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# Effects of Deer Browsing on Soil Nutrients and Regeneration Dynamics in a Carolinian Old-Growth Forest of Ontario

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**Abstract:** Old growth forests are increasingly rare but important carbon sinks which harbour rich biodiversity. Chronic browsing by the white-tailed deer (*Odocoileus virginianus*) is a threat to the sustainability of the services provided by these forests, particularly in northern temperate forests where deer numbers have increased in recent decades (driven by stricter hunting rules and reduced predation) and necessitating local monitoring of vegetation responses. The objective of this study was to determine the effects of deer exclusion on tree regeneration dynamics and soil nutrients in an old growth Carolinian forest. This was performed using exclusion fencing and tip-up mounds at McMaster Forest Nature Preserve and the Sheelah Dunn Dooley Nature Sanctuary in Hamilton Ontario. Tree regeneration was surveyed from thirty 1 m × 1 m quadrats within exclusion plots and another thirty quadrats from deer-browsed areas adjacent to the exclusion plots. Soil samples were taken from each quadrat to analyze browsing impacts on nitrate, phosphate and soil organic matter. Red oak (*Quercus rubra*) was planted at the top and base of tip-up mounds of varying heights and widths and monitored for deer access and browsing activity. Results show a significantly higher density of woody plants within exclosures compared to non-exclosures ($p = 0.0089$) and twice more abundance of highly palatable species within the exclosures. However, species richness ($p > 0.05$) and diversity ($p > 0.05$) were minimally impacted by deer browsing, showing a resilient old growth forest. Soil nitrate was consistently higher in the non-exclosures, while phosphate was consistently higher within deer exclosures. Finally, more seedlings survived at the top of mounds than the bases, showing the potential of tip-up mounds to be a natural method of deer exclusion and a critical avenue for restoring over-browsed forests.

**Keywords:** deer exclusion; Carolinian forests; tip-up mounds; regeneration dynamics; soil nutrients

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## 1. Introduction

The forest is an incredibly complex, interconnected ecosystem of plants, animals, fungi, bacteria and other biotic and abiotic factors. The proper balance of life in a forest allows the ecosystem to function properly as each form of life fulfills a different role [1]. A major factor in the health of a forest is the richness and diversity of the canopy, as trees provide diverse habitats, cycle nutrients, sequester carbon from the atmosphere and perform many other important roles [2,3]. A diverse canopy leads to greater biodiversity and increases a forest's persistence against disturbances, thus promoting the sustainable supply of goods and services [4,5]. That diversity depends on the regeneration phase. Forest regeneration refers to seedlings establishing under the mature canopy [6,7]. Successful regeneration is critical for the formation of future canopy layers [8]. Many factors can imbalance this regenerative process, which often occurs through disturbances (e.g., fire, insect outbreak and herbivory) and limited resources (e.g., soil nutrients and moisture) around seedlings, impacting their ability to grow and replace dying trees [9].

White-tailed deer (*Odocoileus virginianus*) are browsers that are widely distributed in eastern North America whose diet consists of young leaves, buds and branches of seedlings and saplings. They have high fecundity with females in unhunted populations capable of producing 30 offspring in their lifetime [10]. Adult (>2 years) white tailed deer weighs about 65 kg and daily food consumption ranges from 0.37 kg (in winter) to over 2 kg in dry weight (in the spring and summer), preferring a moderate fiber, high protein diet [11,12] and nitrogen rich plants [13].

Deer browsing strongly impacts forest canopy regeneration, primarily in areas where deer populations exceed the carrying capacity of the ecosystem, posing a threat to the sustainable supply of goods and services. One impact of deer browsing is the loss of deer-preferred tree seedlings [7], including northern red oak (*Q. rubra*), black cherry (*Prunus serotina*), eastern hemlock (*Tsuga canadensis*), northern white cedar (*Thuja occidentalis*), eastern white pine (*Pinus strobus*), etc. [14]. When over-browsing occurs, browse-tolerant plant species such as ferns, American beech (*Fagus grandifolia*), and invasive species such as garlic mustard (*Alliaria petiolata*) and buckthorn (*Rhamnus cathartica*) [15–18] come to dominate the understory, leading to a decrease in species richness and diversity. Over a long period of time, over browsing can change the canopy of a forest as it becomes dominated by browse-tolerant tree species such as the American beech tree (*F. grandifolia*) [7].

Over browsing can also impact the sustainability in ecosystem goods and services through its impacts on soil chemical elements. Herbivory may either increase or decrease the nutrient cycling in an area, depending on several factors. Soil nutrients increase when herbivores leave excrement [19]. For instance, the average defecation rate of deer is about 16 fecal clumps per day [11] and the average size pellet pile contributes 1.3 kg $N/m^2$ [20], which is 1000× the atmospheric N deposition. Deer fecal pellets are thus capable of increasing soil nutrients [21]. However, while deer leave fecal pellets, they also compact soil while they walk and selectively browse living palatable tree species. Soil compaction can lower the activity of enzymes in the soil and decrease nutrient cycling [21]. Furthermore, the species they browse are generally high in nutrients, and their loss decreases the nutrient cycling due to the slow decomposition rates of the remaining vegetation [22]. For this reason, high browsing in an area may often deplete the soil nutrient levels and further decrease plant abundance.

Because of limiting resources such as light and nutrients available on the forest floor, competition does impact forest floor plant species composition, which can impact native seedling establishment, growth and survival [8]. Invasive species in the understory tend to outcompete native plants and use up available soil nutrients and light. For instance, garlic mustard (*A. petiolata*) is an invasive plant species that has become dominant in the understory of many forests in North America [23]. One major factor contributing to the success of this plant over others in the understory is its impact on ectomycorrhizal (EM) fungi, which is important for the growth of many tree seedlings [23,24]. Garlic mustard (*A. petiolata*) degrades EM fungi in the soil around it; so, tree seedlings growing in that soil are less likely to survive and grow effectively [23]. Another common invasive species is buckthorn (*R. cathartica*), which is a shade-tolerant shrub that decreases regeneration by outcompeting native tree seedlings [25]. Over the long term and with repeated over browsing, the abundance and richness of invasive species could lead to a decreased abundance and diversity of native tree species.

Within temperate forests where white-tailed deer (*Odocoileus virginianus*) densities have increased dramatically in the last decades, owing to stricter hunting rules and reduced predation [26,27], species regeneration on tip-up mounds has been of significant interest (e.g., [28]). A tip-up mound is the mound formed at the base of trees after a windstorm event pulling up roots and dirt from the ground [28]. While tip-up mounds often experience higher erosion and quicker freezing and thawing rates that can be harmful to seedlings [28], they also provide more light and differing soil conditions that may improve growth [29]. Overall, they create a unique microhabitat which may favor the growth of certain seedlings, decreasing competition against dominant species such as maples (Acer), which do not grow well on tip-up mounds [30]. Furthermore, tip-up mounds provide refuge for plants from deer browsing as deer often cannot access the plants growing on top of the mounds [28].

Carolinian old-growth forests, located only in southwestern Ontario, are incredibly rare, biodiverse ecosystems supporting over 25% of Canada's native tree species [31]. Many of the native trees are endangered or unique to the Carolinian forests making conservation efforts very important [31]. Some of these species include the Eastern flowering dogwood (*Cornus florida*), American sycamore (*Platanus occidentalis*), American chestnut (*Castanea dentata*), sassafras (*Sassafras albidum*), etc. [32]. The deer population in this region (~30 deer/km$^2$) is three times the carrying capacity, which may impact the long-term compositional dynamics of this forest. In addition, Ontario sees catastrophic wind events including the most tornado events of any province in Canada, which includes the Carolinian forest region [33], and thus tip-up mounds are common. It is, however, unclear the extent to which tip-up mounds are mediating the impacts of deer browsing on regeneration. Given how rare and biodiverse this forest type is, this study is critical in determining the contributions of deer browsing, invasive plant abundance and tip-up mounds to native tree regeneration and diversity in the Carolinian old-growth forests. Such insights are critical for more effective conservation efforts.

This study therefore seeks to understand the impacts of deer browsing on native tree species richness and diversity and to clarify the role of tip-up mounds in disrupting deer browsing access. It is hypothesized that deer exclusion plots will have higher native tree regeneration density and species richness than deer-browsed sites. Because the deer-browsed sites are disturbed sites, and invasive species tend to prefer disturbed sites, it is hypothesized that deer-browsed sites will have higher density and richness of invasive species compared to deer exclosures. Given the high density of deer in the Carolinian old-growth forest, it is hypothesized that deer over browsing will lead to higher nutrient concentrations outside rather than inside deer exclosures. Due to potential challenges with accessing plants on tip-up mounds by browsers, it is hypothesized that tip-up mounds will offer an escape from deer browsing.

## 2. Materials and Methods

### *2.1. Project Site Description*

This research was conducted at two sites about 10 km apart: the Sheelah Dunn Dooley (SDD)Nature Sanctuary, which is located in Aldershot, Burlington and owned and managed by the Hamilton Naturalists Club, and the McMaster Forest Nature Preserve (MFNP), which is located in Ancaster and owned and managed by McMaster University (Figure 1).

The SDD Nature Sanctuary is a 32-hectare property that contains a section of Grindstone Creek, with an associated steep-sided (slope = $-19.5°$) ravine with a Dry-Fresh Oak Hardwood Deciduous Forest Type (FOD2-4) that extends from the creek to the upper ravine slope. The land outside of the ravine was predominantly an agricultural cropland until 2005, while the Dry-Fresh Oak Hardwood Deciduous Forest Type (FOD2-4) on the ravine is part of an environmentally sensitive area that has escaped prior agricultural use because of the acute slope. The sanctuary provides important habitats for many unique and endangered species such as the eastern flowering dogwood (*Cornus florida*) and American columbo (*Frasera caroliniensis*). The main trees include white oak (*Quercus alba*), sugar maple (*Acer saccharum*), shagbark hickory (*Carya ovata*), and some red maple (*Acer rubrum*). The understory mainly consists of white ash (*Fraxinus Americana*), black cherry (*Prunus serotina*), and invasive buckthorn (*Rhamnus cathartica*). The soil is mainly Oneida loam, which is well drained with a smooth topography, along with high levels of clay surface soils with low infiltration rates [34]. This area has approximately 45 deer/km$^2$, which is above the 10 deer/km$^2$ carrying capacity of the region [35]. Deer exclusion plots of 20 m $\times$ 20 m were built along the upper ravine slope in the spring of 2023 to monitor the effects of deer browsing. The main invasive species is buckthorn (*Rhamnus cathartica*), which also poses a high threat to biodiversity [34].

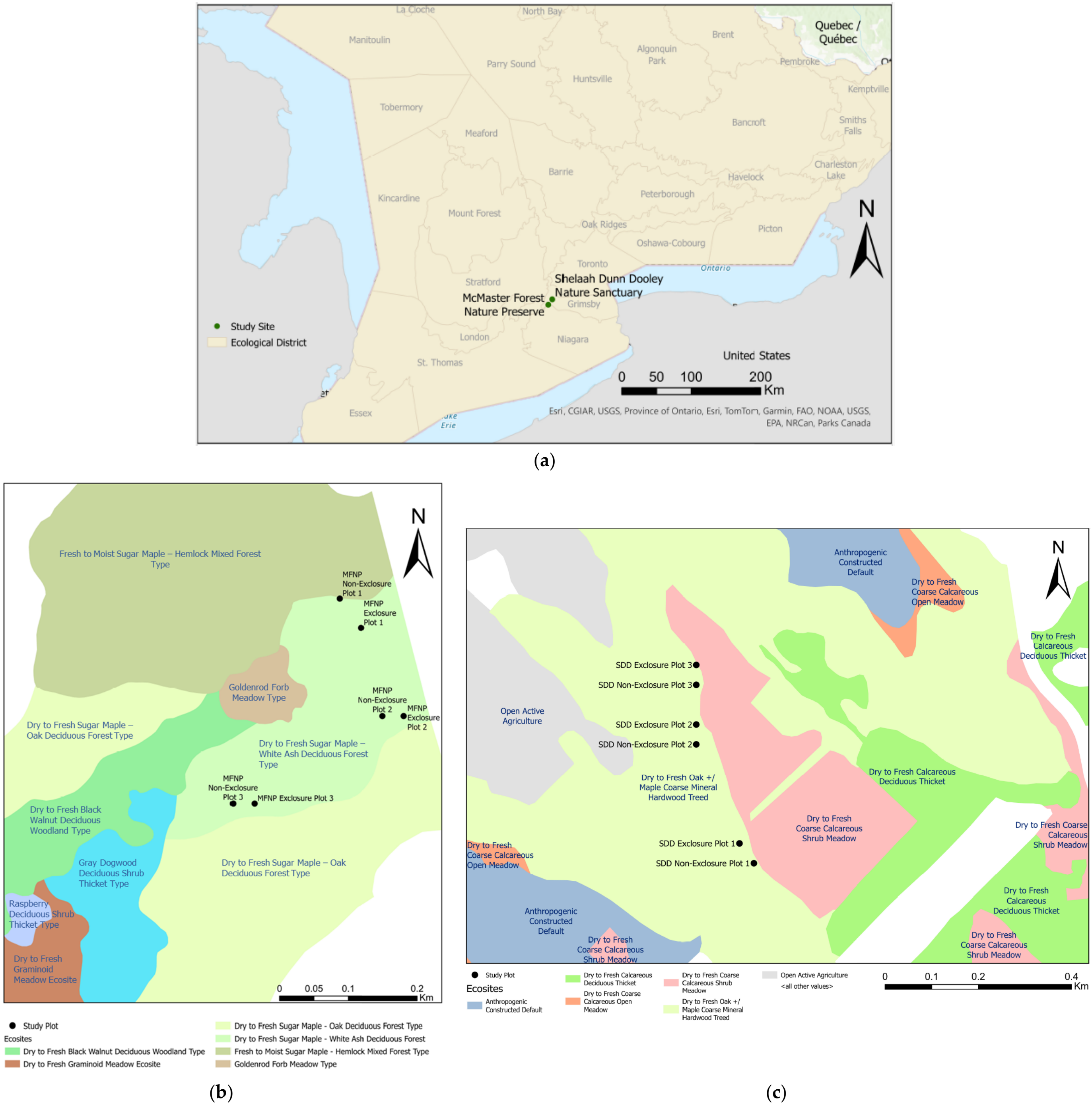



**Figure 1.** The study sites within the Grimsby ecological district. The light blue refers to the lakes in the region i.e. Lake Huron (left), Lake Erie (bottom right), Lake Ontario (right) (**a**), close-up view of the research sites showing the study plots and the ecological land classification types of the McMaster Forest Nature Preserve (**b**) and Sheelah Dunn Dooley Nature Sanctuary (**c**).

The McMaster Forest Nature Preserve is a 48-hectare natural area with relatively flat topography and located within the Niagara Escarpment Protection Area. Much of the area was agricultural crop land until 1964 when McMaster University purchased the property and allowed natural regeneration to take place [36]. However, historical aerial photographs indicate areas of forest that are over 90 years old. The deer population density in forests surrounding the McMaster Forest Nature Preserve was estimated as ~30 deer/km$^2$ for the Dundas Valley Conservation Area and ~36 deer/km$^2$ for the Tiffany

Falls Conservation Area, which is well above the 10 deer/km$^2$ carrying capacity for the region. In 2017, three deer exclusion plots of 14 m × 14 m were established in the Dry-Fresh Sugar Maple–White Ash Deciduous Forest type [36]. Figure 1 shows the ecological land classification types of this forest preserve. The main four species include sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), white ash (*Fraxinus americana*) and eastern hemlock (*Tsuga canadensis*). Garlic mustard (*Alliaria petiolata*) is the main invasive species of concern in this forest preserve. Barker [36] monitored deer browsing activity following the planting of 93 eastern hemlock (Tsuga canadensis), 96 black cherry (*Prunus serotina*) and 96 red oak (*Quercus rubrum*) seedlings within and outside of the exclosures. Results 1 year after planting show that all the black cherry and red oak outside of the exclosures were browsed and 98% of the hemlock seedlings were also browsed, while none of the planted seedlings were browsed within the exclosures. Barker [36] also removed garlic mustard from half of the plot, leaving garlic mustard in the other half of the plot, in order to measure the impact of garlic mustard on the planted seedlings. Barker [36] reported that mustard removal did not impact the planted seedlings. Because the garlic removal treatment was applied to all of the McMaster Forest Nature Preserve study plots, we do not expect it to introduce bias into our observations. In addition, given that garlic mustard has the potential to spread aggressively, we believe that in the 7-years following its removal, the species has re-colonized the studied plots.

### 2.2. Experimental Design and Data Collection

The experiment involved two elements, including (a) deer exclosures and outside of the exclosures and (b) tip-up mounds.

#### 2.2.1. Deer Exclusion Study

The McMaster Forest Nature Preserve contains 3 exclosure plots and 3 exclosure-adjacent plots with 14 m × 14 m dimensions. The Sheelah Dunn Dooley Nature Sanctuary contains 3 exclosures (established in 2023, with no manipulation conducted subsequently) and 3 adjacent plots with 20 m × 20 m dimensions. The adjacent deer-browsed plots were compared to deer exclosures to determine the impact of exclosures on soil nutrients, seedling density and diversity. Data were collected from five 1 m × 1 m quadrats placed at the corners and the centre of the main plots, with a total of 60 quadrats, similar to earlier work conducted by Stephan et al. [37]. The exact coordinates of each study plot were determined using a GPS measurement taken from the centre quadrat within each plot (cross-checked with co-ordinates generated by apple iPhone 14 device). We also measured the elevation and slope from the centre quadrat of each plot. All seedlings (woody plants < 2.5 cm) and saplings (woody plants 1 to 2 m tall) within the quadrats were identified and counted in June 2024 using tree identification guides [38] in conjunction with phone apps [39]. In order to obtain insights into soil nutrients, soil samples were collected in Ziplock bags from each quadrat for soil organic matter and nutrient analyses.

#### 2.2.2. Tip-Up Mound Study

Both study sites were surveyed for suitable tip-up mounds for this study. For a tip-up mound to be suitable, (a) it must not be an inclined mound with the possibility of tipping back up, i.e., there was clear separation between the root plate at the base of the tip-up mound and the pit, (b) the tip-up mound is reasonably tall (≥1 m from the pit) for the potential to limit deer access, (c) there is sufficient mound at the base of the uprooted tree to support seedling growth. We identified 10 tip-up mounds in the McMaster Forest Nature Preserve and marked their location (latitude and longitude co-ordinates). The tip-up mounds provide the opportunity to verify if mounds provide browse-free zones that would promote seedling growth. No suitable tip-up mound was observed at the SDD Nature Sanctuary site. The height and width of each tip-up mound were measured. Soil samples were also collected from each mound for soil organic matter and nutrient analyses. For each of the tip-up mounds, 1-month old red oak (*Quercus rubra*) seedlings (a deer-preferred species [36]) were planted: one at the top of the mound and another at the base of the uprooted tree. We teamed up with a local high school who had embarked on a project to nurse oak seedlings as a way of stimulating interest in tree planting among the youth to obtain the seedlings. Planting took place in May 2024. At the time of planting, the seedlings were on average 10–15 cm tall. Every week following the planting, we counted the total number of leaves on each seedling and measured the length of the longest leaf on each seedling.

### 2.3. Determination of Soil Organic Matter

About 15–30 g of each of the soil samples were oven-dried at 105 °C for 24 h and weighed upon cooling, giving the dried mass. Samples were then placed in ceramic crucibles and heated at 500 °C in a muffle furnace for approximately 24 h [40]. Their placement in the furnace was labelled on paper to keep track of them. Once finished heating, they were cooled for 1–2 h and weighed again to obtain ashed mass. The percentage of organic matter (SOM) in each sample is estimated using Equation (1) below:

$$\%SOM = \frac{Mass\ of\ dry\ soil - Mass\ of\ ashed\ soil}{Mass\ of\ dry\ soil} * 100\% \quad (1)$$

### 2.4. Soil Nitrate and Phosphate Determination

Soil samples from each quadrat were placed into a labelled aluminum foil cup and dried in the oven at 50 ° C for 24 h [41]. The soil from each cup was crushed in a mortar and pestle and sifted through a soil sieve. For nitrate analysis, 1 g of each soil sample was placed in labelled 25 mL Erlenmeyer flasks and 10 mL of KCl was added. A blank with no soil was also made. The solution was placed on a shaker at 160 strokers per minute for 30 min. They were then filtered through Fisherbrand P-5 filter paper into labelled test tubes. A series of $NO_3$—N standards were then made (1.00–8.00 ppm). Using a 100-µL pipette, 20 µL of each sample, standard, and blank were added to wells in a 96-well microplate with their exact placement labelled on paper. A 180 µL of nitrate reduction solution was added to each well using a 1000-µL pipette and the plate was gently shaken. The plate was incubated at 30 °C for 30 min. A colour solution was then made and 20 µL was added to each well. The microplate was left for 10 min for the colour to develop then inserted into a BioTek HT Synergy Microplate Reader for absorbance readings at 543 nm. Using the absorbance values, the nitrate levels of each solution were determined using a standard curve.

Vegetation contributes significantly to soil nutrient quality through, e.g., litter droppings. To verify if the soil nitrate content is significantly correlated with the vegetation nitrate content, we estimated the plot-level vegetation nitrate index, which is an index of the nitrate richness of the seedlings within a plot using Equation (2). We first reviewed 25 papers (Table A1) that report the nitrate content of our observed plant species and based on this assigned nitrate scores to each plant species including 1 (low nitrate content), 2 (medium nitrate) and 3 (high nitrate content). We then computed the vegetation nitrogen index using the Equation (2), as described below.

$$Vegetation\ nitrate\ index = \sum_{i}^{z} V_{ns}\left(\frac{n_{ik}}{N_k}\right) \quad (2)$$

where $V_{ns}$ is the vegetation nitrate score, i.e., 1 is low, 2 is medium and 3 is high amd $n_{ik}$ is the count of species $i$ in plot $k$. $N_k$ is the total seedling count in plot k. Plots with a high abundance of nitrate rich seedlings will have a high vegetation nitrate index.

For phosphate analysis, 2 g of each soil sample was added to a labelled 50 mL Erlenmeyer flask. A total of 20 mL of Mehlich 3 extracting solution was added to each sample under a fume hood, the flasks were corked, and each solution was shaken for 5 min at 160 strokes per minute. These were filtered through Fisherbrand P-5 filter paper into labelled test tubes. A total of 100 µL of each sample was diluted with 10 mL of water. Then, eight PO43—P standards (0.020–0.800 ppm) were made. A total of 40 µL of colour solution was added to each well in a 96-well microplate. A total of 160 µL of each diluted sample, standard and blank were added to the microplate wells and their placements were labelled. After 10 min a BioTek HT Synergy Microplate Reader was used to read the absorbances at 630 nm. Phosphate levels were then determined using absorbances and a standard curve [42].

### 2.5. Vegetation Data

The tree seedling data were coded based on the Ontario vegetation classification codes (Table A2). Based on the species ID, each plant species was grouped into native, non-native or invasive, by deer preference, and by shade tolerance. Plants were identified as either native, non-native, or invasive using the Ontario tree atlas [43], Ontario trees and shrubs [44], and the USDA plants database [45]. The deer preferability of a species was classified on a scale of *1* (highly avoided) to *5* (highly selected) based on several sources [44–52]. The shade tolerance of a species was determined using a scale of 1 (shade intolerant) to 4 (very shade tolerant) based on several sources [53–57]. Total seedling density was calculated in each quadrat along with the density of native, non-native, and invasive species and deer preferred vs. non-preferred species. Seedling species richness was estimated as the number of unique species within each quadrat. Shannon diversity index ($S_h$) was calculated for each plot using Equation (3), as described below.

$$S_h = -\sum_{i=1}^{s} P_i In P_i \tag{3}$$

where $P_i$ is the proportion of species $i$ within a plot and $In$ is the natural log.

### 2.6. Statistical Analysis and Modelling

The impact of deer browsing on nutrient and seedling recruitment dynamics was determined by comparing deer exclosures to non-exclosures using a two-sample *t*-test [58]. The proportion of highly avoided, slightly avoided, neutral, slightly selected and highly selected species was compared between exclosures and non-exclosures using a z-test. The number of seedlings by species was compared between exclosure and non-exclosures using a z-test [58]. A test is significant when the *p*-value is < 0.05 (α = 5%). The Non-Metric Multidimensional Scaling (NMDS) ordination technique was used to visualize and compare the species compositions between exclosures and non-exclosures and between sites. This was performed in R studio (v. 4.3.3) using the following packages: vegan, ggplot2, dplyr, readxl, grid and ggrepel.

In order to explain the variability in regeneration species richness and density, we explored the linear and non-linear (quadratic) effects of potential explanatory variables using the PROC GLM and PROC NLIN procedures in SAS (SAS Institute, Cary, NC, USA). PROC GLM allows for exploring the linear relations between a response variable and a single or a set of explanatory variables, while the PROC NLIN procedure allows for exploring non-linear relations between a response variable and a single or a set of explanatory variables.

$$SR = \overline{SR}\prod_{i=1}^{n} f_i(X_i) \tag{4}$$

where $SR$ is species richness, $\overline{SR}$ is mean species richness observed and $\prod_{i=1}^{n} f_i(X_i)$ designates the product of $n$ modifiers having a value close to unity when the variables $X_i$ are equal to their mean $\overline{X_i}$ and increase or decrease when moving further away from the mean. We define $f_i(X_i)$ as follows:

$$f_i(X_i) = 1 + \beta_{l.xi}\left(\frac{X_i - \overline{X_i}}{\overline{X_i}}\right) + \beta_{q.xi}\left(\frac{X_i - \overline{X_i}}{\overline{X_i}}\right)^2 \tag{5}$$

where $\beta_{l.xi}$ and $\beta_{q.xi}$ represent the linear and quadratic effects of the variable $X_i$ on species richness. Potential $X_i$ explanatory variables include elevation, slope, soil nitrate, soil phosphate, soil organic matter and treatment (exclosure vs. non-exclosure). For each model, the coefficient of determination ($R^2$), the coefficient of variation (CV), the root mean square error (RMSE) and *p*-values were noted. Results show strong linear fits (Tables 1 and A1).

**Table 1.** Species richness model fit statistics.

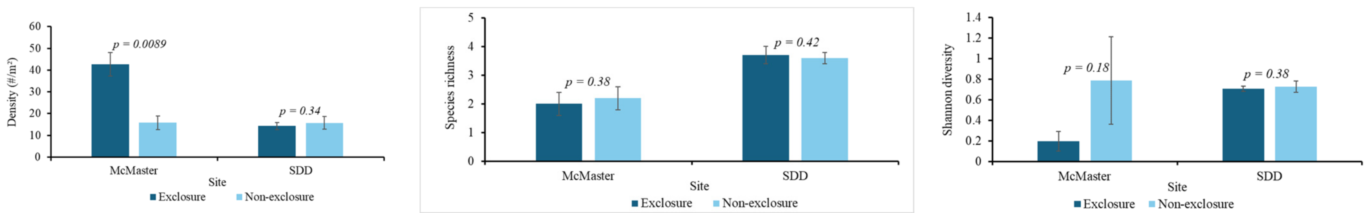
| Species Richness | Explanatory Variables | R-Square | CV | RMSE | *p*-Value |
|---|---|---|---|---|---|
| | Elevation | 0.612 | 19.741 | 0.572 | 0.003 |
| | Slope | 0.334 | 25.856 | 0.750 | 0.049 |
| | SOM | 0.363 | 25.281 | 0.733 | 0.038 |
| | Phosphate | 0.130 | 29.546 | 0.857 | 0.249 |
| | Nitrate | 0.341 | 25.712 | 0.746 | 0.046 |
| | Site | 0.765 | 15.369 | 0.446 | 0.000 |
| | Site, Phosphate | 0.900 | 10.542 | 0.306 | 0.000 |
| | Site, Slope | 0.850 | 12.900 | 0.374 | 0.000 |
| | Site, Nitrate | 0.767 | 16.107 | 0.467 | 0.000 |
| | Elevation, SOM, Phosphate | 0.869 | 12.826 | 0.372 | 0.001 |
| | Site, SOM, Phosphate | 0.905 | 10.900 | 0.316 | 0.000 |
| | Site, SOM, Phosphate, Treatment (Exclosure, Non-exclosure) | 0.912 | 11.207 | 0.325 | 0.001 |
| | Elevation, SOM, Phosphate, Treatment (Exclosure, Non-exclosure) | 0.939 | 7.818 | 0.227 | 0.000 |

## 3. Results

### *3.1. Impact of Deer Browsing on Abundance and Diversity*

#### 3.1.1. Seedling Density, Species Richness and Diversity

Two sample t-test results show that seedling density was significantly higher within exclosures than the non-exclosures at the McMaster Forest Nature Preserve site (Figure 2a, Table A3). This finding was further reinforced by NMDS site-level clustering (Figure 3), which shows a clear distinction between the McMaster exclosures and non-exclosures seedling abundance. This trend was not observed at the Sheelah Dunn Dooley (SDD) Nature Sanctuary site (Figures 2a and 3). Species richness and diversity were comparable between the exclosures and non-exclosures at both sites (Figure 2b,c). Further analysis using species accumulation curves, however, showed that as plots were added, the slope of increase in species richness was higher within deer exclosures compared to outside of the exclosures (Figure 4). When data from both sites were combined, the variability in species richness was mainly explained by slope ($R^2$ = 33%), elevation ($R^2$ = 61%) and SOM ($R^2$ = 36%) (Table 1).




(**a**) (**b**) (**c**)

**Figure 2.** Seedling density (**a**), species richness (**b**) and diversity (**c**) within and outside of exclosures at the MFNP and SDD Nature Sanctuary sites. Error bars represent standard error of the mean.

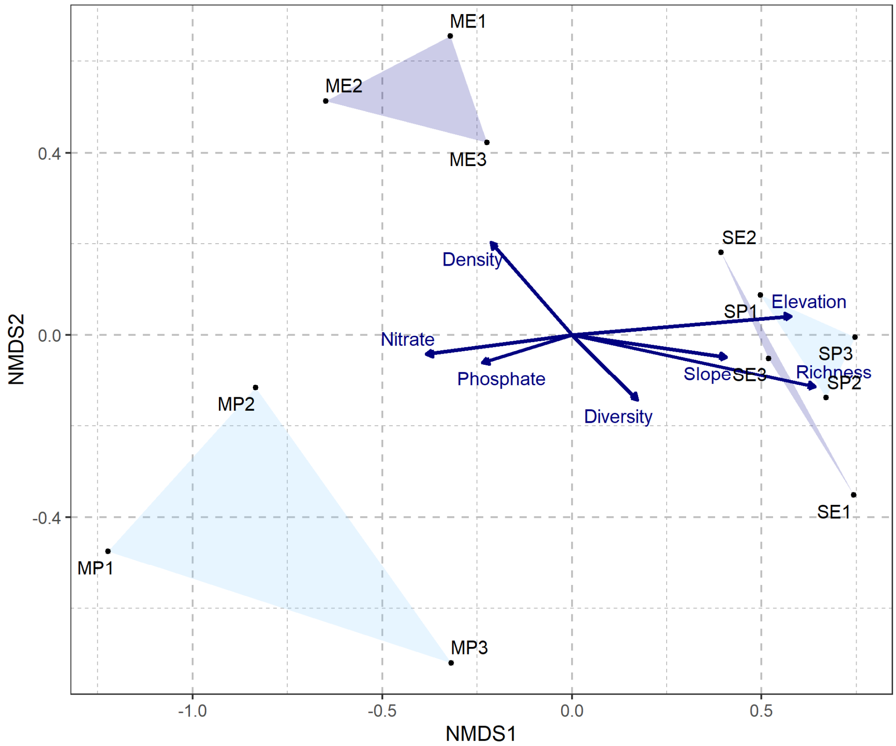



**Figure 3.** NMDS plot showing study sites and their similarity based on the vegetation metrics ($p = 0.007$). The stress value is 0.1041 indicating that this plot is a good representation of the dissimilarity between the sites as reported in other studies e.g., [59]. Darker convex hulls show the exclosures and lighter hulls show the non-exclosures. Trends in seedling density, soil nitrate, soil phosphate, species diversity, elevation, slope and species richness are represented by the arrows in the center. 'M' and 'S' stand for the MFNP and SDD Nature Sanctuary sites, respectively. 'E' and 'P' stand for exclosures and non-exclosures, respectively. As such, ME1, ME2 and ME3 refer to the MFNP exclosure plots 1, 2 and 3, respectively. MP1, MP2 and MP3 refer to the MFNP deer-browsed plots 1, 2 and 3, respectively. SE1, SE2 and SE3 refer to the SDD Nature Sanctuary exclosure plots 1, 2 and 3, respectively, while SP1, SP2 and SP3 refer to the SDD Nature Sanctuary deer-browsed plots 1, 2 and 3, respectively.

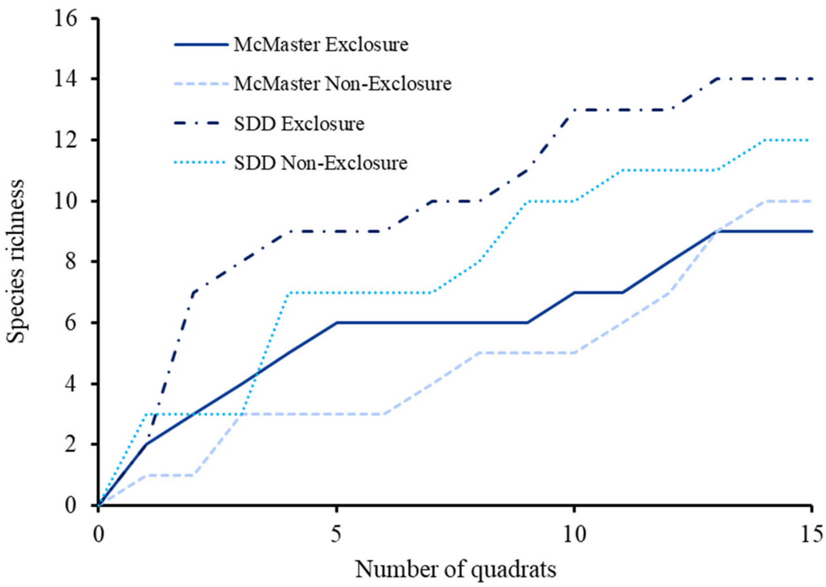



**Figure 4.** Species accumulation plot showing an increase in species richness as more quadrats are accounted for in both exclosures and non-exclosures at the MFNP and SDD Nature Sanctuary sites.

3.1.2. Abundance of Deer-Preferred Species

At the MFNP study site, results show that highly selected/deer-preferred species were far more abundant in the exclosures (70%) than in non-exclosures (30%), as illustrated in Figure 5a. In addition, highly avoided species were far more abundant in the non-exclosures (97%) than exclosures (3%). The middle three categories were relatively similar between exclosures and non-exclosures. At the SDD Nature Sanctuary study site (Figure 5b), highly selected species were slightly higher in the non-exclosure (54%) than in the exclosure (46%). The highly avoided species were also evenly distributed between exclosures (50.5%) and non-exclosures (49.5%). Again, the middle three categories were also very similar between exclosures and non-exclosures.

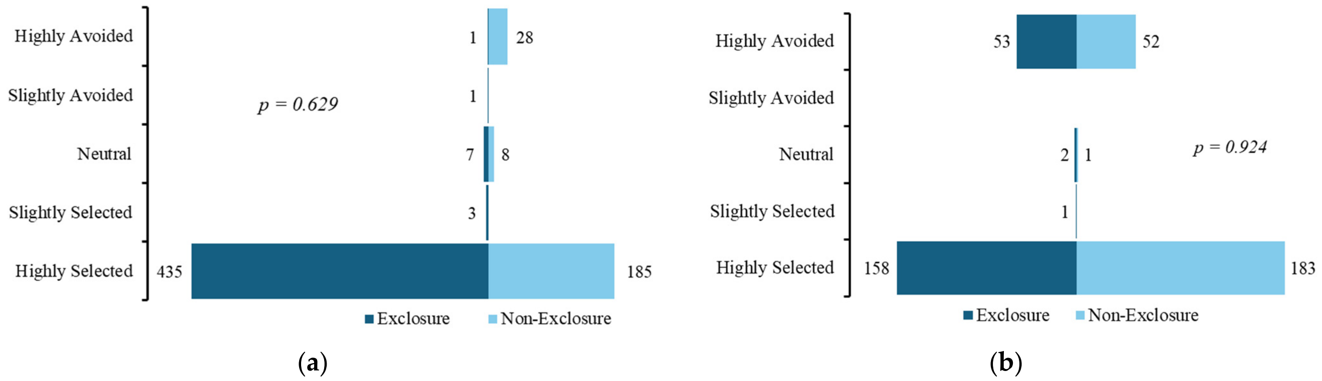



**Figure 5.** Species abundance grouped by deer preference within exclosures and outside exclosures at the (**a**) McMaster Forest Nature Preserve and (**b**) SDD Nature Sanctuary study site.

3.1.3. Species Dominance

The species abundance curves (Figures 6 and A1), the butterfly charts (Figure 7) and the NMDS plots (Figures 3 and 8), indicate a large contrast between the vegetation communities in the MFNP and SDD Nature Sanctuary. The steep lines representing MFNP species abundance show a sugar maple dominance (Figures 6, 7a and A1a,c). The SDD Nature Sanctuary species abundance curves are less steep indicating reduced dominance by a single species ( and Figures 6, 7b and A1b,d). There is a far greater difference in species dominance between MFNP and SDD Nature Sanctuary study sites than between the exclosures and non-exclosures.

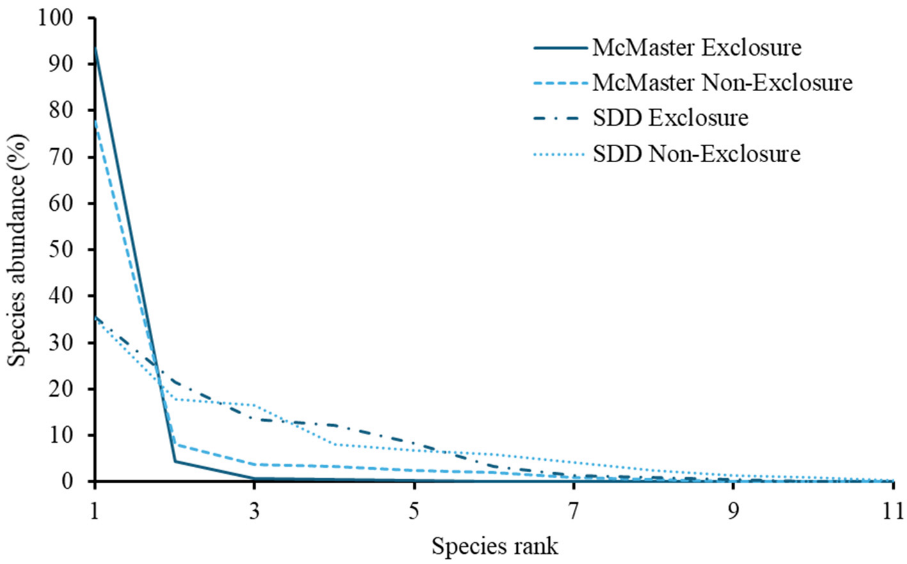
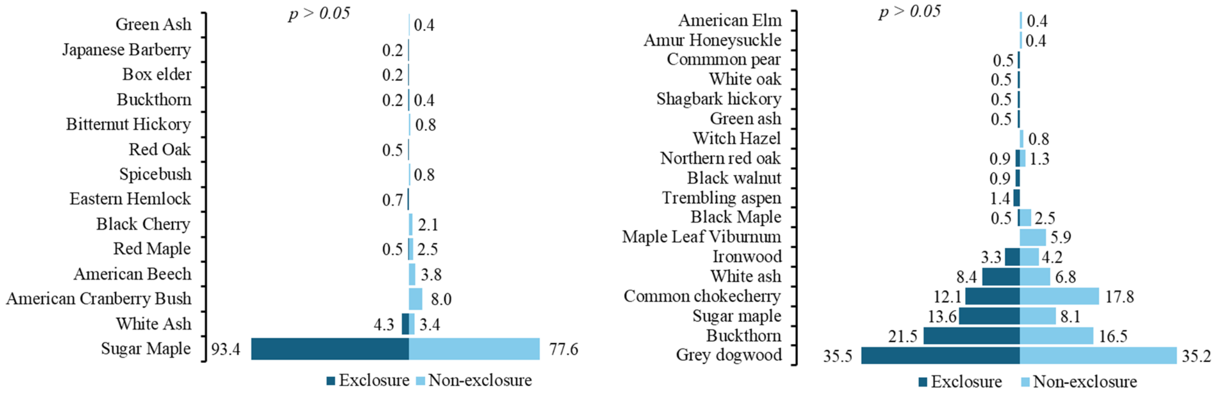
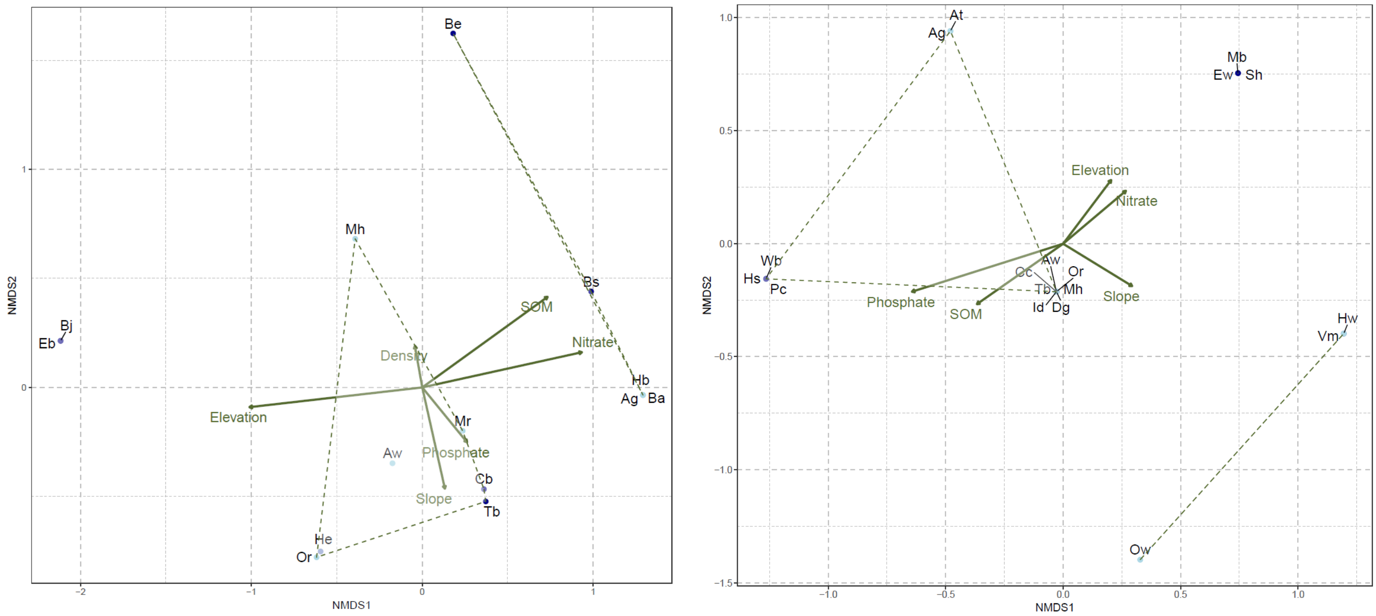



**Figure 6.** Combined species abundance curves for exclosures and non-exclosures at both the MFNP and SDD Nature Sanctuary sites.




(**a**) (**b**)

**Figure 7.** Butterfly charts comparing the abundance of different woody species between the exclosures and non-exclosures in both (**a**) MFNP and (**b**) SDD Nature Sanctuary sites.

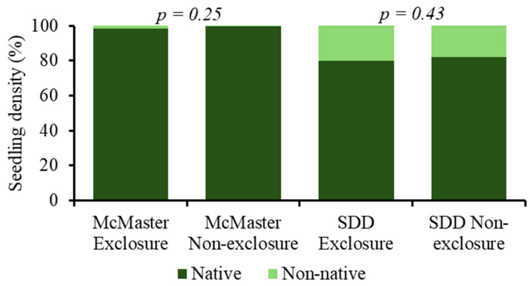



(**a**) (**b**)

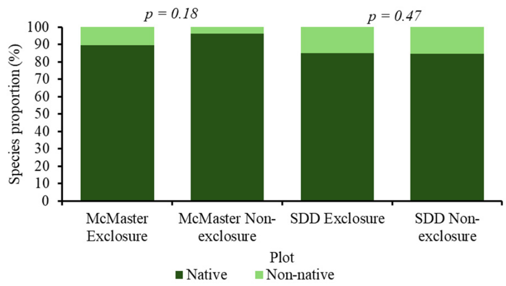
**Figure 8.** NMDS Ordination plot showing the species-level clustering at (**a**) the MFNP site ($p$-value = 0.003) and (**b**) the SDD Nature Sanctuary site ($p$-value = 0.001). Each species is represented by its species code (defined in Table A1). Trends in seedling density, elevation, soil organic matter (SOM), soil phosphate and nitrate content are shown by the arrows in the center (deer preference and species richness were dropped for clarity). The lightness of each point represents the level of deer preference, i.e., lighter blue shows the species is preferred by deer while deeper blue indicate high deer avoidance.

### *3.2. Impact of Deer Browsing on Species Invasion*

Inter-site differences were higher than differences between exclosures and non-exclosure with regards to the proportion of native to non-native species (Figure 9, Table A4). The proportion of native species within the exclosures was statistically insignificantly different from that of the non-exclosures for both sites.




(**a**)

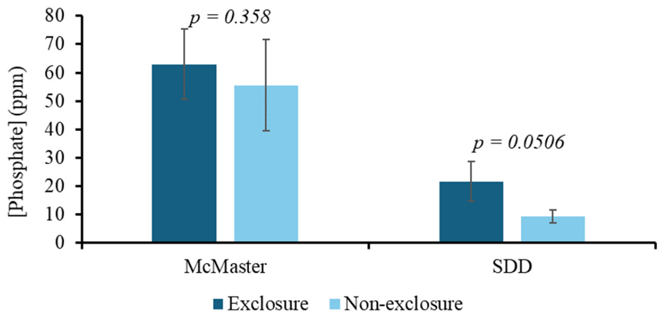



(**b**)

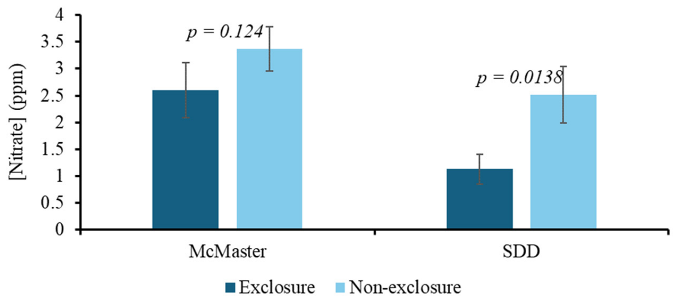
**Figure 9.** Percentage of native species, shown as both (**a**) seedling density and (**b**) species proportion, in the treatment and control plots at the MFNP and SDD Nature Sanctuary sites.

### 3.3. Impact of Deer Browsing on Soil Nutrient Concentration

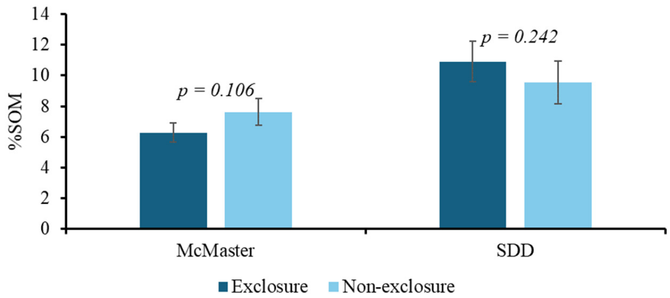
The results of this analysis showed slightly higher phosphate levels in the deer exclosures at both the MFNP study site ($p = 0.358$) and the SDD Nature Sanctuary study site ($p = 0.0506$) (Table A5, Figure 10). Furthermore, nitrate levels were lower in the deer exclosures at both the MFNP ($p = 0.124$) and the SDD Nature Sanctuary study sites ($p = 0.0138$). Finally, soil organic matter was comparable between exclosure and non-exclosures at both the MFNP site ($p = 0.106$) and the SDD Nature Sanctuary site ($p = 0.242$).




(**a**)




(**b**)




(**c**)

**Figure 10.** (**a**) Soil phosphate concentration within and outside of the exclosures at the MFNP and SDD Nature Sanctuary sites. Error bars represent standard error of the mean. (**b**) Soil nitrate concentration

within and outside of the exclosures at the MFNP and SDD Nature Sanctuary sites. Error bars represent standard error of the mean. (**c**) Soil organic matter within and outside of the exclosures at the MFNP and SDD Nature Sanctuary sites. Error bars represent standard error of the mean.

### *3.4. Impact of Tip-Up Mounds on Deer Browsing Activity*

After 9 weeks of monitoring, the seedling count decreased by 90% at the base of the mounds and by 60% at the top of the mounds (Figure 11). The average number of leaves remaining stayed constant at four for seedlings at the base and fluctuated for seedlings planted at the top. Seedling remaining, based on the height of the mound (Figure 11c), shows the greatest decrease for mound height of 0 m (seedlings at the base) and the least decrease in the mounds of 1–1.5 m in height. The two mounds higher than 2 m were omitted from the plots.


(**a**) (**b**) (**c**)

**Figure 11.** The proportion of seedlings remaining at the top and base of the mounds over time (**a**), the average number of leaves remaining on seedlings at the top and base of the tip-up mounds over time (**b**) and the proportion of seedlings in different height categories over time (**c**).

## 4. Discussion

Owing to the rarity and the richness of biodiversity of the Niagara escarpment, the region has been designated a UNESCO Biosphere reserve, thus limiting human interference in order to enhance the sustainability of the ecosystem services provided by this pristine forest. An understanding of how non-human stress factors (such as herbivory by white tailed deer) are impacting this forest will enhance its management. In this study, we examined the impact of deer browsing on the regeneration dynamics and soil nutrient concentration of an old-growth Carolinian forest in south-western Ontario. Because data were collected from two study sites, we discussed the inter-site differences in regeneration dynamics and soil nutrient concentration. We subsequently discussed the impact of deer browsing on seedling attributes (density, diversity and species invasion), the impact of deer browsing on soil nutrients and the impact of tip-up mounds on deer browsing access.

### *4.1. Inter-Site Differences in Regeneration Dynamics and Soil Nutrient*

The species abundance found in this study averaged 16 seedlings per square meter at the MFNP and 14 at SDD Nature Sanctuary site. In a study conducted in North American temperate forests, the average seedling density was found to be 9 with a range of 0–57 in the 1 $m^2$ quadrats [60]. A different study conducted in the MFNP, sampling all saplings above 130 cm in height, found an average of 52.8 stems per 20 m $\times$ 20 m with a large variation in abundance between the plots [61]. This value is equivalent to 0.132 stems per 1 m $\times$ 1 m quadrat; however, as that study only counted saplings and not seedlings, it would be expected to be much lower than the values found in this study. Species richness in this study was found to be on average 2.2 species per square meter at MFNP and 3.6 species per square meter at the SDD Nature Sanctuary study sites (Table A6). An earlier study conducted by Stegman [62] at the same site reported 105 species per square meter, which is higher than our data but may be due to the fact that the earlier study looked at the richness of floral species rather than only woody species. We observed an average Shannon diversity value of 0.79 at the MFNP and 0.73 at the SDD Nature Sanctuary study site (Table A6). As there were only 10 and 12 species in these samples, the maximum diversity value possible is ~1 (log10(10) = 1), indicating significantly high diversity, which is consistent with the high biodiversity known for the Carolinian forests in this region [31].

The average soil nitrate was 3.4 ppm at the MFNP and 2.5 ppm at the SDD Nature Sanctuary sites (Table A6). A study conducted in deciduous forests in Ohio found soil nitrate levels to be ~5.11 ppm [63]. Another study conducted in pine and oak-dominated temperate forests of China reported nitrate values in the range of 12–35 ppm [64]. Our data thus show that soil nitrate levels are low for the deciduous forests of Hamilton (Southern Ontario). Soil phosphate levels in this study were found to be an average of 55.6 ppm at the MFNP and 9.2 ppm at the SDD Nature Sanctuary study site (Table A6). This is comparable to the average soil phosphorus levels of ~37 ppm reported for the Southern Ontario region by the Fertilizer Institute [65]. Our SOM levels were 7.6% at the MFNP and 9.6% at the SDD Nature Sanctuary control sites (Table A6). A typical loam soil in Ontario has a SOM of 4–5% [66] and the Fertilizer Institute's reported average SOM for agricultural soils in southern Ontario is 3.3% [65]. The deciduous forests of Hamilton and Southern Ontario thus have slightly higher SOM values than the agricultural soils of the region.

The two study sites differ most notably in species dominance, with the MFNP site dominated by mainly sugar maple (*Acer saccharum*) species while the SDD Nature Sanctuary site was dominated by Gray dogwood (*Cornus racemose*), albeit to a lesser extent. The two sites differ significantly in slope and elevation; the SDD Nature Sanctuary site has greater elevation (135 m) and steeper slopes ($-19.5^{\circ}$) than the MFNP site (Table A6), which explains the significant variability in species richness. A higher elevation with steeper slopes would drive more erosion events leading to phosphate levels that are 6× lower on this site [67]. Phosphate is more susceptible to erosion than nitrate as it has been found to decrease soil stability by impacting the soil charge [68]. He et. al. [69] reported over 7× higher loss in available phosphorus on 20° slope compared to 10° slope. The higher SOM values of this site reflect the dense old growth closed canopy forest with high litter turnover rate [34].

### *4.2. The Impact of Deer Browsing on Seedlings*

We observed a significantly higher density of regeneration within the exclosures (e.g., [37]) after 7 years of deer exclusion. Though differences in species richness and Shannon diversity between exclosures and non-exclosures were not significant even after 7 years of deer exclusion, the nature of the result is similar to the results reported by Stephan et al. [37]. Furthermore, density may increase richness if there is high species evenness, but the species evenness was quite low in some of the exclosures, meaning that increased density did not lead to significantly higher richness or diversity [70]. However, species accumulation curves reveal a higher slope of increase in species richness in the deer exclosures at both study sites (Figure 4), which signals a positive correlation between deer exclusion and species richness. We also observed that after 7 years of deer exclusion (MFNP), deer-preferred species were far more abundant in the exclosures and deer-highly avoided species were more abundant in the non-exclosures, which supports the hypothesis that deer browsing will lead to the predominance of deer-tolerant species. This trend is because selective browsing on preferred species allows unpalatable species to proliferate in non-exclosures, while lack of browsing will have the opposite effect (e.g., [71]). It was also hypothesized that more invasive species would be present on the browsed sites relative to deer exclosures [72]; however, the opposite was observed at the MFNP site. This may be because there were very few invasive tree species recorded overall at the MFNP site, meaning no strong trends could be obtained. The SDD Nature Sanctuary site had slightly more invasive species; however, the impact of browsing was insignificant, perhaps because the exclusion plots at SDD Nature Sanctuary were established only 1-year prior to the study. Hemlock (*Tsuga canadensis*) was the only conifer species observed; further studies could examine the impact of deer browsing on conifer regeneration.

### 4.3. The Impact of Deer Browsing on Soil Nutrient

We expected higher nutrient concentration on browsed sites relative to non-exclosures, which was true for nitrate and SOM. However, phosphate levels were higher in the exclosures. A study on the effects of deer exclosures monitored over 18 years showed that PO4-P and NO3-N concentrations were not impacted and that SOM increased in the exclosures [37]. Another study found that deer browsing led to decreased soil nitrate levels [22]. A study conducted in Canadian temperate forests showed that the exclusion of deer decreased soil compaction, which increased soil phosphate levels [73]. Overall, these results vary, which may indicate that deer may play a role but other factors in the biotic and abiotic environment, such as soil microbes and plant makeup in the understory, may make large differences and cause the impact of deer to be less straightforward [37,74]. In our case, further analysis of the vegetation nitrate content shows that vegetation nitrate is comparable across sites and between exclosures and non-exclosures (Figure 12), which implies that the observed differences in soil nitrate were not driven by vegetation nitrate content but rather most likely due to the direct impacts of deer fecal droppings.

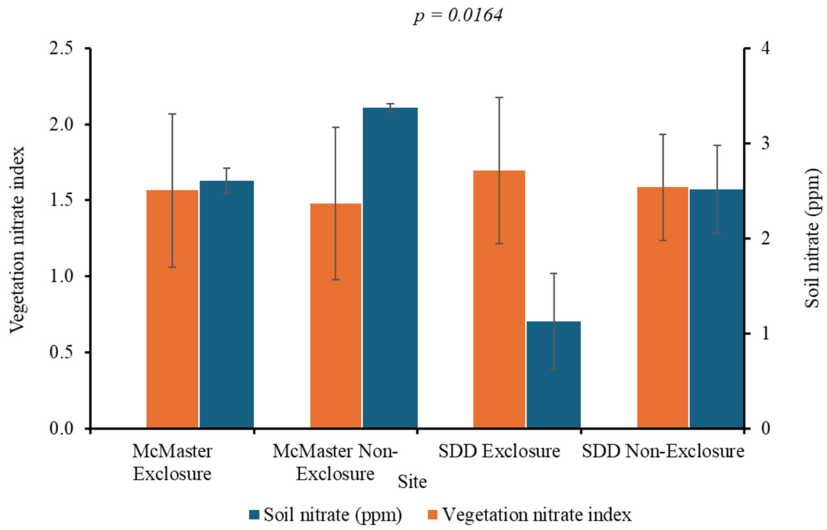



**Figure 12.** Vegetation nitrate index and soil nitrate by site and for exclosures and non-exclosures. Error bars represent standard error of the mean.

### 4.4. The Impact of Tip-Up Mounds on Deer Browsing Activity

Finally, we verified if seedlings growing on tip-up mounds tend to be inaccessible to deer. As expected, the tip-up mounds appeared to give some protection to the seedlings growing on top, as more seedlings at the bases of the mounds were found missing over the 9-week period. There did not appear to be useful insights in terms of leaf growth in relation to seedling position on mound (top vs. base of mound) and limited correlation between the height of mound and the seedlings remaining overtime, most likely due to the limited number of tip-up mounds considered in this study.

## 5. Conclusions

The results of this study show how deeply deer browsing impacts both soil and vegetation dynamics in an old growth Carolinian forest. Exclusion of deer led to a significant increase in vegetation density, insignificant differences in richness and diversity and a greater abundance of deer-preferred species, reinforcing the notion of deer as careful selective browsers. Furthermore, as more time passes, the differences between exclosures and non-exclosures became evident, as demonstrated by the differences in the results from the MFNP and SDD Nature Sanctuary sites. Further work could track the growth of specific seedlings over time because some studies reported that certain plant species in exclosures may outcompete native seedlings due to the lack of browsing. Finally, results regarding the tip-up mounds indicate that this may be a promising, natural method of deer exclusion.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su162310589/s1.

**Author Contributions:** Conceptualization, K.A.A. and S.A.D.; methodology, K.A.A., S.K.D. and S.A.D.; validation, K.A.A., S.A.D. and S.K.D.; formal analysis, K.A.A. and S.K.D.; data curation, K.A.A. and S.K.D.; writing—original draft preparation, K.A.A. and S.K.D.; writing—review and editing, K.A.A., S.K.D. and S.A.D.; visualization, K.A.A. and S.K.D.; supervision, K.A.A.; funding acquisition, K.A.A. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

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## Appendix A

**Table A1.** Leaf nitrate classification into low (1), medium (2) and high (3) and the reference literature.

| Common Name | Scientific Name | Nitrate Class | Reference |
|---|---|---|---|
| American beech | *Fagus grandifolia* | 3 | [75] |
| American cranberry bush | *Viburnum americanum* | 3 | [76] |
| American elm | *Ulmus americana* | 3 | [77] |
| American witch hazel | *Hamamelis virginiana* | 3 | [78] |
| Amur honeysuckle | *Lonicera maackii* | 3 | [79] |
| Bitternut hickory | *Carya cordiformis* | 3 | [80] |
| Black cherry | *Prunus serotina* | 3 | [81] |
| Black maple | *Acer nigrum* | 1 | [82] |
| Black walnut | *Juglans nigra* | 2 | [83] |
| Box elder | *Acer negundo* | 2 | [84] |
| Buckthorn | *Rhamnus cathartica* | 3 | [85] |
| Common chokecherry | *Prunus virginiana* | 1 | [86] |
| Common pear | *Pyrus communis* | 3 | [87] |
| Eastern hemlock | *Tsuga canadensis* | 1 | [88] |
| Green ash | *Fraxinus pennsylvanica* | 3 | [89] |
| Grey dogwood | *Cornus racemosa* | 1 | [90] |
| Ironwood/hophornbeam | *Ostrya virginiana* | 2 | [91] |
| Japanese barberry | *Berberis thunbergii* | 3 | [92] |
| Mapleleaf viburnum | *Viburnum acerifolium* | 1 | [93] |
| Red maple | *Acer rubrum* | 1 | [94] |
| Red oak | *Quercus rubra* | 2 | [95] |
| Shagbark hickory | *Carya ovata* | 3 | [80] |
| Spicebush | *Lindera benzoin* | 1 | [96] |
| Sugar maple | *Acer saccharum* | 1 | [82] |
| Trembling aspen | *Populus tremuloides* | 3 | [97] |
| White ash | *Fraxinus americana* | 3 | [98] |
| White oak | *Quercus alba* | 2 | [99] |

**Table A2.** Tree species codes (following the Ontario Vegetation Classification).

| Common Name | Scientific Name | Species Code |
|---|---|---|
| American beech | *Fagus grandifolia* | Be |
| American cranberry bush | *Viburnum americanum* | Ba |
| American elm | *Ulmus americana* | Ew |
| American witch hazel | *Hamamelis virginiana* | Hw |
| Amur honeysuckle | *Lonicera maackii* | Sh |
| Bitternut hickory | *Carya cordiformis* | Hb |
| Black cherry | *Prunus serotina* | Cb |
| Black maple | *Acer nigrum* | Mb |
| Black walnut | *Juglans nigra* | Wb |
| Box elder | *Acer negundo* | Eb |
| Buckthorn | *Rhamnus cathartica* | Tb |
| Common chokecherry | *Prunus virginiana* | Cc |
| Common pear | *Pyrus communis* | Pc |
| Eastern hemlock | *Tsuga canadensis* | He |
| Green ash | *Fraxinus pennsylvanica* | Ag |
| Grey dogwood | *Cornus racemosa* | Dg |
| Ironwood/hophornbeam | *Ostrya virginiana* | Id |
| Japanese barberry | *Berberis thunbergii* | Bj |
| Mapleleaf viburnum | *Viburnum acerifolium* | Vm |
| Red maple | *Acer rubrum* | Mr |
| Red oak | *Quercus rubra* | Or |
| Shagbark hickory | *Carya ovata* | Hs |
| Spicebush | *Lindera benzoin* | Bs |
| Sugar maple | *Acer saccharum* | Mh |
| Trembling aspen | *Populus tremuloides* | At |
| White ash | *Fraxinus americana* | Aw |
| White oak | *Quercus alba* | Ow |

**Table A3.** Seedling density and species richness and diversity in both the control and treatment groups at the MFNP and SDD Nature Sanctuary sites.

| | McMaster | | | Sheelah Dunn Dooley | | |
|---|---|---|---|---|---|---|
| | **Treatment** | **Control** | ***p*-Value** | **Treatment** | **Control** | ***p*-Value** |
| Density | 42.6 ± 5.4 | 15.8 ± 3.1 | 0.0089 | 14.3 ± 1.6 | 15.7 ± 2.9 | 0.34 |
| Species Richness | 2.0 ± 0.40 | 2.2 ± 0.4 | 0.38 | 3.8 ± 0.3 | 3.6 ± 0.02 | 0.42 |
| Shannon Diversity | 0.20 ± 0.096 | 0.79 ± 0.43 | 0.18 | 0.71 ± 0.03 | 0.73 ± 0.05 | 0.38 |

**Table A4.** Percentage of native seedlings (seedling density) and native species (species proportion) in the treatment and control groups at the MFNP and SDD Nature Sanctuary sites.

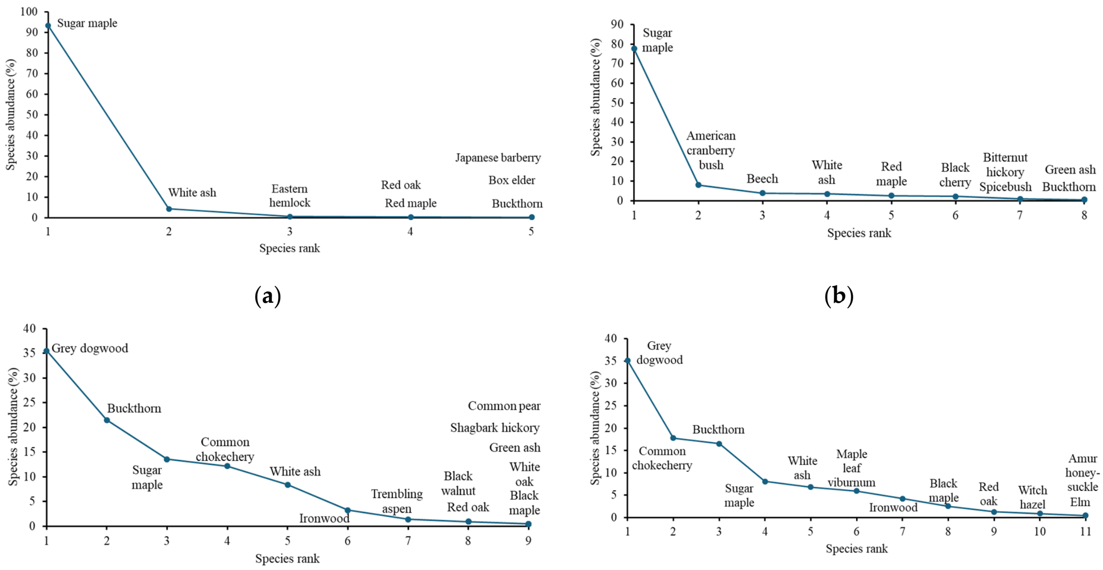
| | McMaster | | | Sheelah Dunn Dooley | | |
|---|---|---|---|---|---|---|
| | **Treatment** | **Control** | ***p*-Value** | **Treatment** | **Control** | ***p*-Value** |
| Seedling Density | 98.2 ± 1.5 | 99.4 ± 0.6 | 0.25 | 79.8 ± 8.5 | 82.1 ± 9.3 | 0.43 |
| Species Proportion | 89.7 ± 5.2 | 96.3 ± 3.7 | 0.18 | 85.2 ± 3.7 | 84.9 ± 2.6 | 0.47 |

**Table A5.** Soil phosphate, nitrate and organic matter in the treatment and control plots at the MFNP and SDD Nature Sanctuary sites.

| | McMaster | | | Sheelah Dunn Dooley | | |
|---|---|---|---|---|---|---|
| | **Treatment** | **Control** | ***p*-Value** | **Treatment** | **Control** | ***p*-Value** |
| Phosphate | 63.0 ± 12.3 | 55.6 ±16.0 | 0.358 | 21.6 ± 7.0 | 9.2 ± 2.3 | 0.0506 |
| Nitrate | 2.6 ± 0.5 | 3.4 ± 0.4 | 0.124 | 1.1 ± 0.3 | 2.5 ± 0.5 | 0.0138 |
| SOM | 6.3 ± 0.6 | 7.6 ± 0.9 | 0.106 | 10.9 ± 1.3 | 9.6 ± 1.4 | 0.242 |

**Table A6.** Inter-site summary of biotic and abiotic attributes.

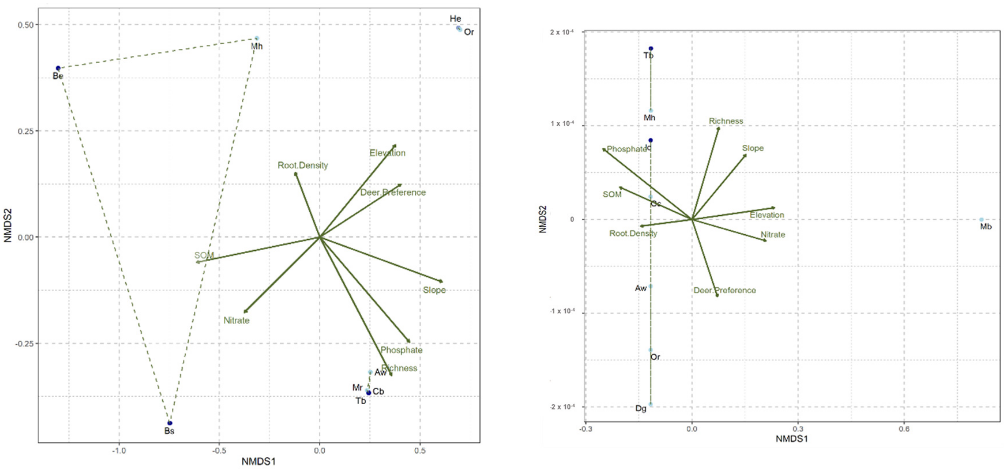
| Metric | McMaster Forest Preserve Average (Min, Max) | Sheelah Dunn Dooley Nature Sanctuary Average (Min, Max) |
|---|---|---|
| Species density (/m$^2$) | 16 (4, 60) | 14 (4, 33) |
| Species richness (/m$^2$) | 2.2 (1.8, 3) | 3.6 (3.2, 3.8) |
| Shannon diversity index | 0.79 (0.24, 1.64) | 0.73 (0.64, 0.83) |
| Elevation (m) | 115 (110, 120) | 135 (130, 140) |
| Slope (degrees) | −5 (−2, −14) | −19.5 (−12, −27) |
| Soil phosphate (ppm) | 55.6 (2.0, 229) | 9.2 (3.0, 28) |
| Soil nitrate (ppm) | 3.4 (2.6, 4.2) | 2.5 (1.8, 3.0) |
| Soil Organic Matter (%) | 7.6 (5.0, 17.6) | 9.6 (4.8, 28) |




(**a**) (**b**)

(**c**) (**d**)

**Figure A1.** Species abundance curves for McMaster (**a**) exclosures and (**b**) non-exclosures and Sheelah Dunn Dooley (**c**) exclosures and (**d**) non-exclosures.




(**a**) (**b**)

**Figure A2.** NMDS plot showing the species distributions/associations at (**a**) the McMaster site ($p$ = 0.004) and (**b**) the Sheelah Dunn Dooley site ($p$ = 0.144). Each species is represented by its species code (Table A1). Only species that occurred on at least two plots were included in this analysis.

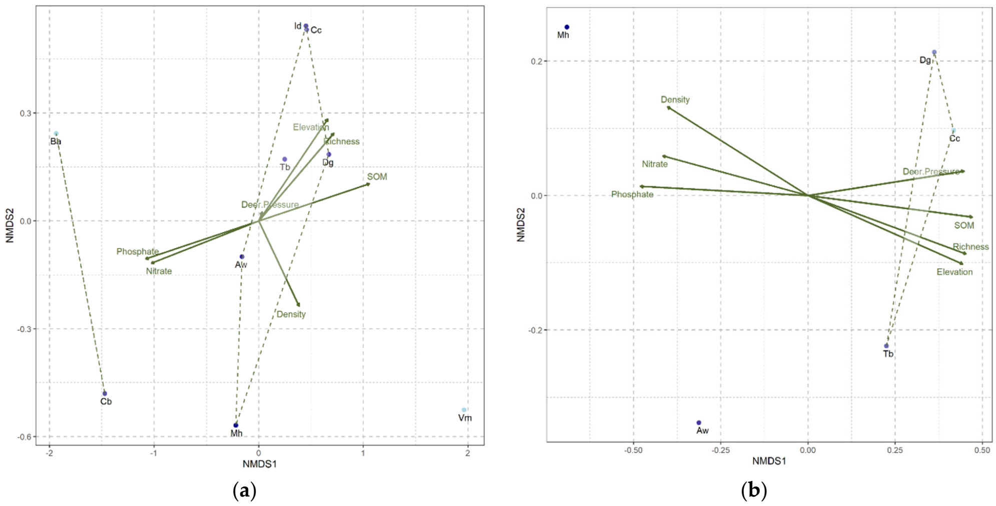



**Figure A3.** NMDS plot showing the species-level clustering with all sites combined and removing rare species. (**a**) Species with total abundance < 10 removed (1%) and (**b**) species with total abundance < 50 removed (5%).

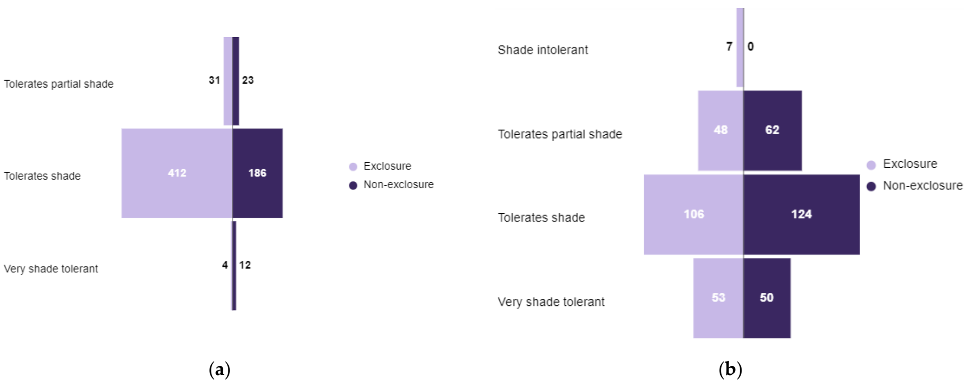



**Figure A4.** Species abundance by shade tolerance class for (**a**) the McMaster Forest Nature Preserve and (**b**) the Sheelah Dunn Dooley Nature Sanctuary.

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