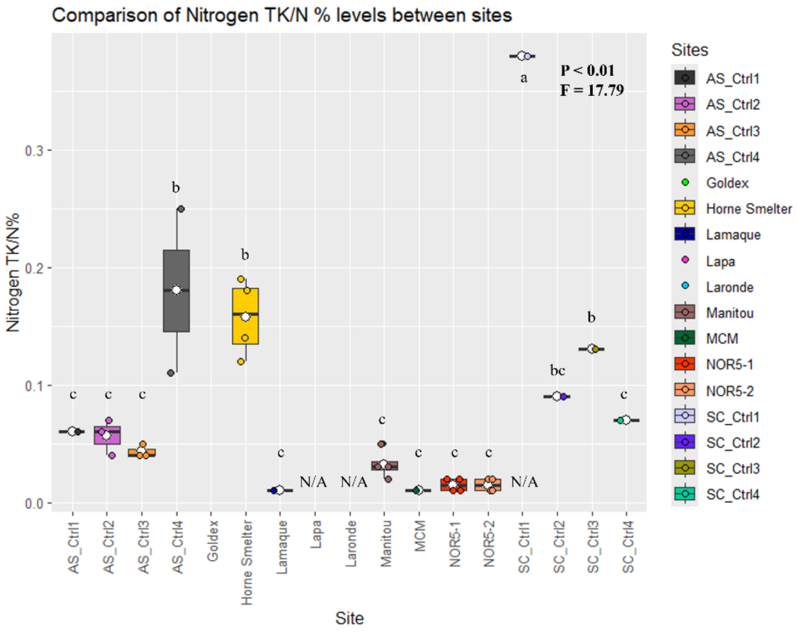


Figure 22

Comparison of soil pH (a), total concentrations of Sulfur (b) and Sodium (c), electrical conductivity (d), organic matter concentration (e), and total Nitrogen elemental concentration (f) among mining and control sites

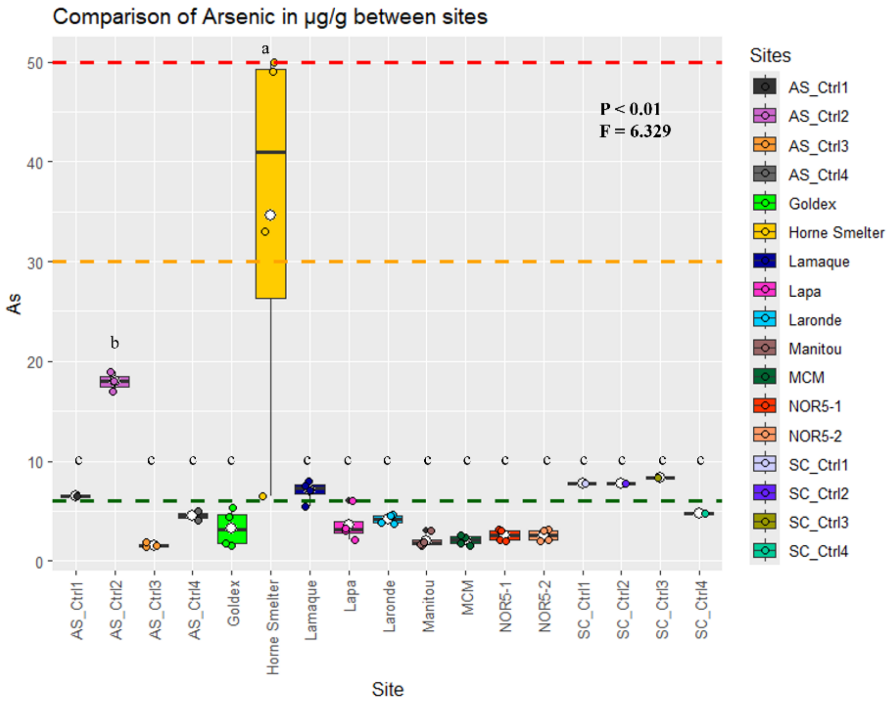
Soil physicochemistry analysis for a mining revegetation experiment. There were 9 mine sites, 4 roadside control sites with naturally occurring *A. syriaca* plants and 4 roadside control sites with naturally occurring *S. canadensis* plants. Horizontal hashed green line represents Criteria A (acceptable for domestic use) soil concentrations while the horizontal hashed red line represents Criteria C, the maximum allowable concentration for industrial use according to the province of Québec. Sites that yielded statistically significant different results according to an ANOVA analysis followed by a TukeyHSD analysis are identified with *a, b, c,* etc.

6.4.2 Soil chemistry analysis for Arsenic, Copper, Lead and Phosphorus

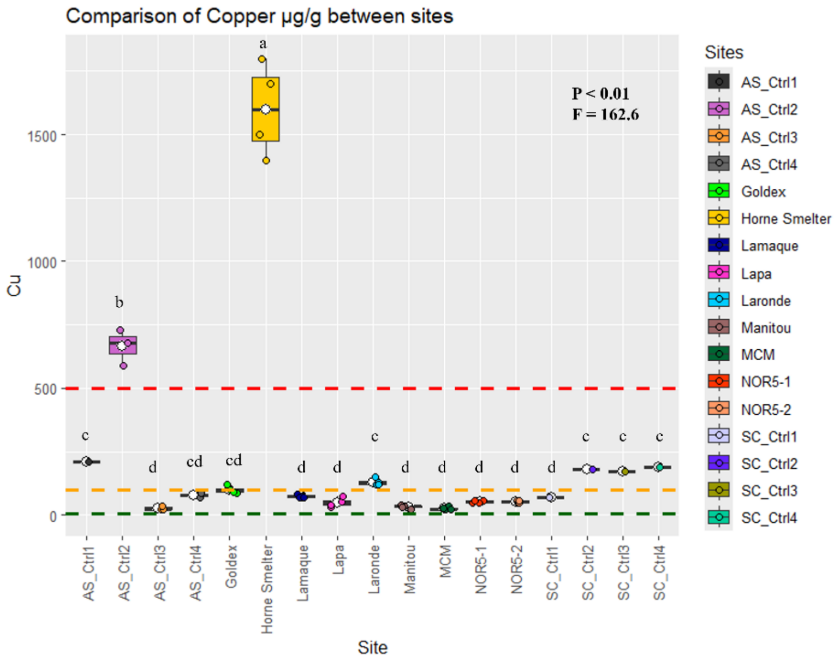
The soil chemistry analyses for As, Cu and Pb indicate that there is minimal variation between mining sites and control sites regarding the total concentrations of these trace elements in the soil (Figure 23).

There are two sites, however, that differ from all other mining and control sites and yielded comparable results between themselves for As (F = 6.329, $p < 0.01$), Cu (F = 162.6, $p < 0.01$) Pb (F = 18.88, $p < 0.01$), and P (F = 10.26, $p < 0.01$) with a 95% confidence interval in the total concentrations: *A. syriaca* Control 2 and the Horne Smelter mining site. These two sites also differ from each other for As and Cu. The Horne Smelter mining site had higher total concentrations of As, Pb, Cu and P than all other sites, as well as *A. syriaca* Control 2 (except for P). Sites were strong predictors for the levels of As, Cu, Pb and P in the soil.

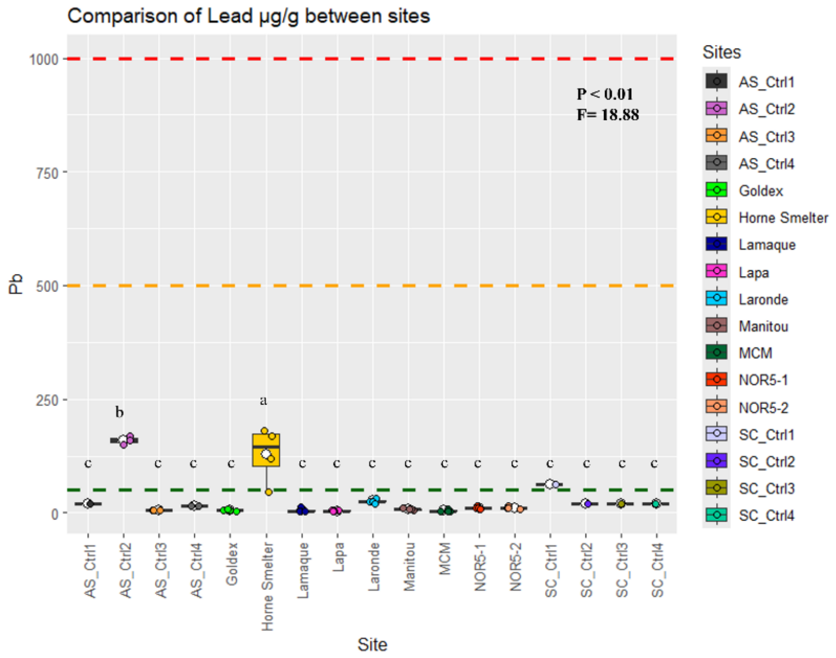
(a) Arsenic



(b) Copper



(c) Lead



(d) Phosphorus

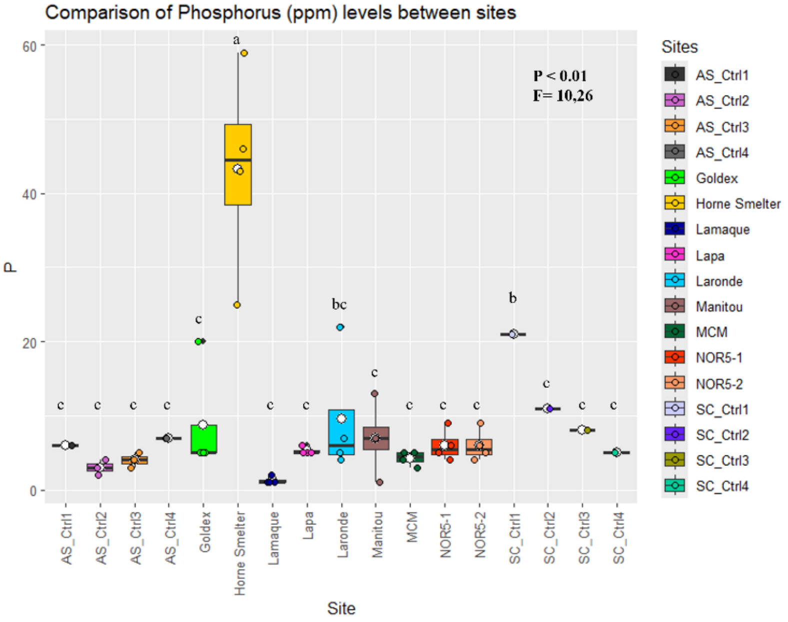


Figure 23

Total soil concentrations of Arsenic (a), Copper (b), Lead (c) and Phosphorus (d) in soils for mining and control sites

Soil physico-chemistry analysis for a mining revegetation experiment. There were 9 mine sites, 4 roadside control sites with naturally occurring *A. syriaca* plants and 4 roadside control sites with naturally occurring *S. canadensis* plants. Horizontal hashed green line represents Criteria A (acceptable for domestic use) soil concentrations, horizontal hashed orange line represents Criteria B (max permitted for domestic use), while the horizontal hashed red line represents Criteria C, the maximum allowable concentration for industrial use according to the province of Québec. Sites that yielded statistically significant different results according to an ANOVA analysis followed by a TukeyHSD analysis are identified with *a, b, c,* etc.

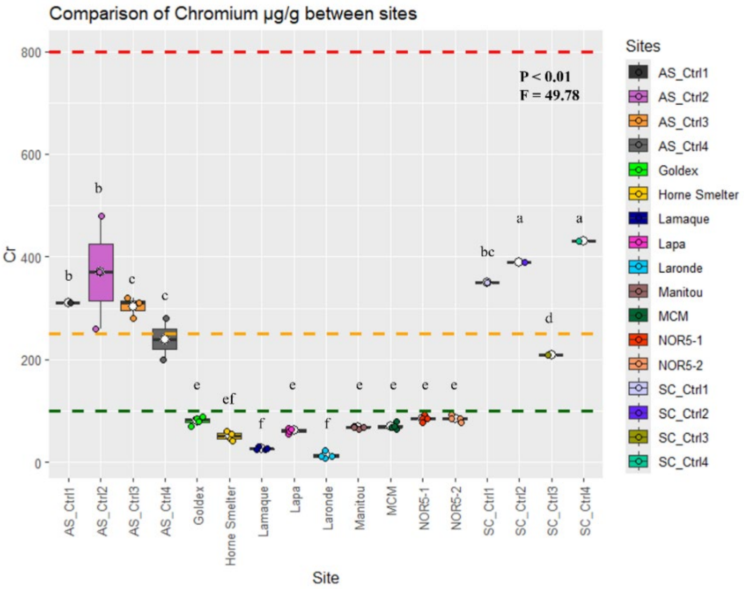
6.4.3 Soil chemistry analysis for other elements surpassing Criteria B: Chromium, Manganese and Molybdenum

The soil chemistry analysis revealed that there were no statistically significant differences between the various mining sites for total elemental concentration of chromium, and they all had values of Cr total concentrations below Criteria A (Figure 24). However, most of the roadside control sites had Cr total concentrations that surpassed Criteria B. There were statistically significant differences comparing the mining sites to the roadside control sites for Cr ($F = 49.78$, $p < 0.01$), including both the *A. syriaca* and *S. canadensis* control sites.

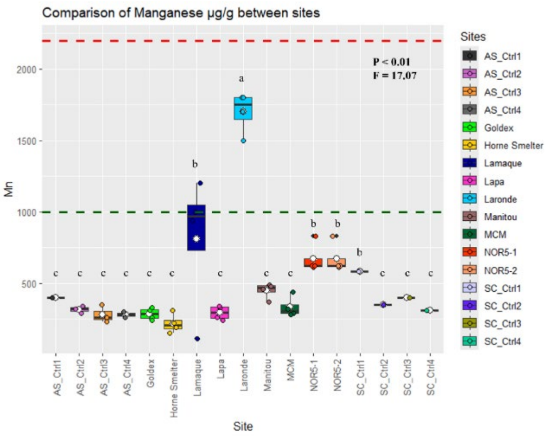
Manganese only had Criteria A or Criteria C thresholds for soil trace element concentrations (Figure 24). The LaRonde mine site surpassed Criteria A for its Mn total concentrations and had statistically greater concentrations compared to all other sites. The Lamaque Eldorado Gold mine site had some sample concentrations that surpassed Criteria A but a mean value below Criteria A, closely resembling the two Noranda 5 sites as well as *S. canadensis* Control 1. The four *A. syriaca* control sites and three remaining *S. canadensis* control sites closely resembled the Goldex, Lapa, Manitou, Mine Canadian Malartic and Horne Smelter mine sites. Sites were a significant predictor for differences between sites for Mn (F = 17.07, $p < 0.01$).

There was a noticeable difference between control and mining sites for Molybdenum soil concentrations. All *A. syriaca* control sites and *S. canadensis* controls 1 and 2 all closely resembled each other with greater concentrations above criteria B. The mining sites all closely resembled each other as well as *S. canadensis* Control 3. Finally, *S. canadensis* Control 4 did not resemble any of the other sites. Sites were thus also a significant predictor for concentrations of Mo in the soil (F = 25.48, $p < 0.01$).

(a) Chromium



(b) Manganese



(c) Molybdenum

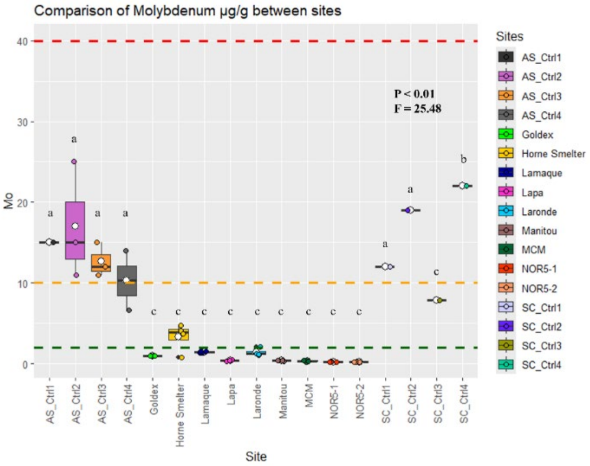


Figure 24

Total soil concentrations of Chromium, Manganese and Molybdenum in soils for mining and control sites

Soil physico-chemistry analysis for a mining revegetation experiment. There were 9 mine sites, 4 roadside control sites with naturally occurring *A. syriaca* plants and 4 roadside control sites with naturally occurring *S. canadensis* plants. Horizontal hashed green line represents Criteria A (acceptable for domestic use) soil concentrations, horizontal hashed orange line represents Criteria B (max permitted for domestic use), while the horizontal hashed red line represents Criteria C, the maximum allowable concentration for industrial use according to the province of Québec. Sites that yielded statistically significant different results according to an ANOVA analysis followed by a TukeyHSD analysis are identified with *a, b, c,* etc.

Additional analyzed elements are included in the Annexes section as graphs without further discussion. Linear regression tests comparing the number of *D. plexippus* for all stages of development to the soil physicochemical properties did not yield significant results.

7. DISCUSSION

7.1 Danaus plexippus and Asclepias syriaca

7.1.1 Survival of *D. plexippus*: mine sites vs. control sites

We reject the hypothesis that the survival rate of *D. plexippus* would be similar between mining sites and the survival rate found on the control roadside sites, as all of the roadside sites had a positive survival rate in 2021 whereas only 2 mine sites had a positive survival rate. In 2022 only one stage 5 instar larva was observed (Table 4), so the findings for 2022 are inconclusive for this hypothesis.

Sites were an important factor in observing *D. plexippus* eggs and larvae in 2021, as illustrated in Figure 9. LaRonde mine was the only mining site with *D. plexippus* observations during both 2021 and 2022. Lamaque Eldorado Gold had no *D. plexippus* observations during either year. Goldex had evidence of a missed cohort during the 2021 season and *D. plexippus* was present in 2022 although not on the T1 plots.

The Noranda 5-1 and Mine Canadian Malartic sites both sustained *D. plexippus* to the 4th and 5th instar larvae stages in 2021, demonstrating that the site as factor had a significant impact on survival during the 2021 season. Unfortunately, there were too few mine sites with surviving instar larvae in 2022 to be able to compare between mine and control sites as a metric of success (Table 8). All the control sites had instar larvae surviving to stages 4-5.

There is no clear indication why both Noranda 5 sites, Mine Canadian Malartic, and *A. syriaca* Control 1 had several observations in 2021 but no observations in 2022. There were too few observations in 2022 to repeat the ANOVA test using the sites as a predictor. The physiochemical soil composition was statistically different for the Noranda 5-1, Mine Canadian Malartic and Control 1 sites in pH levels, sodium, % of organic matter, copper, chromium, manganese, and molybdenum, compared to the other sites, further confirming that mine sites are not a homogeneous group. We were unable to conclude why *D. plexippus* instar larvae survived to the mobile stages in 2021 at these sites when the same locations were not successful the following year.

7.1.2 Phenology of *D. plexippus* in Abitibi-Témiscamingue

The phenological activity of *D. plexippus* in Abitibi-Témiscamingue has not been extensively reviewed in the literature thus far. Cohorts can overlap (Nail et al., 2015), with considerable variation year over year (Semmens et al., 2016; Flockhart et al., 2017; Thogmartin, Wiederholt, et al., 2017). We anticipated one cohort per year based on the literature, which shows that the northern limit of the *D. plexippus* migration is 50° north (Committee on the Status of Endangered Wildlife in Canada, 2016) while the area of Abitibi-Témiscamingue studied is situated at 48° north.

Population modelling (Flockhart et al., 2019) using citizen science data indicates that *D. plexippus* does migrate to Abitibi-Témiscamingue and this population may be phenotypically distinct from non-migratory populations in the southern United States (Freedman & Kronforst, 2023). The migratory population in Abitibi-Témiscamingue may have genetic differences in their circadian rhythm (Nguyen et al., 2021) and responsiveness to environmental cues (Freedman & Kronforst, 2023). Furthermore, the *A. syriaca* plant traits in latex production and cardenolide concentrations could be genotypically adapted to their local conditions (Freedman et al., 2020), and these were plant-level variables not measured during this study.

Physiological factors such as temperature, light-intensity thresholds (Barker & Herman, 1976; Chapman et al., 2015), nectaring plant availability or suitable *A. syriaca* presence for ovipositing (Crewe et al., 2019), as well as shifting directional daylight cues (Guerra & Reppert, 2013; Guerra et al., 2014; Yang et al., 2022) trigger the instincts to migrate north, reproduce, or migrate south.

The mean temperatures for the region were higher in 2021 than 2022 for both Rouyn-Noranda and Val-d’Or, with 2021 also receiving less total annual precipitation in both cities (Table 2). The literature has already correlated that each average degree Celsius of temperature increase impacts when *A. syriaca* blooms, with 1°C increase equating to a mean flowering time 3.93 days earlier (Howard, 2018). The 1.85°C difference in Rouyn-Noranda between 2021 and 2022 translates to a mean flowering time potentially 7.27 days earlier in 2021 compared to 2022, and the 1.38°C in Val-d'Or means a flowering time potentially 5.42 days earlier in 2021 than 2022. This could indicate a physiological mismatch between when *A. syriaca* was available for ovipositing compared to the arrival date of the migratory *D. plexippus* reproductive population. This aligns with potential temporal matches or mismatches already being studied (Culbertson et al., 2022; Boyle et al., 2023).The observed decline between 2021 and 2022 in Abitibi-Témiscamingue aligns with Wilcox et al.'s (2019) explanation that a change in suitable abiotic environmental conditions (Brower et al., 2017; Hunt & Tongen, 2017) and matches the diminished Mexican overwintering colony size between 2020-2021 and 2021-2022 (Rendón-Salinas, Fernández-Islas, et al., 2023).

7.1.3 Mission Monarque vs. the observed phenology of *D. plexippus* in Abitibi-Témiscamingue

Mission Monarque was used to monitor the arrival of the *D. plexippus* spring migration in southern Québec and Ontario, and compare the arrival with our data.There were two distinct cohorts of *D. plexippus* on the mine sites and control sites in 2021, which aligns with Mission Monarque's vetted sightings for Abitibi-Témiscamingue, using a date range of April 1, 2021, through October 31, 2021, for search parameters. There were fewer sightings for 2022 reported on Mission Monarque's dataset for the April 1, 2022, through October 31, 2022, search parameters.

Mission Monarque's 2021 dataset documents ovipositing adult females as of June 7 in Rouyn-Noranda and June 8 in Val-d'Or. There is a peak of egg and instar larvae sightings in Rouyn-Noranda from June 29-30, and another peak from July 5-12. There is a final cluster of stage 5 instar larvae and adults documented from July 29-August 1, demonstrating 2 distinct cohorts with the possibility of one overlapping cohort in

Rouyn-Noranda. Most of the sightings in Rouyn-Noranda were on *A. syriaca* stems, however, there were also sightings of *D. plexippus* instar larvae on *Asclepias verticillata*, which is not native to this latitude. Several data points from Mission Monarque were on private properties in the area adjacent to the Horne Smelter site, separate from our study site. There were also several data points from the Pont Kinojévis area on the southwest side of the bridge, while the *A. syriaca* roadside control site was situated on the northeast side of the Pont Kinojévis bridge.

The 2022 Mission Monarque dataset showed evidence of one cohort in 2022, with eggs documented as of July 7. The most comprehensive data for Rouyn-Noranda in 2022 involved captive rearing of *D. plexippus* on *Asclepias incarnata*. That data point had various stages of development as of July 19, with eggs, stages 3 through 5 instar larvae and several chrysalids. As captive individuals comprised most of the Rouyn-Noranda data, it is not possible to determine the number of cohorts in 2022 for this city.

Val-d'Or had fewer reported sightings, with adults cited on June 8, 2021, and eggs, stage 5 instar larvae and chrysalids reported between July 20-26, indicating there were likely two cohorts. Table 1 indicates that from ovipositing to emergence from the chrysalid, the cycle can take between 19 to 40 days total. Some of the reported sightings in Val-d'Or are from the *A. syriaca* roadside control site on Boul. Barrette, although it is not possible to know if the sightings are from the stems included in this study or adjacent stems, as this control site had a dense cluster of *A. syriaca* with several hundred stems on both the north and south side of the road.

The dataset for Val-d'Or from Mission Monarque is sparce for 2022, but it indicates that there were two distinct cohorts. The first sighting of the season is an adult *D. plexippus* butterfly on June 22. This sighting is not geotagged to a specific area of Val-d'Or as it was submitted to Mission Monarque by my user profile. There are additional data points between August 21-29 in Val-d'Or of caterpillars, there are no photos attached to the data point so it is not possible to know the stage of instar larval development. If this data is accurate, this indicates that there were at least two distinct cohorts of *D. plexippus* in Val-d'Or. The second cohort is late in the season, when we would anticipate the migration towards Mexico to be well underway at the studied latitude (Davis & Dyer, 2015; Culbertson et al., 2022).

Malartic had the fewest reported sightings on Mission Monarque for 2021. The only reported sightings were on July 20 on *A. incarnata* stems found in a pollinator garden maintained by Mine Canadian Malartic within the town, not affiliated with this thesis. This is not in the same location as our mine site at Mine Canadian Malartic. Due to the limited number of sightings, it is not possible to know how many cohorts were present in Malartic in 2021.There were zero reported sightings in Malartic on Mission Monarque for 2022.

Although the self-reported data on Mission Monarque aligns with the data from our research for 2021 and 2022, it is important to note that there are other factors that may influence the difference between the two different years. Table 2 demonstrates that 2021 was a hotter year with significantly less precipitation than 2022, which may explain a natural fluctuation in the *D. plexippus* population.

The phenological activity in Table 6 and Figure 8 indicate that the first cohort in 2021 would have arrived between June 3 and June 17, with a second cohort arriving between July 5 and July 19. The 2022 peak is less precise due to fewer observations but appears to have occurred during the period of July 4-15.

7.1.4 Impacts of soil physicochemistry on *D. plexippus*

It is difficult to define agronomical trends in the mine sites as there is a wide range of soils, adjacent environment, active vs. inactive sites. The mines are heterogenous. For this reason, we cannot group all of the mine sites used in this study together as one group; they are too different from each other. The investigated physicochemical parameters (pH, electrical conductivity, trace elements including trace metal element concentrations, and organic matter) in the soil did not have any influence on either the number of *D. plexippus* larvae or the total number of pollinator insects collected during either portion of this experiment according to linear modelling.

LaRonde had unique results both in the *D. plexippus* and *S. canadensis* portions of the study, and this site stands out with uniquely high concentrations of Mn and S, as well as a basic pH close to the limit of 8 and higher conductivity. This site is surrounded by forest, much like the Noranda-5 sites. LaRonde and the Noranda-5 sites are both physically and chemically different from all the other mine sites, as well as from each other.

The revegetated mine sites selected for this thesis are subject to much inter-variability, as research was conducted at an active copper smelter, various tailings ponds and tailings management facilities, land at two underground gold mines, land at an open-pit gold mine, and a defunct mine undergoing revegetation. There was also a considerable amount of intra-site variability year over year. It was not possible to analyze the mines as a single group compared to the roadside control sites in this case; we opted to discuss the results on a site-by-site basis as there was too much variability between mine sites.

A limitation of this study is the roadside nature of the control sites. It would have been interesting to have more “natural” and less disturbed control sites, but it was not possible with the nature of the region (long distances between sites, few farmlands, and the northern limits of the current *A. syriaca* range). The roadside control sites were found in situ, which prevented our ability to control the age of the plants, the number of stems, or the ability to have a standard number of replicates per control site.

7.1.5 Effect of height vs. number of stems of *Asclepias syriaca* (quality of quantity)

Our study found that the mine sites had a greater total number of *A. syriaca* stems per plot than the control sites per plot during both years. While there were more *A. syriaca* stems present on the mine sites, they were smaller than on control sites (except at LaRonde). Thus, we were not able to conduct a plant material-level chemical comparison between repetitions as intended, as some of the plants were dwarf-sized on mine sites, and enough leaf material could not be collected without compromising the integrity of the habitat for *D. plexippus*. Conclusions about the quality of the plant material thus cannot be drawn from this portion of the experiment.

It is widely agreed upon in the literature that if you plant *Asclepias spp.* stems, there will be a positive impact on the number of *D. plexippus* eggs and larvae found (Flockhart et al., 2012; Flockhart et al., 2015; Thogmartin, López-Hoffman, et al., 2017; Thogmartin, Wiederholt, et al., 2017; Grant et al., 2018). There was a positive linear relationship between the number of *A. syriaca* stems and the number of larvae observed (Figure 11) in 2021 ($p = 0.00018$), but this was not the only plant-related variable we tested. There were sites such as the Lamaque and Goldex mines where we had 20-30 stems per plot in 2021, but zero observations of *D. plexippus* larvae. There were also sites with far fewer stems per plot, such as Mine Canadian Malartic, LaRonde, and Control 3, where we had between 3 and 15 stems per plot but many *D. plexippus* larvae.

We tested the pooled total number of *A. syriaca* leaves per plot in 2021 (Figure 11). This also had a significant correlation ($p = 0.03$) between the number of *A. syriaca* leaves and the number of observed *D. plexippus* larvae. Dwarf *A. syriaca* stems will typically have between one and four pairs of leaves and do not attract ovipositing *D. plexippus* females even when there are many available stems.

The most important plant-level variable in this study was the pooled total height of a plot of *A. syriaca* stems ($p = 9.188^{e-06}$) (Figure 11). This is a small departure from the literature (Flockhart et al., 2012; Flockhart et al., 2015; Thogmartin, López-Hoffman, et al., 2017; Thogmartin, Wiederholt, et al., 2017; Grant et al., 2018) but is still correlated to the number of stems in a plot and the number of leaves. This indicates that an area of robust and healthy *A. syriaca* stems is the most important variable in observing the presence of *D. plexippus* larval presence.

7.1.6 Dwarf plants: size of plant matters for *D. plexippus*

The mean height and mean number of leaves per *A. syriaca* stem at LaRonde more closely resembled the mean height and mean number of leaves of the control sites for both years (Table 4). All the study sites began with 16 stems per plot in 2019 (Figure 4), whereas the age of the *A. syriaca* stems at the control sites are unknown. *Asclepias syriaca* has a perennial clonal nature, permitting the rhizomatic roots to resprout in the same place for extended periods, in addition to reproducing sexually through seeds.

Non-chemical attempts to eradicate *A. syriaca* in the wild, such as via mowing or pulling regimes, can actually create anthropogenic disturbances in the soil conducive to increase the spread of *A. syriaca* stems through its rhizomes (Bankó et al., 2002; Klimešová & Herben, 2015; Klimešová et al., 2018; Bakacsy & Bagi, 2020). Recent research focusing on eradication of *A. syriaca* focuses on the plant outside of its native range, such as in Europe where it is aggressively invasive (Bakacsy & Bagi, 2020) and mortality risk is low, as death only occurs when both the shoots and the bud bank of the plant are simultaneously destroyed (Bankó et al., 2002; Xu et al., 2016; Bakacsy & Bagi, 2020). There is evidence that rhizome clonal species such as *A. syriaca* can transport heavy metals and contaminants along the clonal network of the plant (Bankó et al., 2002; Xu et al., 2016; Bakacsy & Bagi, 2020).

Strategic mowing regimes of *A. syriaca* can increase *D. plexippus* ovipositing (Knight et al., 2019). The Canadian study in Sarnia took place on a two-lane paved highway with a speed limit of 80km/h (Knight et al., 2019), conditions similar to the *A. syriaca* control sites located by the Kinojévis bridge in Rouyn-Noranda and along Boul. Barrette in Val-d'Or. Their study found that the most eggs were laid per plant in plots that were mowed between the second and third weeks of July (Knight et al., 2019); our study found that there was a peak of eggs and instar larvae observed during the second week of July with no mowing treatment used. Their observations noted that *D. plexippus* preferred to lay eggs on the regenerated *A. syriaca* stems, as well as the tallest *A. syriaca* stems and the stems in good condition (Knight et al., 2019).

There were two groupings of sites based on the *A. syriaca* height: Group A > 40 cm and Group B < 10 cm. There were four sites in Group A in 2021; from tallest to shortest: Control 2, LaRonde, Control 3, and Control 1 (47 cm). The control sites each had stage 5 *D. plexippus* instar larval presence (Control 1 = 24, Control 2 = 22, Control 3 = 28), while LaRonde had no stage 5 instars but it did have 4 stage 4 instar larvae.

The remaining mine and control sites are Group B. The five sites with the shortest *A. syriaca* plants in 2021 in Group B, from shortest to tallest: Lapa, Noranda 5-2, Goldex, Manitou, and Mine Canadian Malartic. Two of these dwarf sites were able to sustain *D. plexippus* larvae including 5th stage instar larvae: Lapa (9) and Mine Canadian Malartic (7). Noranda 5-2 had no 5th stage instar larvae, while Manitou had no *D. plexippus* presence on the T1 plots. Goldex had evidence of a missed cohort in June 2021.

Bergström et al. (1994) performed a greenhouse-controlled study on *D. plexippus* ovipositing behaviour, and showed that females preferred the younger *A. syriaca* leaves surrounding the flower heads or cluster of buds. This same study found no eggs and only a single larva on 400 full-grown *A. syriaca* plants bearing flowers or seed pods, while on younger plants measuring approximately 20 cm in height, 74 eggs and 12 larvae were observed (Bergström et al., 1994). On the mine sites, no flowering of planted *A. syriaca* was observed in 2021 or 2022 (except on LaRonde) even if the plants had reached 3 years of age in 2021, which corresponds to the age of first flowering. The control sites had inflorescences during both seasons; the age of the control *A. syriaca* stems are unknown.

Recent literature suggests that patch density, quantified by the number of stems and number of leaves within a polygon, in addition to the presence of a diversity of nearby blooming flowers for nectaring both greatly influences the likelihood of *D. plexippus* ovipositing (Fisher & Bradbury, 2023). Indeed, during both seasons, the presence of *D. plexippus* was observed at Goldex outside of T1 in the mixed-species plots of T4, indicating that these plots of varied sizes and species were possibly beneficial to ovipositing females (Nestle et al., 2020). At the Manitou site, the *A. syriaca* stems were only visible if one moved aside the diverse competing vegetation to locate the stems, while at the Horne Smelter site there was heavy competition from graminoids, particularly in 2022. These were not conducive conditions to attract an ovipositing female *D. plexippus*. One of the earliest studies on *D. plexippus* by Urquhart (1960) supports this finding, as Urquhart believed that in the field vision plays a significant and decisive role in the selection of a plant for ovipositing.

Unlike the clear relationship between the presence of additional flowering plants adjacent to *A. syriaca* stems and the likelihood of *D. plexippus* ovipositing, there does not appear to be any connection in the literature between the presence or absence of graminoids and the presence of *D. plexippus*. Graminoids are herbaceous plants with small, inconspicuous flowers; their pollen is carried by the wind and by pollinator insects (Jones, 2014).

7.2 Soil physicochemistry

7.2.1 Inter-site variation of pH, Sulfur, Sodium, Electrical conductivity, Organic Matter and Nitrogen in the soil

All the mine sites had near neutral to lightly basic soil, except for the Horne Smelter site, which had acidic soil. The roadside control sites are all slightly acidic, and while there was some variability at the mine sites, most of the sites were neutral or basic. The roadside control sites also had elevated chromium concentrations, although the specific chromium oxide was not characterized. These roadside chemical compositions align with the expected elements associated with transport truck circulation that is characteristic of the Trans-Canada corridor from western Québec towards the larger population centres of the south. The roadside control sites were more statistically homogeneous in their chemical composition than the mine site group. We found that there was considerable variation between open pit mines, underground mines, and tailings ponds in terms of the physicochemical composition of the soil that was not conducive to generalizations in the mining site group. Even among the various sub-groupings of mine sites of similar nature, we did not see comparable results in this experiment.

There was thus a measurable difference between the mine sites and the control sites for pH values. The pH of Lamaque Eldorado Gold was statistically similar to the pH of LaRonde, Lapa, and both Noranda-5 sites, we found zero evidence of *D. plexippus* presence at Lamaque Eldorado Gold during the study.

It is worth noting that soil pH in the reference boreal forest ecosystem varies based on the biochemical properties and microbial communities linked to the vegetation; these differences arise from the interaction of plant community composition, soil pH and the nitrogen content of boreal forest floors (Högberg et al., 2007; Macdonald et al., 2012) and at different gradients (Giesler et al., 1998). The soil pH from the boreal forest of western Québec at UQAT'S Forêt d'enseignement et de recherche du lac Duparquet (FERLD) situated in Rapide-Danseur in western Abitibi-Témiscamingue, has an acidic pH ranging from a 4.83 pH in *P. banksiana* stands to a more neutral 6.23 pH in *P. tremuloides* stands (Ste-Marie & Paré, 1999). The soil samples in Ste-Marie & Paré's study came from well-drained lacustrine clay soils, with forest stands originating from wildfires (Ste-Marie & Paré, 1999).

The total Nitrogen concentration expressed as TK / N% in the soil was surprisingly not a determining factor in *D. plexippus* presence or pollinator insect presence on *S. canadensis*. We expected there to be a strong correlation between TK / N % and the number of *D. plexippus* stage 4-5 instar larvae, the results did not support this prediction. Sites were a strong predictor of TK / N%, Goldex, Lapa and LaRonde mine sites had concentrations below the detection threshold. These sites had very different results in the number of *D. plexippus* eggs and larvae. The Horne Smelter site had comparable results of TK / N% to the *A. syriaca* control 4 with radically different results. The remaining mine sites and *A. syriaca* control sites were statistically like each other; thus, we can say that there were no significant differences between the mine sites and the control sites for TK / N% and the impact on *D. plexippus* larvae.

As for the *S. canadensis* control sites, the TK / N% was significantly higher than the values for the two Noranda-5 sites. We can therefore say that there were important differences between the mine sites and control sites for TK / N%, and we had more pollinator insects on the plots where TK / N% was lower.

Sulfur is typically associated with more acidic soils (Kabata-Pendias, 2011). In this experiment, an ANOVA test followed by a post-hoc TukeyHSD test showed that sites were a significant predictor of S concentrations in the soil. There were no significant differences between the *A. syriaca* control sites, most mining sites, and the *S. canadensis* control sites, except for two mining sites: Lamaque and LaRonde. These two mine sites had S concentrations that exceeded the provincial guidelines (Beaulieu, 2021). LaRonde significantly exceeded the upper threshold of 2000 µg/g of S with a mean value of 11,350 µg/g, while the mean pH at LaRonde was basic at 7.95. High concentrations of S are associated with potential acid generation in metalliferous mine wastes due to the oxidation of their sulfide minerals (Plante et al., 2020). However, all of the mine wastes (tailings and waste rocks) used in the experimental settings of this study were classified as not acid generating, thanks to the presence of enough neutralizing minerals in the rocks (Plante et al., 2020).

Total electrical conductivity values, measured as [dS/m] were under the CCME guidelines (2007) of 2 dS/m for agricultural and residential/parkland soils. There were no significant differences between the mining sites and either group of control sites for soil conductivity, except for the values at LaRonde mine. While the values at LaRonde surpassed the lower limit of 2 dS/m in some of the plots, the mean conductivity at LaRonde was 1.69 dS/m, still well below the threshold of 2 dS/m. Total electrical conductivity measured the total dissolved salts in the soil, sodium was measured as a separate value. We found that there were significant differences between the roadside control sites and the mine sites for Na soil concentrations. With the exceptions of the Goldex and LaRonde mine sites, total Na soil concentrations were typically below > 50 µg/g, while the roadside control sites had mean values in excess of < 200 µg/g. The Goldex experimental plots were situated on the side of the highway on the Goldex property, so this was to be expected. Research already supports the hypothesis that anthropogenic increases in sodium in *A. syriaca* do not impact ovipositing or larval foraging behaviour of *D. plexippus* (Mitchell et al., 2019), and in our study we had the most *D. plexippus* eggs and larvae on LaRonde and our *A. syriaca* control sites, all of which had higher Na values.

We can state that total salinity did not have a significant impact on the survival of *D. plexippus* eggs to the larval stages, nor did salinity have a significant impact on the number of pollinator insects collected from *S. canadensis* in this study on mine sites or control sites. We expected to find a higher concentration of salinity and electrical conductivity at the mine sites compared to the control sites. This was not the case in this study; thus we must reject this hypothesis.

The percentage of (OM) had a mean value of 2.76% for the mine sites and a mean value of 4.15% for the control sites. The soil samples from the mine sites were collected from each corner of the experimental setting outside of the treatments to avoid bias. As illustrated in Figure 20, the mine sites typically had a low mean percentage of OM in the soil, while the *A. syriaca* and *S. canadensis* control sites typically had a higher mean percentage of OM in the soil. Note that *A. syriaca* control site 4 is topsoil. While statistical analyses showed that sites were an important predictor in the percentage of organic matter in the soil, this did not translate into a significant impact on the presence of *D. plexippus* larvae.

The mine site with the highest range of organic matter (7.9% to 9.3%) was the Horne Smelter site, and this site did not have any larval presence of *D. plexippus*. Furthermore, there were no statistically significant differences between the two Noranda-5 sites, LaRonde, Manitou and *A. syriaca* Control 3 for mean percentages of organic matter nor the range of values. Furthermore, the *S. canadensis* control sites had higher mean percentages of soil organic matter than the mine sites.

7.2.2 Inter-site variation of Arsenic, Copper, Lead and Phosphorus in the soil

The Horne Smelter site and *A. syriaca* control site 2 did not resemble the other mining or control sites for soil. As, Cu and Pb, since they had greater concentrations than regulatory thresholds. The concentration of As is above threshold A for *A. syriaca* control site 2, while the concentration is above threshold B for the Horne Smelter site. Concentrations of Pb are both above threshold A. For Cu, the concentration is above threshold C for both sites, with the *A. syriaca* control site 2 having a mean concentration value of 667 µg while the Horne Smelter site has a mean concentration value of 1600 µg.

We hypothesized that there would be a difference in trace metals concentrations that would be found at mine sites compared to the roadside sites, with a higher amount of heavy metal concentrations in the soil of mine sites. The soil chemical analysis for arsenic and copper tells a very different story in this study, where one mine site (Horne Smelter) and one *A. syriaca* control site have significantly different results from all other mine sites or control sites.

The Horne Smelter site (1600 µg/g) and the *A. syriaca* Control 2 (667 µg/g) both had mean Cu values that exceeded the provincial Criteria C of 500 µg/g (Beaulieu, 2021). The illustrations in Figure 1.19 show that these two sites stood out compared to all other mine or control sites in regard to the soil chemistry results for Cu, As and Pb, with the copper values surpassing provincial Criteria C. Arsenic and copper are known to limit plant growth and establishment (Young et al., 2013).

Soil chemistry results for arsenic indicated that *A. syriaca* Control 2 (18 µg/g) and the Horne Smelter (35 µg/g) site both exceeded Criteria A for their mean values, while only the Horne Smelter site exceeded Criteria B (30 µg/g). No soil chemistry values exceeded Criteria C for As, but the highest mean and greatest range of values could both be found at the Horne Smelter site. This means that all the mine sites and control sites had acceptable concentrations of arsenic in the soil for industrial, commercial and non-sensitive institutional use.

The Horne Smelter site is an urban site situated on the grounds of the smelter site but is separated from the smelter's main activities by train tracks, while *A. syriaca* Control 2 is located approximately 16 km southeast of the smelter, on a high traffic stretch of the Trans-Canada Hwy 117 near the municipal airport. Many transport trucks use this route as it is one of four highway access points to Rouyn-Noranda, and the only one directly connecting Val-d'Or to Rouyn-Noranda via Malartic to the east of the city. Copper is transported to the smelter via transport truck passing by this control site, or via train directly to the smelter. The presence of copper in the soil at these two locations corresponds to the wind patterns of the region, as well as documented presence of smelter dust in the humus layer of soil surrounding Rouyn-Noranda (Knight & Henderson, 2006).

The soil chemistry values for lead were encouraging as all of the sites except for the Horne Smelter site (129 µg/g) and *A. syriaca* Control 2 (160 µg/g) had mean values under Criteria A (50 µg/g), and all of the values including these two sites were well under Criteria B (500 µg/g). This means that all of the mine sites had lead values well within the maximum permitted value acceptable for residential use or within the first metre of soil for playgrounds in municipal parks (Beaulieu, 2021).

Phosphorus is grouped with As, Cu and Pb in this section as the concentration of phosphorus in ppm is significantly higher at the Horne Smelter site than all other sites, just like the As, Cu and Pb concentrations at this site. There are no provincial or federal recommendations on the concentration of P in ppm in soil. The presence of phosphorus is not problematic as it is a macronutrient necessary for plants (Chapin et al., 2012; Guittonny, 2020).

7.2.3 Inter-site variation of Chromium, Molybdenum and Manganese in the soil

Chromium, manganese, and molybdenum are grouped together as the soil chemistry analysis indicates concentrations that exceed Criteria B. We can also see a greater distinction between mine sites and control sites based on the results of the soil chemistry analyses for Cr, Mn and Mo.

Chromium and molybdenum had almost identical patterns in the soil. For these elements, there was a difference between the mine sites and the control sites, with the control sites for both *A. syriaca* and *S. canadensis* having higher concentrations of Cr and Mo in the soil than the mine sites. For both elements, the mine sites generally had mean values below Criteria A, except for the Horne Smelter site for Mo. Even though the Mo value at the Horne Smelter site surpassed Criteria A, it was not statistically different from the other mine sites according to an ANOVA test and TukeyHSD.

The elevated presence of Cr and Mo aligns with the literature review expectations that traffic patterns have a higher impact on elemental contamination of the soil than adjacent land use (Shephard et al., 2022) and diminished bumble bee colony growth (Scott et al., 2022), with chromium and molybdenum shed from vehicular brake pad wear and tear (Apeagyei et al., 2011; Cowan et al., 2021). Chromium may also lead to oxidative stress in plants (Zulfiqar et al., 2023), while molybdenum may limit plant nitrogen fixation (Chapin et al., 2012).

Manganese demonstrated a difference between some mine sites and control sites, with Lamaque having values that exceeded Criteria A and B (1000 µg/g for both) in its range of values (110 µg/g to 1200 µg/g). LaRonde's mean (1700 µg/g) exceeded Criteria A and B, but inferior to Criteria C (2200 µg/g). At Lamaque, the experimental setting was constructed on tailings, while it was on waste rock at LaRonde. On the other mine sites, the experimental settings were constructed on natural soils.

Here we can say that there is indeed a difference between the mine sites and the control sites and that we did find different metals to be present in higher amounts (Cr, Mo) at the control sites compared to the mine sites, but the reverse for Mn.

7.3 Solidago canadensis pollinators

A study in New Hampshire examined bee assemblages in managed early-successional habitats in the Southeastern portion of the state, using pan traps to collect bee specimens on a variety of plants (Milam et al., 2018) and found *S. canadensis* as the plant species with the third-highest abundance of wild bees. This study documented the species-level abundance of Colletidae, Halictidae, Andrenidae, Megachilidae, and Apidae, with the greatest number of individuals in the Halictidae and Apidae families (Milam et al., 2018). The New Hampshire study had $N = 968$ for the five bee families, compared to a value of $N = 185$ for native bees in our study. There were higher abundances of the rarer bee families (Colletidae 5.7%, Andrenidae 4%, Megachilidae 1.8%), while the two more common bee families had very similar abundances to our study on *S. canadensis* (Apidae 20.0% compared to 21.1% in our study, Halictidae 68.6% compared to 71.9% in our study) (Milam et al., 2018). The diversity results are very similar to our study, while the abundance is much higher in the New Hampshire study, likely due to the more southern latitude.

Observations on iNaturalist (Canadian Wildlife Federation et al., 2024) in Abitibi-Témiscamingue during the 2021 and 2022 seasons also included all the same pollinating bee families and syrphids as in our studied region, thus we can say that *S.*

canadensis appears to capture the diversity of larger pollinator insects at the family level. However, citizen science data from iNaturalist had sparse entries for native bees and Syrphidae in the Abitibi-Témiscamingue region for 2021 and 2022. The data from iNaturalist identified 9 species of Apidae and 23 species of Syrphidae, with Colletidae, Andrenidae and Megachilidae being somewhat rare families, much like our study. Our study found Halictidae to be the dominant family, which we did not predict based on the data found in iNaturalist; there were zero observations of the Halictidae family of bees on iNaturalist in Abitibi-Témiscamingue from April 1, 2021, to October 31, 2022. We found that iNaturalist was good at documenting the presence of larger, easier to identify taxa of native bees while data on smaller, harder to identify taxa was severely lacking.

The decision was made to limit identification of the *A. syriaca* pollinators to family level due to timing constraints. The functional traits of the various bee species and genera (Normandin et al., 2017) was not the objective of this thesis.

7.3.1 Impact of *S. canadensis* density on pollinator abundance and diversity

Solidago canadensis often occurs in dense monospecific stands in nature due to its high growth rate and its ability to spread locally by means of rhizomes (Groot et al., 2007). The study plots on revegetated mines thus closely resembles the natural conditions for *S. canadensis* stands. In attempts to use wildflowers as a revegetation technique, previous studies have found that pre-seeding treatments influenced the initial plant establishment, however, this relationship becomes less apparent over time (Perkins et al., 2024). In Perkins's study, the pre-seeding treatments used a completely randomized design and included a frequent mowing regime (2x per month with the mower deck set to 2 inches), infrequent mowing (1x per month with the mower deck set to 5.5 inches), a herbicide application that included a 2% concentration glyphosate applied 1x per month in the summer, or a soybean cover crop using Roundup Ready seed with glyphosate spot spraying 1x per month. Wildflower seed mixes were applied in the late fall of 2017, and vegetation assessments were done in June and September from 2018-2020. Seed density was also very important for the establishment of new wildflower habitats (Perkins et al., 2024).

The number of *S. canadensis* stems in a plot, or the density of stems, had a significant effect on the number of pollinators present in that plot. The number of stems most strongly influenced the number of Apidae and Syrphidae individuals collected in this study, while the number of *S. canadensis* stems did not strongly influence the number of Halictidae collected. Syrphidae is a very diverse family of generalist pollinators (Horiuchi et al., 2022) and their overall abundance has been demonstrated to be highest during August (Groot et al., 2007).

Many existing studies on pollinator abundance and diversity on *S. canadensis* focus on the species as an invasive or alien species outside of its natural range (Dudek et al., 2016; Ustinova & Lysenkov, 2020; Zubek et al., 2020; Qiang et al., 2021) whereas this study uses *S. canadensis* well within its defined range in Canada (Melville & Morton, 1982). One Canadian study (Fukase & Simons, 2016) linked the late-blooming *S. canadensis* with the presence of Andrenidae and Megachilidae bees, while smaller bees such as Halictidae did not show a preference for native flowers later season. This could explain why there did not appear to be a significant effect of the number of stems on the number of Halictidae in our study. This same study found that the independent effect of native plant density, regardless of species, had a significant impact on pollinator activity (Fukase & Simons, 2016).

Halictidae are ground nesting bees, but nesting preferences vary between species. Some prefer to nest in sparsely vegetated ground while others prefer denser vegetation so that nests are not as easily detected (Packer et al., 2007). This variability can account for the results that differentiated Halictidae from Apidae preference for the number of *S. canadensis* stems in a plot.

We can thus conclude that the higher the number of *S. canadensis* stems are positively correlated with the number of pollinator insects for Syrphidae and the native bee families (Apidae and Halictidae). We hypothesized that there would be more *S. canadensis* stems on the control sites than on the mine sites and this was incorrect as we found the highest stem density at the LaRonde mine site, therefore we must reject this hypothesis. The LaRonde mine site had the highest concentration of manganese in the soil, and *S. canadensis* is phytoaccumulator of various trace metals (Bielecka & Królak, 2019), including Mn and Zn. Bielecka's (2019) study took place in the Olkusz region of Poland, a region characterized by high concentrations of heavy metals connected with the mining industry in Olkusz. They found concentrations of manganese in some of the morphological parts of *S. canadensis* was lower than its concentration in the soil (Bielecka & Królak, 2019), which may explain why *S. canadensis* was very successful at the LaRonde site.

7.3.2 Surprise! So many Halictidae!

Bee species richness knowledge is closely related to larger population centres (Williams et al., 2014; Carril & Wilson, 2021), which are found further south in Québec. We were expecting to find a variety of Apidae species, and indeed most of the Apidae we found belonged to the *Bombus* genus. The abundance of Halictidae initially became apparent during visual observations of *S. canadensis* plots prior to conducting net sweeps: the stems were buzzing with small black bees. We did not predict that Halictidae would be the dominant family found in this study as no individuals were identified on iNaturalist during the period covering April 1, 2021, to October 31, 2022. However, a recent study on wild bees in the Nearctic and Palearctic Regions of northern Ontario and Akimiski Island, Nunavut did identify *Halictus virgatellus* Cockerell as the most common bee species on Akimiski Island (Vizza et al., 2021), so this family is more common than anticipated in higher latitudes.

Halictidae are small to medium-sized bees, and the family covers a wide range of colours from the black colour we observed in situ to metallic green (Carril & Wilson, 2021; Marshall, 2023). Halictidae individuals tend to be abundant in number when they occur, and generally nest in the ground (Carril & Wilson, 2021). Some species of Halictidae have a distribution as far north as James Bay in Québec (Carril & Wilson, 2021), with the Abitibi-Témiscamingue region well within their expected distribution. To the naked eye, Halictidae can closely resemble some Andrenidae or Colletidae species: small black bodies, some with fine hair, and challenging to see if subantennal sutures are present or not. By examining the wing structure under the microscope and determining if subantennal sutures were present or not, it was possible to identify to family level using taxonomic keys (Carril & Wilson, 2021; Marshall, 2023).

Halictidae were evenly distributed across the mining sites, with N = 40 to 42 individuals identified per mine site. Future research on pollinator insects on revegetated mine sites should include a greater examination of the Halictidae family and its abundance on mine sites undergoing revegetation, as well as Halictidae presence on other pioneer flowering plants used in revegetation. Halictidae have been documented in early-successional habitats using visual observations and pan traps as a method of collection (Milam et al., 2018), much like our study. Milam's research in southeastern New Hampshire (2018) found that Halictidae were the most common family followed by Apidae, and while they did collect Andrenidae, Colletidae and Megachilidae, these were far more rare in the overall species richness. This aligns very closely with the results we obtained in our study, although they collected bees on a greater variety of primary succession plants (Milam et al., 2018).

7.3.3 Taxonomic level of identification and other limits of the study

One of the limitations of this study was that the pollinator insects were identified to the family level instead of genus or species level. This is a potential limitation on fully representing the true level of pollinator diversity found on *S. canadensis* on revegetated mine sites in Abitibi-Témiscamingue. Identification to the species level would have been challenging in terms of analyzing diversity, due to sample size issues as only 203 pollinator specimens were identified across six families.

Family-level taxonomic identification is not without precedent in pollinator insect studies (Hall et al., 2022) which used one of the same taxonomic keys used in this thesis (Marshall, 2017).

To conduct robust statistical analysis on the diversity of pollinator insects, we decided to use the same level of taxonomy for all identified specimens to avoid skewing results towards rarer species or genera.

We recognize that the Shannon index may not be an accurate representation of diversity for some sites (Table 10) such as *S. canadensis* Control 3, as this site only has 1-2 pollinator insect individuals per family for a total of 7 pollinator insects. This is because the Shannon index also considers equitability in the number of individuals belonging to different families, by using relative abundance of species.

An additional limitation was the quantity of mine sites used in the *S. canadensis* portion of the study. The *S. canadensis* plants experienced a high level of mortality at the Horne Smelter site and it was withdrawn from this portion of the study. The mine sites in Val-d'Or and Malartic were not used due to constraints of distance and the requirement of a larger research team for feasibility.

7.3.4 Citizen Science Comparison

To compare our data with some citizen science data to see if the native bee assemblages were similar, we pulled observations from iNaturalist (Canadian Wildlife Federation et al., 2024). Citizen science data of pollinator insects surrounding the city of Rouyn-Noranda, QC were logged in iNaturalist (Canadian Wildlife Federation et al., 2024), and compared to the family assemblages found in early-successional habitats in New

Hampshire (Milam et al., 2018) as well as known pollinator family visitors to *S. canadensis* (Fenesi et al., 2015).

For the period between April 1, 2021, and October 31, 2022, the broad category of “Honey Bees, Bumble Bees, and Allies” has 49 observations belonging to a total of 9 species on iNaturalist (Canadian Wildlife Federation et al., 2024), including *Bombus ternarius*, *B. perplexus*, *B. borealis*, *B. impatiens*, *B. sandersoni*, *B. terricola*, *Bombus* spp. unknown, and the non-native honeybee, *Apis mellifera*.

There were 46 observations of the Syrphidae family (hoverflies – not a family of bee) during this period on iNaturalist (Canadian Wildlife Federation et al., 2024), for a total of 23 species, including *Toxomerus geminatus*, *Eristalis tenax*, *E. arbustorum*, *E. dimidiata*, *E. nemorum*, *Sericomyia chrysotoxoides*, *S. militaris*, *S. lata*, *Syrphidae* spp. unknown, and *Merodon equestris*.

There was one observation of the Colletidae family (*Hylaeus modestus*), two in the Andrenidae family (*Andrena vicina* and *A. clarkella*), seven in the Megachilidae family (*Megachile inermis*, *M. rotunda, M. melanophaea,*and *Megachile* spp. unknown) during this period on iNaturalist (Canadian Wildlife Federation et al., 2024). There were zero observations of the Halictidae (sweat bee) family were made on iNaturalist (Canadian Wildlife Federation et al., 2024) during the period of April 1, 2021, to October 31, 2022. Halictidae tend to be small to medium-sized bees that are abundant where they occur, with numerous individuals of the same species occupying an area (Carril & Wilson, 2021). Halictidae species are not numerous in the studies in the Abitibi-Témiscamingue region, but species such as *Halictus confusus* and *H. rubicundus* have been documented as far north (Carril & Wilson, 2021) as the Eeyou Istchee James Bay area of northern Québec in the Boreal Shield ecozone and *H. virgatellus* have been documented in Akimiski Island, Nunavut (Vizza et al., 2021).

CONCLUSION

We did succeed in finding *D. plexippus* eggs and larvae on some revegetated mine sites and on all the roadside control sites, with sometimes elevated survival rates. The results were not homogeneous between mine sites. The revegetated mine sites with a higher number of *D. plexippus* eggs and larvae had taller *A. syriaca* stems, more leaves available for larval food sources, and a greater density of stems, in addition to having a greater variety of wildflowers of other species available for nectaring adults. For those wishing to create appropriate habitats for *D. plexippus* and other insect pollinators on revegetated mine sites, it is recommended to consider a higher density of *A. syriaca* seedlings of between 49 stems/m^2 and 81 stems/m^2, both perfect square numbers. This will create higher quality habitat patches or corridors on the revegetated mine sites, as well as planting a surrounding meadow-like habitat of wildflowers. Additional research at the LaRonde mine site is recommended, as this site was different from all other mine sites we tested. The health of *D. plexippus* larvae was not the focus of this study. Future research may wish to examine *D. plexippus* caterpillars for trace metal transfer and compare it to the soil and *A. syriaca* foliar concentrations. The overall health of *D. plexippus* could be assessed in further studies, including comparing the prevalence of *O. elektroscirrha* and other parasites compared to control sites in the region.

We successfully found pollinator insects on *S. canadensis* on mine sites that align with the same wild bee families found on other restoration projects. There were more individual insects and more families of pollinator insects present on the mine sites than the roadside control sites. Halictidae was the predominant family we found; future research into mine site revegetation could examine why the habitat is particularly attractive to this family of mostly solitary bees. Future research on this subject should also examine the overall health of the pollinator insects, as previously suggested for *D. plexippus*. One could evaluate trace metals from pollen collected from corbicula of collected insects, evaluate the trace metals in the stomach contents, and compare body sizes to the literature for that species. There is a lot of room to expand our understanding of which pollinator insects use revegetated mine sites for foraging and reproduction, and additional taxonomic exploration is possible with the existing specimens from this research project.

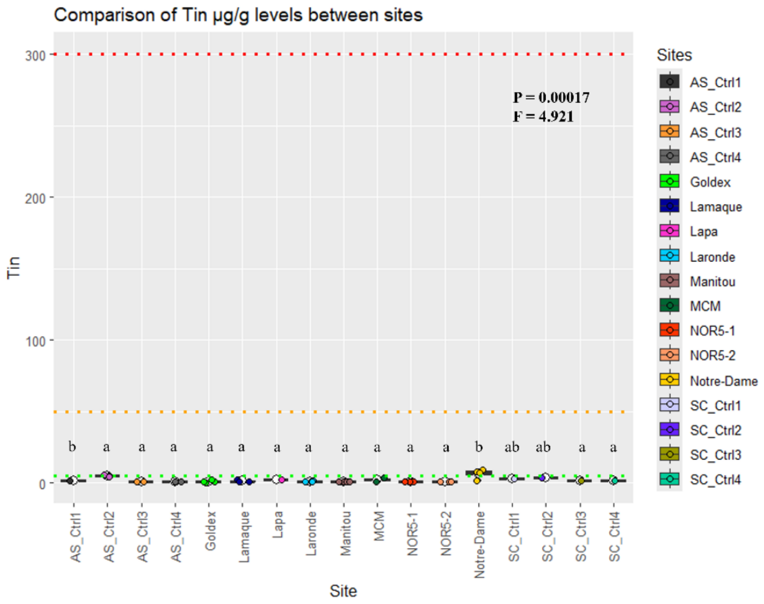
Future research on *S. canadensis* as a pioneer plant for revegetating mine sites should include an analysis of trace metal concentrations on the roots, rhizomes, stems, leaves, and inflorescences of the plant, in addition to the soil trace element concentrations. These data could be compared with the above suggested evaluation of trace metals from insects to further explore the health of the specimens on mine sites.

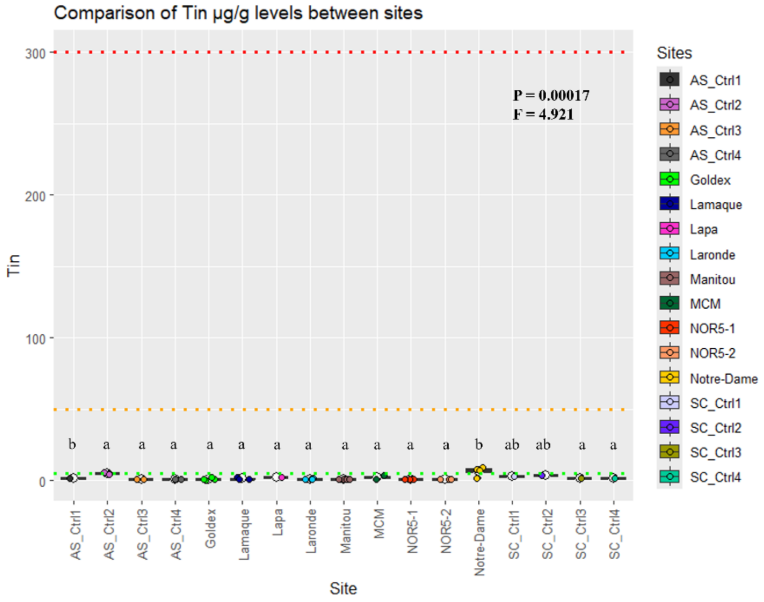
The concentrations of contaminants were not homogeneous across the mine sites. Greater Cr and Mo total concentrations were consistently found in roadside soils, while As and Cu total concentrations appeared higher at the urban Horne Smelter site and on one control site compared to all other sites. All soil concentrations of trace elements respected at least criteria C for industrial use, except for Cu.

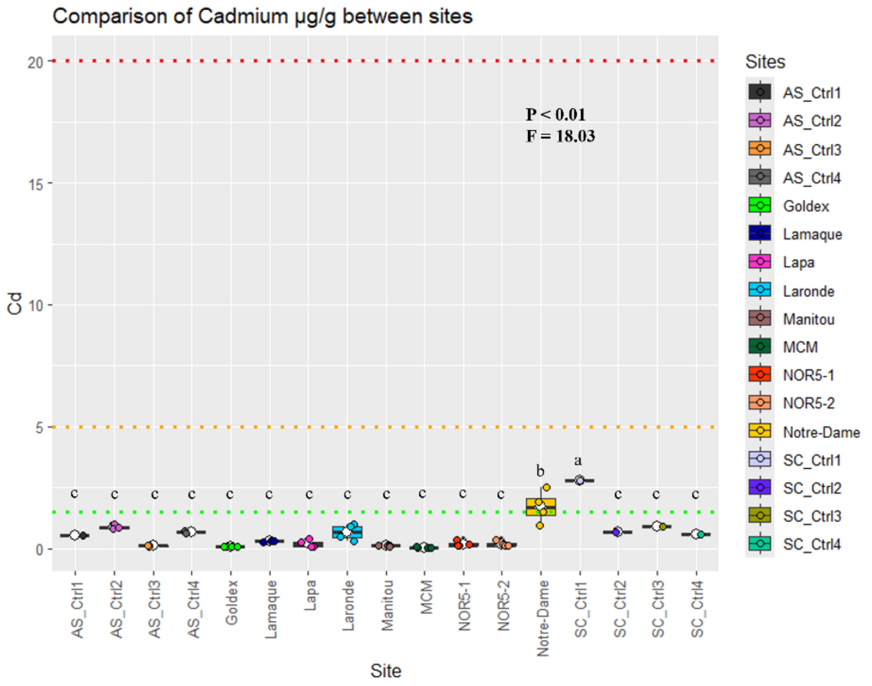
ANNEX A - TREATMENTS T2-T4

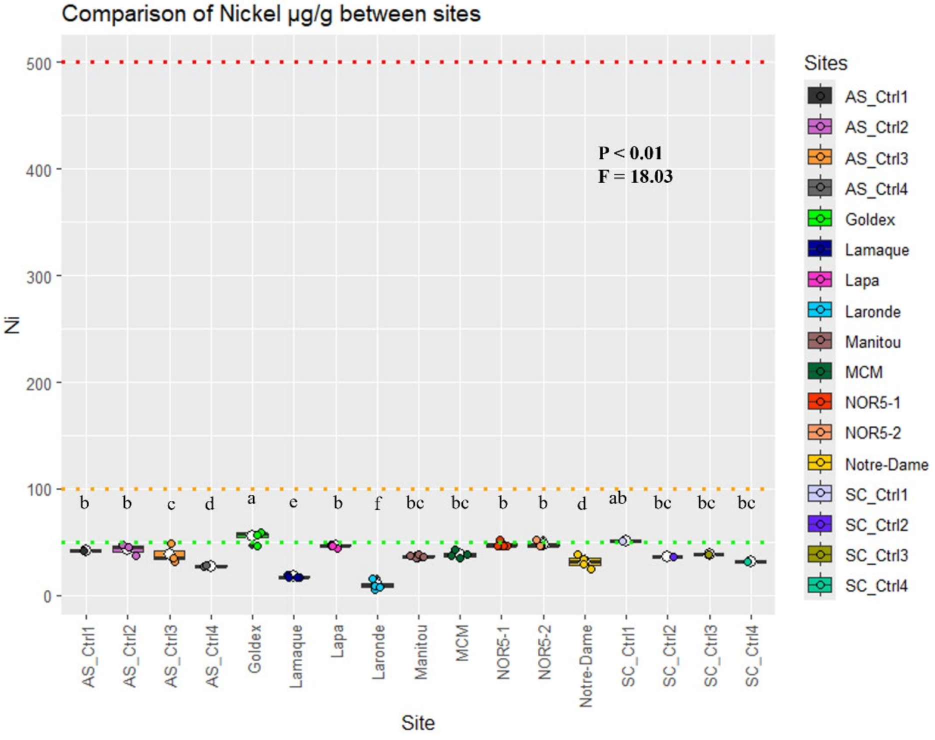
Treatment 2 received seeds of *Achillea millefolium* L. (common yarrow), *A. syriaca, S. canadensis*, and the nurse plant *A. sativa*. Treatment 3 consisted of a custom seed mix that made up 60% of the mix mass and a nurse plant that consisted of 40% of the mix mass, along with the application of mineral fertilizer. Within the 60% custom mix, the species breakdown was 85% *Phleum pratense* L. (Timothy grass) and 15% *Trifolium hybridum* L. (Alsike clover). T4 received the same seeding mixture as T2 and an amelioration treatment including organic material (topsoil or wood chips) or *Melilotus officinalis* L. (field melilot) seeds in the mix (M. Guittonny, personal communication, 23 February 2022).

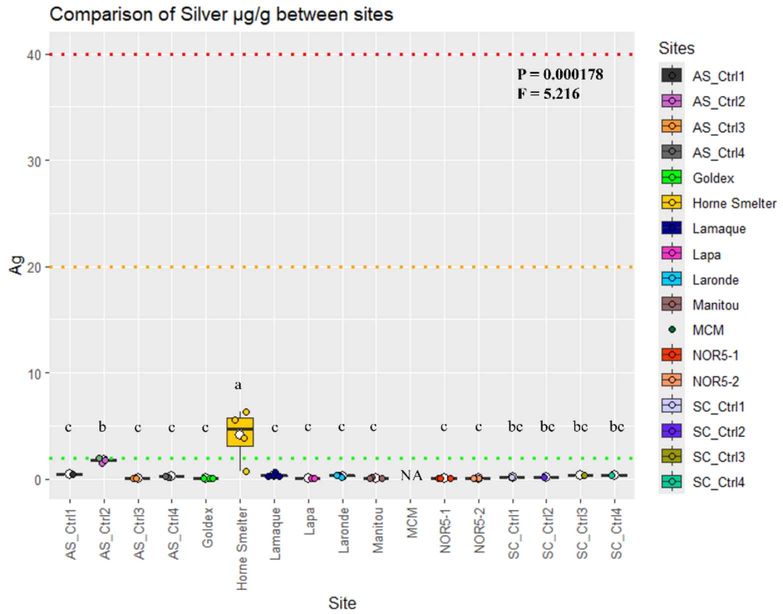
ANNEX B – ADDITIONAL SOIL CHEMICAL ANALYSIS

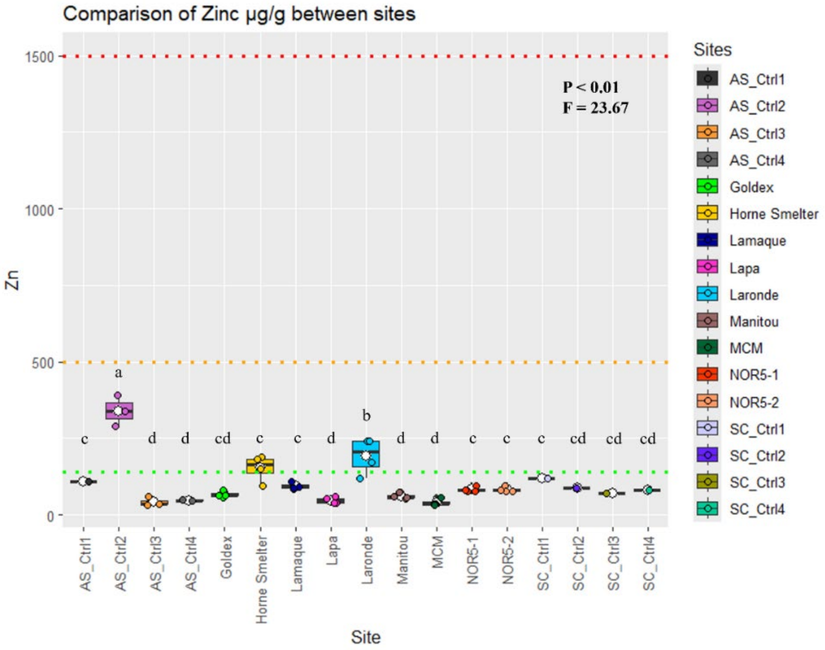





Comparison of Cadmium µg/g between sites
P < 0.01
F = 18.03
Cd
20
15
10
5
0
c
c
c
c
c
c
c
c
c
c
c
c
b
a
c
c
c
Site
AS_Ctrl1
AS_Ctrl2
AS_Ctrl3
AS_Ctrl4
Goldex
Lamaque
Lapa
Laronde
Manitou
MCM
NOR5-1
NOR5-2
Notre-Dame
SC_Ctrl1
SC_Ctrl2
SC_Ctrl3
SC_Ctrl4
Sites


Comparison of Nickel µg/g between sites
P < 0.01
F = 18.03
Ni
500
400
300
200
100
0
b
b
c
d
a
e
b
f
bc
bc
b
b
d
ab
bc
bc
bc
Site
AS_Ctrl1
AS_Ctrl2
AS_Ctrl3
AS_Ctrl4
Goldex
Lamaque
Lapa
Laronde
Manitou
MCM
NOR5-1
NOR5-2
Notre-Dame
SC_Ctrl1
SC_Ctrl2
SC_Ctrl3
SC_Ctrl4
Sites





Total soil concentrations of Tin, Cadmium, Nickel, Silver and Zinc in soils for mining and control sites

Soil physico-chemistry analysis for a mining revegetation experiment. There were 9 mine sites, 4 roadside control sites with naturally occurring *A. syriaca* plants and 4 roadside control sites with naturally occurring *S. canadensis* plants. Horizontal hashed green line represents Criteria A (acceptable for domestic use) soil concentrations, horizontal hashed orange line represents Criteria B (max permitted for domestic use), while the horizontal hashed red line represents

Criteria C, the maximum allowable concentration for industrial use according to the province of Québec. Sites that yielded statistically significant different results according to an ANOVA analysis followed by a TukeyHSD analysis are identified with *a, b, c,* etc. This grouping of sites were generally below the threshold of Criteria A.

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