OXFORD

Institute of Chartered Foresters

Forestry: An International Journal of Forest Research, 2023, 1–10

https://doi.org/10.1093/forestry/cpad052

Original Article

Changes to the understory vegetation community of the Acadian Forest a decade after herbicide use

Jennifer Xiao[1], Sarah Yakimowski[1], Marika Brown[2], Shane Heartz[3], Amy L. Parachnowitsch[2] and Christopher B. Edge [3,*]

[1]Biology Department, Queen's University, Kingston, Ontario K7L 3N6, Canada
[2]Department of Biology, University of New Brunswick, Fredericton, New Brunswick E3B 5A3, Canada
[3]Atlantic Forestry Centre, Natural Resources Canada–Canadian Forest Service, 1350 Regent St, Fredericton, New Brunswick E3B 5P7, Canada

*Corresponding author. E-mail: christopher.edge@nrcan-rncan.gc.ca

Abstract

Herbicides are commonly used in forestry to enhance conifer tree growth by reducing the abundance of competitive, undesired, early successional species. Reducing the abundance of understory species could also lead to changes in community composition that need to be documented to aid the understanding of any downstream ecosystem changes. We examined the effects of glyphosate-based herbicides on the abundance, diversity, and community composition of the understory vegetation community of forests located in the temperate-boreal transition zone. We sampled 37 blocks in two ecoregions of the Acadian Forest in eastern Canada that were harvested over the last 15 years. Species richness, Shannon's diversity, or evenness did not differ among blocks with different herbicide history. However, community composition differed between the non-herbicide and herbicide blocks in both ecoregions. Overall, 26.5% of the plant community variation was explained by the factors herbicide use (10.6%), Biomass Growth Index/site quality (8.6%), time since harvest (3.6%), and ecoregion (1.7%). We found 16 indicator species that differentiated the non-herbicide (9 species) and herbicide blocks (7 species). Indicator species for non-herbicide blocks included two blueberry species, three shrubs (two flowering), and two ferns, whereas indicator species for herbicide blocks were largely perennial forbs. Together, our results indicate that herbicide use does not alter species richness but does reduce shrub abundance, a change that persists throughout the 10 years post-herbicide application captured in our study. The reduced shrub layer likely leads to other changes in the plant community. Herbicide use is associated with subtle changes to the understory plant community, and these changes are missed when only alpha diversity is used to examine the effects of herbicides use on community composition.

Keywords: glyphosate; succession; silviculture; clearcutting; plant community

Introduction

Forest regeneration, following both anthropogenic and natural disturbance, is fundamental to stand development and is influenced by many factors, including local site characteristics, climate, and biotic interactions (Bell *et al.* 2011, Bartels *et al.* 2016). Resource competition is a biotic interaction that can have a large effect on the growth of crop trees and the regenerated post-harvest vegetation community (Balandier *et al.* 2006). When competition is likely to prevent a stand from reaching a desired state, a variety of silvicultural techniques can be used to guide the regeneration trajectory (Spence 2001, Wiensczyk *et al.* 2011). However, the tools and techniques used to guide the trajectory of crop trees also affect the performance of other species, which could, in turn, lead to effects on plant community structure and other components of the forest ecosystem.

In Canadian forestry, herbicides are commonly used to reduce competition between conifer trees and other vegetation (Sullivan *et al.* 1996, Thiffault and Roy 2011, Rolando *et al.* 2017). The most commonly used herbicides in Canadian forestry, and around the world, are glyphosate based (Wagner *et al.* 2004, Rolando *et al.* 2017). Glyphosate-based herbicides are non-selective, broad spectrum, and often applied 1–3 years post-planting of coniferous trees. Applications are made in late summer when coniferous trees have developed a waxy cuticle protecting them from the herbicide (Rolando *et al.* 2017, Comeau and Fraser 2018). Typical applications of glyphosate-based herbicides reduce competition for conifers from co-occurring grasses, herbs, broadleaf trees, and shrubs, leading to an increase in coniferous wood volume and greater basal area in fir and spruce (MacLean and Morgan 1983, Wagner *et al.* 2004, Olson *et al.* 2012, Comeau and Fraser 2018). In addition to the direct effects of herbicide on the forest vegetation community, reducing the abundance of vegetation that competes with conifer species could lead to indirect effects on other components of the ecosystem (Edge *et al.* 2020). Indirect effects are most likely to arise from broad changes in the vegetation community rather than changes in alpha diversity or the abundance of individual species which have been the focus of prior research.

The application of glyphosate-based herbicides modifies the relative abundance of plant species, but not species richness or diversity (Sullivan *et al.* 1996, Boateng *et al.* 2000, Comeau and Fraser 2018, Deighton *et al.* 2021). Few species disappear from treated sites for three reasons: First, the intent of herbicide application in forestry is to reduce the abundance of

Handling editor: Dr. Dominik Thom
Received: May 17, 2023. **Revised:** October 5, 2023. **Accepted:** October 11, 2023

competing vegetation, not eliminate it (Sullivan and Sullivan 2003, Thompson and Pitt 2003). Second, application is rarely homogeneous because herbicides are applied at the minimum effective volume by aircraft (Pitt *et al.* 1992, Sullivan and Sullivan 2003). Finally, most of the spray cloud is intercepted by tall vegetation leaving low-growing vegetation untreated or partially treated (Sullivan and Sullivan 2003, Wood 2019, Botten *et al.* 2021). Consistent with the goal of reducing competing vegetation, herbicide application reduces the abundance of most non-conifer species by up to 60% the year after application (Freedman *et al.* 1993, Sullivan *et al.* 1998). Overall, the loss of abundance is greater for taller species, such as deciduous trees and lower, but this is still significant for shrubs and herbs (Santillo *et al.* 1989, Freedman *et al.* 1993, Sullivan *et al.* 1996, 1998, Comeau and Fraser 2018). Shortly after glyphosate application (3–5 years), plant species diversity is either the same or increases in treated sites relative to control sites (Freedman *et al.* 1993, Boateng *et al.* 2000, Lautenschlager and Sullivan 2004, Comeau and Fraser 2018). Differences in abundance generally persist for 5–10 years post herbicide application after which the abundance of near-ground herbaceous and woody vegetation is similar (Lautenschlager and Sullivan 2004, Ristau *et al.* 2011, Comeau and Fraser 2018, Ulappa *et al.* 2020). Increases in plant diversity have also been detected and are attributed to herbicide application reducing the abundance of dominant/taller species, providing opportunities for the establishment of early successional forbs and grasses (Freedman *et al.* 1993, Boateng *et al.* 2000).

The effects of herbicide use on forest structure have been well established (e.g. Lautenschlager 1993, Sullivan *et al.* 1998, Boateng *et al.* 2000, Lautenschlager and Sullivan 2004, Ristau *et al.* 2011, Stoleson *et al.* 2011, Comeau and Fraser 2018, Ulappa *et al.* 2020). There is also abundant evidence that herbicides are effective at shifting stand composition to coniferous dominance and increasing merchantable volume in the long term (MacLean and Morgan 1983, Olson *et al.* 2012, Bataineh *et al.* 2013). However, changes in forest understory composition have not been well studied (Bataineh *et al.* 2014) and are of particular interest in areas where herbicides are commonly used and forest composition may have shifted due to past silvicultural practices, such as the Acadian Forest of Atlantic Canada.

The Acadian Forest region in Canada, comprising most of the provinces of New Brunswick, Nova Scotia, and Prince Edward Island, features a distinct history and ecology derived from its land use, forest impacts, and physiographic and biological diversities (Loo and Ives 2003, Taylor *et al.* 2017). The Acadian Forest is a transitional forest composed of both northern boreal and southern temperate tree species (Noseworthy and Beckley 2020). Natural forest types in the Acadian Forest region include deciduous-dominated forests, spruce-fir forests, and a variety of mixed/intermediate forests (Loo and Ives 2003). However, since European settlement 400 years ago, forest management has shifted the composition of the Acadian Forest to a larger percentage of coniferous than deciduous tree species (Noseworthy and Beckley 2020). Since the early 1970s, forest management for increased conifer growth in Atlantic Canada has relied heavily on the use of herbicides, such as glyphosate, to control competitive vegetation (Loo and Ives 2003, Thompson and Pitt 2003). Over the last 10 years, herbicides have been applied to as much as 30% of forested land in some watersheds (Edge *et al.* 2023), having the potential to alter the composition of the understory forest vegetation community at a large spatial scale. Here, we use a retrospective analysis to examine the effects of herbicide use on understory plant communities within New Brunswick's Acadian Forest region. Specifically, we investigate whether trends in community richness, diversity, evenness, and composition differ between blocks that were and were not treated with herbicide.

Table 1. Number of forest harvest blocks in each of the two ecoregions and herbicide categories in New Brunswick, Canada.

Ecoregion	Control blocks	Herbicide blocks
Valley Lowland	2	8
Eastern Lowland	13	14

Materials and Methods

Study site and data collection

The study was conducted in the Valley Lowlands and Eastern Lowland ecoregions of New Brunswick, Canada. The two ecoregions have a similar climate, but slightly different vegetation communities. The Valley Lowlands region contains hardwood and mixed forest, where red pine (*Pinus resinosa*), white pine (*Pinus strobus*), red oak (*Quercus rubra*), aspen (*Populus spp*), yellow birch (*Betula alleghaniensis*), American beech (*Fagus grandifolia*), and white ash (*Faxinus americana*) are the dominant tree species (Zelazny 2007). Violets (*Viola spp*), hay-scented fern (*Dennstaedtia punctilobula*), sensitive fern (*Onoclea sensibillis*), and Christmas fern (*Polystichum acrostichoides*) dominate the understory (Zelazny 2007). By contrast, the Eastern Lowlands is dominated by conifers such as balsam fir (*Abies balsamea*), red spruce (*Picea rubens*), jack pine (*Pinus banksiana*), white pine (*P. strobus*), and black spruce (*Picea mariana*); the understory is dominated by sheep laurel (*Kalmia angustifolia*), mountain-holly (*Ilex mucronate*), goldthread (*Coptis trifolia*), bunchberry (*Cornus canadensis*), bristly club-moss (*Spinulum annotinum*), and sphagnum (*Sphagnum spp*) (Zelazny 2007).

A total of 37 harvest blocks clear cut between 2005 and 2016 were selected for the study (Table 1), 22 of which received at least one application of a glyphosate-based herbicide (hereafter herbicide blocks) and 15 did not receive an herbicide application (hereafter non-herbicide blocks) (Fig. 1). As glyphosate-based herbicides are typically applied to high quality blocks in which there is considerable competitive vegetation it is reasonable to assume that some differences in the vegetation communities existed between the non-herbicide and herbicide blocks at the time of herbicide application. None of the blocks are categorized as poor sites by the New Brunswick Department of Energy and Resource Development. To control for site quality we used the Biomass Growth Index (BGI); a prediction from a forest productivity model (20 m grid) that includes climate, lithology, soils, and topographic metrics for the Acadian Forests (Hennigar *et al.* 2017). For each harvest block we calculated mean BGI and included it in the analyses below as an indicator of local site quality. No site preparation was performed on blocks, and they were planted with either uniform or mixed coniferous tree species 1–3 years post-harvest. In the herbicide blocks, a glyphosate-based herbicide was applied using a helicopter 1–4 years post-planting. The formulated herbicide product differed between years and blocks and included Weed-Master™, Vantage™, and Vision™ applied at rates ranging from 2.26 to 2.54 kg acid equivalent/ha. Nine of the 22 herbicide blocks received a second herbicide application, 2–4 years after the initial application.

Within each of the harvest blocks a 1 ha study plot was established 150 m from the edge nearest to road access. We confirmed that study plots within herbicide treated blocks received herbicide

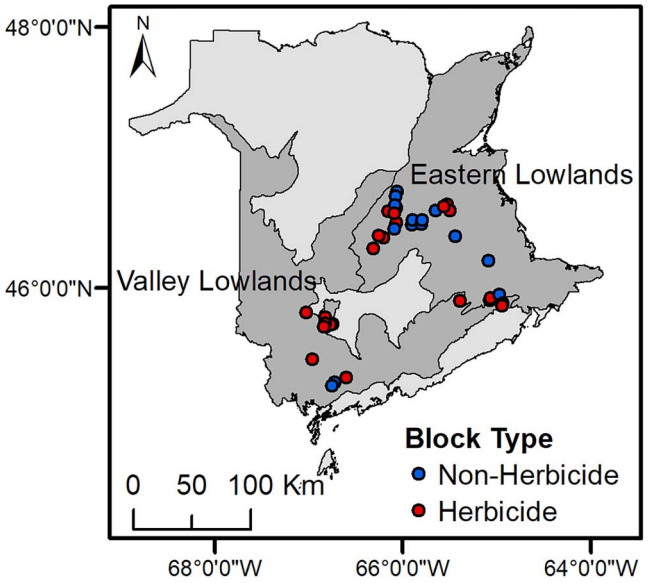

Figure 1. Map of harvest block locations in the two ecoregions (Eastern Lowlands and Valley Lowlands) in New Brunswick Canada.

Table 2. Braun-Blanquet cover-abundance scale with species percent cover conversions, and the midpoint values for each of the categories.

Braun-Blanquet Scale	Range of cover (%)	Midpoint of cover range (%)
5	75–100	87.5
4	50–75	62.5
3	25–50	37.5
2	5–25	15.0
1	<5; many individuals	2.5
+	<5; few individuals	0.1

application by overlaying global positioning system (GPS) points from transect locations on herbicide treatment polygons. Within each study plot three transects were established: one transect was located in the center of the study plot and the other two were located 50 m away from the center in a random direction and orientation. Transects were 30 m long, 1 m wide, and divided into 5 m segments. All vascular plants were identified and their abundance in each segment was estimated using the Braun-Blanquet method (Westhoff and Van Der Maarel 1978). The Braun-Blanquet method uses categories of percent cover to estimate the abundance of species (Table 2). Surveys were conducted in July and August 2020.

Most species were identified in the field; in some cases, a representative sample and/or photograph was identified in the laboratory. It was not possible to identify some plants to species with certainty because they lacked identifying features during surveying. Grass species generally lacked reproductive structures during surveying and were identified to family (Poaceae). Plants of *Alnus*, *Carex*, *Equisetum*, *Galium*, *Juncus*, *Picea*, *Pinus*, *Salix*, and *Viola* were grouped into their respective genera. For the Asteraceae family, the species *Nabalus trifoliolatus, Tussilago farfara, Lactuca canadensis, Cirsium vulgare, Eupatorium perfoliatum, Erigeron hyssopifolius and Erigeron annuus* were identified as species; however, plants from the genera *Aster, Hieracium* and *Solidago* were identified as genus. For each segment on each transect, the range in percent cover from the Braun-Blanquet Scale was converted to the midpoint (Table 2), and the average of midpoint value from all segments in a harvest block was used in the analyses.

Data analysis

We evaluated whether sampling was sufficient to capture most species present in the study area by creating species accumulation curves for all blocks combined, and for each of the non-herbicide and herbicide blocks individually. Species accumulation curves were constructed by randomly selecting blocks with replacement over 1000 permutations using the R package "vegan" (Oksanen *et al.* 2020).

For each harvest block we calculated three measures of alpha diversity: species richness, Shannon diversity index, and evenness. Species richness was the count of all species identified in each harvest block across all transects. Shannon's diversity index was calculated using the midpoint (Table 2) of the cover range for each species (Equation (1)):

$$H' = -\sum_{i=a}^{h} p_i \ln(p_i) \quad (1)$$

where p_i represents the proportion of total percent cover for each species. Evenness was calculated using species richness for each harvest block (Equation (2)):

$$J' = {}^{H'}\!/_{\ln(S)} \quad (2)$$

where H' is Shannon's diversity and S represents species richness. We fitted General Linear Models (GLM) with a Gaussian distribution for each of the three biodiversity response variables with block type (non-herbicide or herbicide), time since harvest, BGI, and the interactions as fixed factors using R and the "stats" package (R Core Team, 2021). If the interactions were not significant, we ran a GLM without interactions to increase statistical power to detect main effects.

The measures of alpha diversity calculated above do not account for species identity or differences in species composition, so we conducted two additional tests. First, we assessed broad changes in forest structure by grouping species into five categories: coniferous trees, deciduous trees, forbs, graminoids, and shrubs. We tested whether the percent cover of each group differed using a GLM with a quasi-binomial distribution that included herbicide treatment, time since harvest, BGI, and the interactions as fixed factors. As above, if the interactions were not significant, we ran a GLM without interactions.

Second, we investigated changes in community composition due to herbicide use and time since last harvest. Nonmetric Multidimensional Scaling (NMDS) with two axes was conducted on the mean percent cover for each species in each of the harvest blocks using the "vegan" package (Oksanen *et al.* 2020). A Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted with the fixed factors treatment type, time since harvest, BGI, and the interactions. A second PERMANOVA was conducted with ecoregion only because two replicate control blocks in the Valley Lowland Ecoregion prevented using a full factorial design. Variance partitioning was conducted to assess the proportion of variance among vegetation data attributed to herbicide use, time since last harvest, and ecoregion. In all cases, Hellinger's transformation was used to minimize effect differences. Finally, to identify species associated with factors deemed significant by the PERMANOVA, we estimated indicator species using the

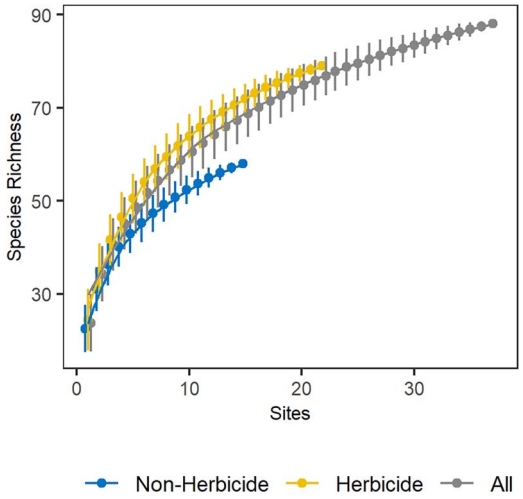

Figure 2. Species accumulation curves for all harvest blocks, and each of the non-herbicide, and herbicide blocks; estimates are the mean of 1000 permutations +/− SD.

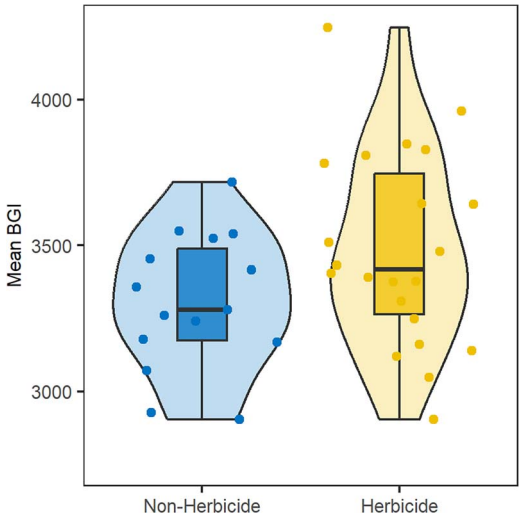

Figure 3. Box and violin plots for mean BGI in the non-herbicide and herbicide blocks; BGI did not differ significantly between the non-herbicide and herbicide blocks ($t = -1.92$, $P = .063$).

point biserial correlation coefficient (Chytrý *et al.* 2002) in the "indicspecies" package (De Cáceres and Legendre 2009). To assess the robustness of our results to the unbalanced design (only two replicate non-herbicide blocks in the Valley Lowland Ecoregion), we conducted two supplementary NMDS, PERMANOVA, and variance partitioning analyses (Supplementary Material 2). First, we repeated the main analyses using all blocks within the Eastern Lowlands Ecoregion only. Second, we compared only the herbicide blocks to test for an effect of ecoregion.

To gain insight into whether rare species might have a statistical and/or biological influence on our analyses (Poos and Jackson 2012), we conducted two variations of the main analyses (Supplementary Material 3): (1) excluding taxonomic groups that occur at only one site and (2) excluding taxonomic groups that occur at ≤ 5% of sites (i.e. translates to <=2 sites). See Supplementary Material 3 for complete results. We also investigated whether the understory vegetation community was influenced by the number of herbicide applications (1 or 2), by repeating the main analyses including number of herbicide applications as a fixed categorical factor (Supplementary Material 4). We discuss the general results of the supplemental analyses and highlight any qualitative differences in the main text; see supplementary material for complete results.

All data management, statistics, and figures were performed and created in R (R Core Team, 2021) using the "dplyr" (Wickham and Francois 2014) and "ggplot2" (Wickham 2016) packages.

Results

Species accumulation curves indicate that, overall, sampling was likely minimally sufficient to capture the number of species found in the study area (Fig. 2). We identified 88 taxonomic units across all harvest blocks (Table S1). Of these, 49 were common between non-herbicide and herbicide blocks, 9 were found only in non-herbicide blocks, and 30 were found only in herbicide blocks. Plants found only in one block type were rare and present in fewer than five blocks (Table S1). The site quality index, BGI, did not differ ($t = 1.92$, $P = .063$, Fig. 3) between the non-herbicide (mean = 3306, SD = 234) and herbicide blocks (mean = 3485, SD = 332) at a significance level of 0.05.

Alpha diversity

Neither herbicide use nor time since harvest were good predictors of plant community diversity measures. Species richness increased with BGI, with one species being added for every 139 increase in BGI ($P = .0470$, Table S2), but did not differ with respect to herbicide use and was not associated with time since harvest (Fig. 4A, Table S2). None of the main effects or interactions had a significant effect on Shannon's diversity ($P > .05$, Fig. 4B, Table S3) or evenness ($P > .05$, Fig. 4C, Table S4).

Cover

In general, we did not observe consistent effects of herbicide use, time since harvest, or BGI on plant cover. The percent cover of tree species—coniferous or deciduous—was not related to any of the main effects regardless of whether or not interactions were included in the model (Fig. 5, Tables S5 and S6). For forb species, percent cover increased with time since last harvest (Estimate = 0.0840, $P = .004$), but did not differ between herbicide and non-herbicide blocks (Estimate = 0.276, $P = .176$), or with BGI (Estimate = −0.0002, $P = .454$, Fig. 5, Table S7). For graminoid species, cover was positively related to BGI (Estimate = 0.002, $P = .004$), but cover was not associated with time since harvest (Estimate = 0.0760, $P = .152$) and did not differ between the control and herbicide blocks (Estimate = 0.419, $P = .322$, Fig. 5, Table S8). The percent cover of shrub species was significantly lower in the herbicide treated blocks (Estimate = −0.693, $P = .006$, Fig. 5), but there was no relationship with time since last harvest (Estimate = −0.0200, $P = .59$) or BGI (Estimate = 0.0004, $P = .309$, Table S9).

Community composition

We found differences in the community composition between herbicide and non-herbicide blocks, despite the lack of strong effects of herbicide on alpha diversity and percent cover (Fig. 6). A NMDS solution was reached after 15 runs with a stress test of 0.159, which indicates good representation (< 0.2) of the data. The PERMANOVA test of the effects of herbicide use, time since harvest, and BGI (Table S10) found vegetation community differed between the herbicide and non-herbicide blocks ($r^2 = 0.186$, $P = .001$), and was related to BGI ($r^2 = 0.104$, $P = .002$). However, the

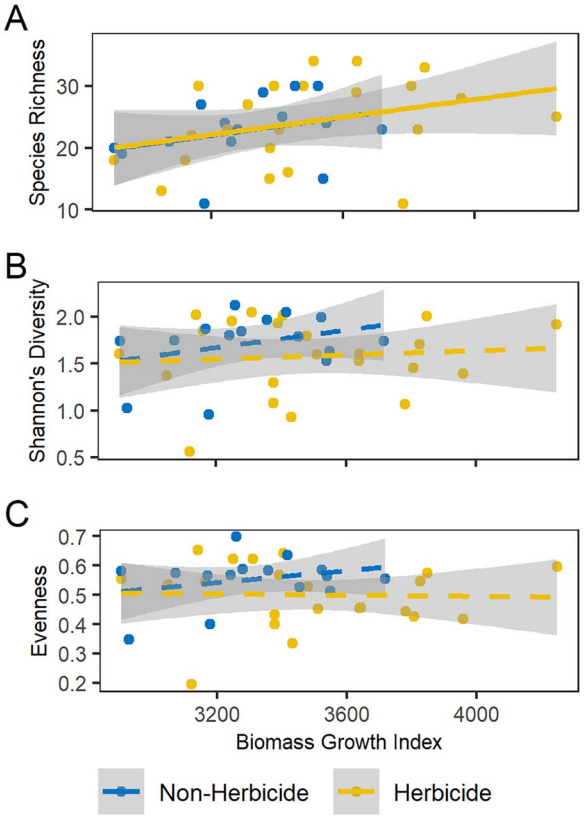

Figure 4. Relationship between three measures of alpha diversity; (A) Species Richness, (B) Shannon's Diversity, and (C) Evenness; and the BGI in the non-herbicide and herbicide blocks of forest in New Brunswick, Canada; shaded areas indicate 95% confidence intervals; the relationship between species richness and the BGI was significant (B: $P = .047$, solid line), but all other relationships are not ($P > .05$, dashed lines).

vegetation community did not change with time since last harvest ($r^2 = 0.032$; $P = .147$) and none of the interactions were significant ($P > .05$). The PERMANOVA test of the effect of ecoregion found that the vegetation community differed between the ecoregions ($r^2 = 0.0578$, $P = .051$, Table S11). Overall, 26.5% of the plant community variation was explained by the four explanatory variables, with 10.6% explained by herbicide application, 8.6% by BGI, 3.6% by time since last harvest, and 1.7% by ecoregion. We identified 16 taxonomic units that differentiated the non-herbicide (9 species) or herbicide (7 species) blocks, 14 of which were not included as indicators of ecoregion (Table 3).

Site clustering, rare species, and multiple herbicide applications

Supplemental analyses exploring the influence of site clustering in the ecoregions were largely consistent with the main results (Supplementary Material 2). Herbicide use, BGI, and ecoregion all had significant effects on the vegetation community and the relative rank of variation explained by the factors was the same in all analyses (Table S12, Fig. S1). The NMDS plot showing the vegetation community (Fig. S2) in the herbicide blocks indicated that there is some differentiation in the vegetation community between the ecoregions (Table S13), but ecoregion explained a relatively small amount of variation, 2.6% at most. Results of the main analyses and inferences drawn on the importance of herbicide use are likely robust as herbicide use explained approximately five times as much variation as ecoregion in all analyses.

Rare species did not have a large role in shaping the forest community patterns observed across herbicide treatment or ecoregion (Supplementary Material 3). Extremely similar patterns were observed when all species are included and when rare species were removed in supplemental analyses (Table S14 and S15, Fig. S3 and S4). We also found that rare species were not playing a substantial role in the detection of species associations: 20 of the 21 taxonomic groups found to have significant roles ($P < .05$) in shaping the multivariate space among study site communities in the main analyses remained significant after rare species were removed. Bull thistle (*C. vulgare*) was the only rare species significant in the main analyses.

The number of herbicide applications did not have a large influence on the general pattern of the results (Supplementary Material 4). The NMDS indicated that the vegetation community in the non-herbicide treated blocks is different than that in the herbicide blocks, and the vegetation community in the blocks treated twice is less variable and appears to be a subset of that in the blocks treated once (Table S16 and S17, Fig. S5). We caution about drawing strong inferences from this result due to the limited sample size of blocks treated twice, sampling additional blocks treated twice is likely to increase the observed variation in vegetation communities.

Discussion

Using a suite of forest blocks that were harvested over the last 15 years, we demonstrate that herbicide use has likely resulted in subtle changes to the understory community that persist for at least 10–15 years—the abundance of shrub species is reduced and is associated with an increase in abundance of non-woody flowering plants. While herbicides are used to reduce competition and increase the growth and yield of planted coniferous trees (Bell *et al.* 2011, Olson *et al.* 2012, Comeau and Fraser 2018), we show that herbicides could have much broader effects on the understory plant community.

Overall, there were no differences in three alpha diversity indices (Species Richness, Shannon Diversity, and Evenness) for the understory vegetation between the herbicide and non-herbicide blocks. Our results are consistent with the intention of herbicide use to reduce the abundance of competitive species but not to remove species from the application area (Ristau *et al.* 2011). However, there were differences in the abundance of individual species which lead to a subtle difference in understory community composition between the herbicide and non-herbicide blocks. Moreover, the difference in community composition persisted for 15 years after harvest. Prior work has emphasized that no change in alpha diversity indicates minimal effects on the understory vegetation community (Sullivan and Sullivan 2003, Ristau *et al.* 2011, Bataineh *et al.* 2014, Ulappa *et al.* 2020). Because alpha diversity measures do not account for species identity, the previous studies were unable to detect the subtle differences in the understory community highlighted here.

A short-term reduction in understory vegetation after herbicide application has been noted in several studies (Sullivan and Sullivan 2003, Lautenschlager and Sullivan 2004, Ristau *et al.* 2011,Comeau and Fraser 2018 , Ulappa *et al.* 2020) but some conclude that the understory vegetation community composition is resilient to the impact of herbicides (Ristau *et al.* 2011, Bataineh *et al.* 2014). In our study, of the five coarse plant groups (coniferous, deciduous, forb, graminoid, and shrub), shrubs were the only group that demonstrate persistent change. Lower shrub abundance with herbicide application is maintained, up to

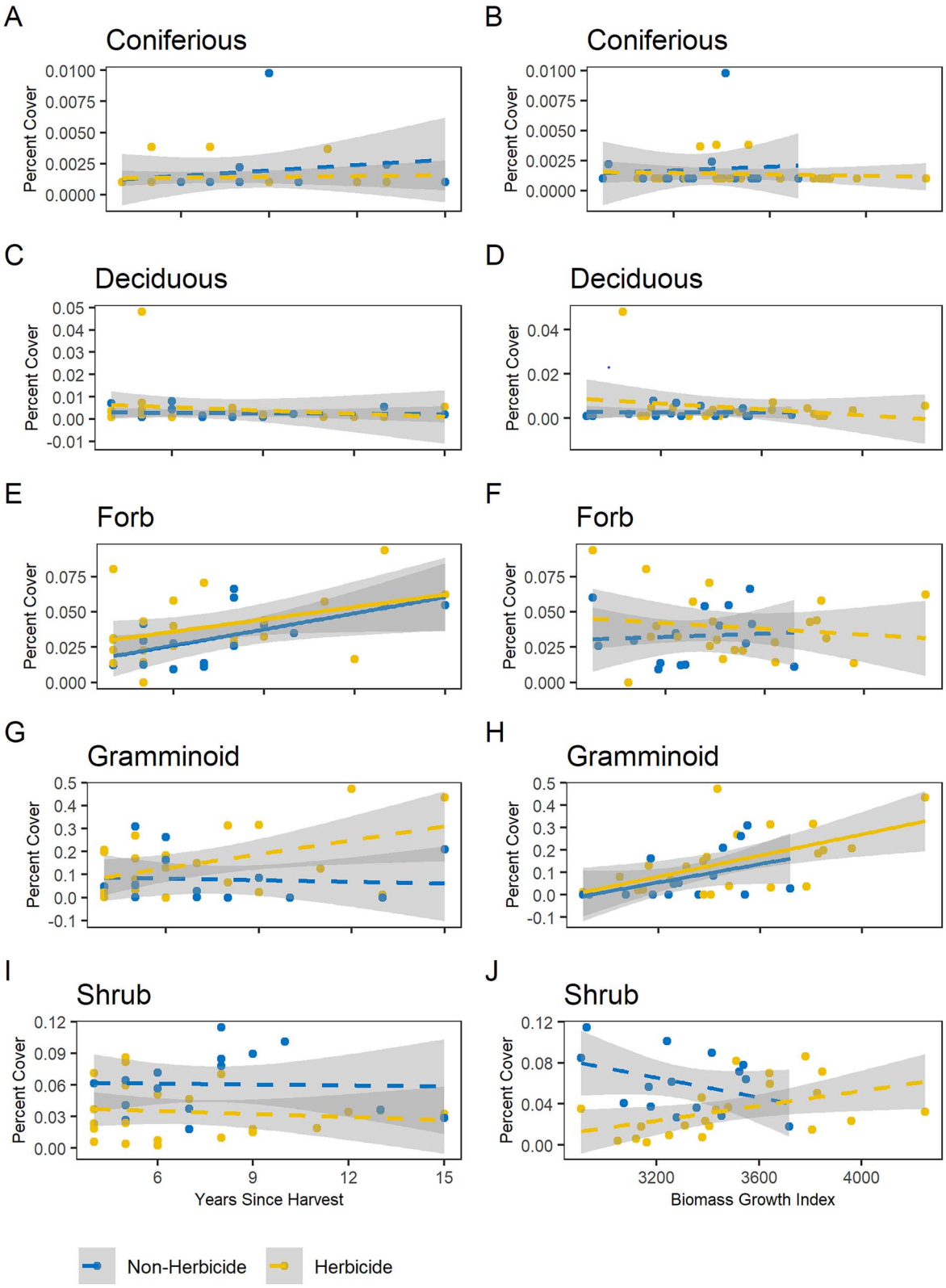

Figure 5. Percent cover of five different groups of vegetation, showing linear relationship with years since last harvest (A, C, E, G, and I) and BGI (B, D, F, H, and J) in 37 New Brunswick forest harvest blocks. Shaded areas indicate 95% confidence intervals. Significant relationships ($P < .05$) between response and predictor variables shown with solid lines, non-significant ($P > .05$) relationships shown with dashed lines.

Table 3. Results of the indicator species analysis for non-herbicide and herbicide blocks in two ecoregions of New Brunswick, Canada.

Group	Species	Statistic	P-value	Non-herbicide (15 total blocks)	Herbicide (22 total blocks)
Valley Lowland	*Solidago* spp.	0.440	.008	7 (46.7%)	15 (68.2%)
	Rubus idaeus	0.440	.018	9 (60.0%)	21 (95.5%)
	Quercus rubra	0.365	.027	0 (0%)	4 (18.2%)
	Fallopia convolvulus	0.342	.029	1 (6.7%)	4 (18.2%)
Eastern Lowland	*Maianthemum canadense*	0.401	.027	8 (53.3%)	19 (86.4%)
	Comptonia peregrina	0.363	.018	8 (53.3%)	5 (22.7%)
	Carex spp.	0.350	.033	6 (40.0%)	5 (22.7%)
Non-herbicide blocks	*Vaccinium angustifolium*	0.689	<.001	15 (100%)	14 (63.6%)
	Vaccinium myrtilloides	0.620	<.001	14 (93.3%)	12 (54.5%)
	Kalmia angustifolia	0.511	.002	12 (80.0%)	9 (40.9%)
	Pteridium aquilinum	0.497	<.001	13 (86.7%)	13 (59.1%)
	Comptonia peregrina	0.412	.003	8 (53.3%)	5 (22.7%)
	Rhododendron canadense	0.409	.003	9 (60.0%)	4 (18.2%)
	Pinus spp.	0.387	.020	15 (100%)	16 (72.7%)
	Betula papyrifera	0.374	.019	14 (93.3%)	18 (81.8%)
	Ilex mucronata	0.340	.041	10 (66.7%)	8 (36.4%)
Herbicide blocks	*Nabalus trifoliolatus*	0.522	<.001	2 (13.3%)	14 (63.6%)
	Cornus canadensis	0.411	.014	13 (86.7%)	20 (90.9%)
	Maianthemum canadense	0.395	.017	8 (53.3%)	19 (86.4%)
	Anaphalis margaritacea	0.353	.005	2 (13.3%)	12 (54.5%)
	Chamaenerion angustifolium	0.351	.011	6 (40.0%)	19 (86.4%)
	Hieracium spp.	0.342	.005	1 (6.7%)	13 (59.1%)
	Aster spp.	0.340	.041	4 (26.7%)	12 (54.5%)

Only species shown to be indicators ($P < .05$) for each category (Group) are listed. The number of blocks containing the species is listed (Non-Herbicide, Herbicide) and the percent of blocks with the plant present is in parentheses.

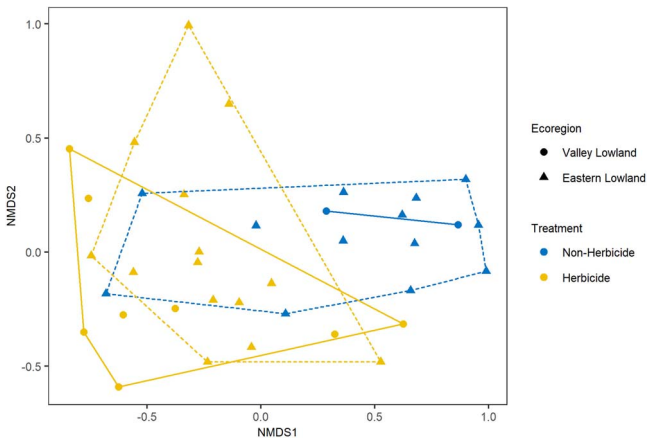

Figure 6. Ordination showing results of NMDS analyses of ecoregion and treatment type for 37 forest blocks in New Brunswick, Canada. Individual points represent study blocks. Solid lined polygons surround the Valley Lowland Ecoregion and dotted lined polygons surround the Eastern Lowland Ecoregion.

15 years after harvest. Reduced shrub cover is consistent with the expectation that the majority of the herbicide spray cloud is intercepted by the tallest vegetation (Pitt *et al.* 1992, Wood 2019, Edge *et al.* 2021).

The most striking differences associated with herbicide application was the community composition, which is likely facilitated by the reduced shrub layer with herbicide use. Non-herbicide blocks were distinguished from herbicide blocks by the higher frequency of occurrence of ferns, blueberry species, and other shrubs and trees (Table 3). In addition to being indicator species, Low bush blueberry (*Vaccinium angustifolium*) and Velvet leaf blueberry (*V. myritilloides*) were the most abundant species in the non-herbicide blocks. Both blueberry species are important food resources for several species of birds, mammals, and insects. Glyphosate typically reduces the abundance of blueberry species, with recovery expected ~4 years after herbicide application (Sullivan and Sullivan 2003, Lautenschlager and Sullivan 2004). However, our results contradict this expected recovery, as time since harvest was not a significant predictor of shrub cover, indicating that the vegetation shifts may persist for much longer than previously thought.

Herbicide application is expected to reduce the overtopping shrub and herbaceous tree layer, allowing other species to flourish (Comeau and Fraser 2018, Ulappa *et al.* 2020). In our study, indicator species of herbicide treated blocks were all forbs and the majority (four of six) are associated with disturbance and early succession (Panebianco and Willemsen 1976). These species were all found in lower frequency (range 6.7%–40%) in non-herbicide blocks than in herbicide blocks (54.5%–86.4%). The two indicator species associated with late succession, *C. canadensis* and *Maianthemum canadense*, were present at moderate rates in non-herbicide blocks (53.3 and 86.7%), but occurred in almost all herbicide blocks (86.4 and 90.9%), possibly due to reduced competition. All of the species associated with herbicide treatment are perennial. This is somewhat surprising given that early stages of succession and invasion are often associated with rapidly growing and reproducing annual species

(Seabloom et al. 2003, Leffler et al. 2011). However, very few of the species found in our study were annuals, suggesting that these dynamics may differ in regenerating forests. Moreover, the perennial nature of the indicator species associated with herbicide use may play a critical role in the observed longevity of the vegetation shift.

Our results suggest that herbicide use has a disproportionately large negative effect on shrub species abundance, and that fast-growing, perennial, herbaceous species take advantage of the reduction in shrub cover. To more directly test this hypothesis, future studies should (i) characterize the plant community prior to herbicide treatment to capture the natural variation in forest communities that exists prior to herbicide disturbance and (ii) include early successional forests created by other non-herbicide forms of disturbance to estimate the effect of herbicide disturbance relative to other forms of disturbance.

Not surprisingly, ecoregion helped explain variation in our plant communities; however, ecoregion explained a relatively small amount of variation in the vegetation community (1.2%). Because climate and environmental conditions play a very important role determining species composition (Zhang et al. 2014, Taylor et al. 2017, Harrison et al. 2020), it was surprising that such a small amount of variation was explained by ecoregion. The site quality index we used, BGI, is likely a better representation of local site growing condition than ecoregion because it is a continuous rather than categorical variable and is derived from climate, lithology, soils, and topographic metrics and has been shown to be a reasonably good predictor of forest productivity in the Acadian Forests of New Brunswick (Hennigar et al. 2017). Indeed, BGI explained more variation in community composition (8.6%) than ecoregion, and there is considerable overlap in the plant communities in herbicide blocks from the different ecoregions (Fig. 6). Unfortunately, only two non-herbicide blocks were sampled from the Valley Lowland ecoregion, preventing the inclusion of all variables in one analysis. It appears that herbicide use shifted the communities in both ecoregions in the same direction (Fig. 6), but this inference requires future validation due to the small sample size for the Valley Lowland ecoregion.

Local site characteristics, such as soil (Zhang et al. 2014), can exert a strong influence on the vegetation community that grows post-harvest. BGI explained a significant amount of variation in the vegetation community and grass cover was positively associated with BGI. The BGI is likely confounded with herbicide use because herbicides are used to reduce competition and competition is generally higher in high quality sites. Although we found no average difference in BGI with between herbicide treatment, six of the herbicide blocks were higher in BGI than the highest of the non-herbicide blocks. Further complications arise because some of the herbicide blocks were treated twice and others only once. Generally, a second herbicide application occurs when the first did not result in sufficient control of competitive vegetation, which is likely correlated with site quality in some way. The supplementary analyses conducted suggests that the understory vegetation community in blocks treated twice is a subset of those treated once. However, this result should be treated with caution to the small sample size ($n = 8$) of blocks treated twice. If more blocks are sampled, more variation in plant community would be expected. A more robust study design would include herbicide and non-herbicide blocks that span a similar range in BGI and had consistent herbicide application or better replication of blocks treated once and twice. We choose harvest blocks that were as similar as possible for study. However, it is likely that competition was lower in the non-herbicide blocks and did not require an herbicide treatment to optimize conifer growth. These initial site conditions likely contribute to differences in the vegetation community found later in the regeneration cycle. Therefore, future studies that control for spatial location, quantify the vegetative community pre- and post-herbicide treatment, and document changes through time are much-needed to increase our understanding of herbicide use in complex and dynamic forest communities.

Conclusion

The use of glyphosate-based herbicides in forestry is associated with subtle, but longer lasting changes to the understory vegetation community than previously expected. The largest differences are associated with reduced shrub abundance in herbicide treated plots. Herbicides are used in Canadian forestry to reduce the abundance of species that compete with coniferous trees and improve tree growth, results presented here demonstrate that herbicides are effective at reducing the abundance of competitive shrub species. In addition to altering the abundance of shrub species, herbicide application likely alters the composition of the understory community as well. Past work that focused on measures of alpha diversity did not detect these important shifts in community composition. We found that blueberry species were suppressed, and the abundance of some herbaceous forbs associated with disturbance were higher in the herbicide-treated plots compared to untreated plots. In some regions, herbicides are applied to large percentages of the regenerating forest, potentially leading to broad-scale shifts in the understory vegetation community and potential indirect effects on other components of the ecosystem. We recommend that these potential indirect effects be considered within forest management plans for areas where herbicides are commonly used as they could lead to effects on non-timber related ecosystem objectives.

Acknowledgements

We thank the New Brunswick Department of Energy and Resource Development for assistance. Lucie Vézina and Benjamin Dow provided field and data management assistance. The study was conducted in New Brunswick on the unsurrendered and unceded traditional Wolastoqey land.

Supplementary data

Supplementary data is available at *FORESJ Journal* online.

Conflict of interest statement: None declared.

Funding

The study was funded by Natural Resources Canada—Canadian Forest Service and the University of New Brunswick.

Data availability

Data and R scripts for analyses are available via open science framework https://osf.io/w2smq/?view_only=cf53a2fc21bb472e85cd39edf08016b0.

References

Balandier P, Collet C, Miller JH. *et al.* Designing forest vegetation management strategies based on the mechanisms and dynamics of crop tree competition by neighbouring vegetation. *Forestry* 2006;**79**:3–27.

Bartels SF, Chen HYH, Wulder MA. *et al.* Trends in post-disturbance recovery rates of Canada's forests following wildfire and harvest. *For Ecol Manage* 2016;**361**:194–207.

Bataineh MM, Wagner RG, Olson MG. *et al.* Midrotation response of ground vegetation to herbicide and precommercial thinning in the Acadian Forest of Maine, USA. *For Ecol Manage* 2014;**313**: 132–143.

Bataineh MM, Wagner RG and Weiskittel AR. Long-term response of spruce-fir stands to herbicide and precommercial thinning: observed and projected growth, yield, and financial returns in Central Maine, USA. *Can J For Res* 2013;**43**:385–395.

Bell FW, Thiffault N, Szuba K. *et al.* Synthesis of silviculture options, costs, and consequences of alternative vegetation management practices relevant to boreal and temperate conifer forests: introduction. *For Chron* 2011;**87**:155–160.

Boateng JO, Haeussler S and Bedford L. Boreal plant community diversity 10 years after glyphosate treatment. *West J Appl For* 2000;**15**:15–26.

Botten N, Wood LJ and Werner JR. Glyphosate remains in forest plant tissues for a decade or more. *For Ecol Manage* 2021;**493**: 119259.

De Cáceres M and Legendre P. Associations between species and groups of sites: indices and statistical inference. *Ecology* 2009;**90**: 3566–3574.

Chytrý M, Tichý L, Holt J. *et al.* Determination of diagnostic species with statistical fidelity measures. *J Veg Sci* 2002;**13**: 79–90.

Comeau PG and Fraser EC. Plant community diversity and tree growth following single and repeated glyphosate herbicide applications to a white spruce plantation. *Forests* 2018;**9**:1–14.

R Core Team. 2021. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing, Available from https://www.r-project.org/.

Deighton HD, Bell FW, Thiffault N. *et al.* Trade-offs among release treatments in jack pine plantations: twenty-five year responses. *Forests* 2021;**12**:1–23.

Edge CB, Baker LF, Lanctôt CM. *et al.* Compensatory indirect effects of an herbicide on wetland communities. *Sci Total Environ* 2020;**718**:137254.

Edge CB, Brown MI, Heartz S. *et al.* The persistence of glyphosate in vegetation one year after application. *Forests* 2021;**12**:1–13.

Edge CB, Haines W, Blaney M. *et al.* Low detection of glyphosate in rivers following application in forestry. *Pest Manag Sci* 2023;**79**: 2951–2958. https://doi.org/10.1002/ps.7473.

Freedman B, Morash R and MacKinnon D. Short-term changes in vegetation after the silvicultural spraying of glyphosate herbicide onto regenerating clearcuts in Nova Scotia, Canada. *Can J For Res* 1993;**23**:2300–2311.

Harrison S, Spasojevic MJ and Li D. Climate and plant community diversity in space and time. *Proc Natl Acad Sci U S A* 2020;**117**: 4464–4470.

Hennigar C, Weiskittel A, Allen HL. *et al.* Development and evaluation of a biomass increment based index for site productivity. *Can J For Res* 2017;**47**:400–410.

Lautenschlager RA. Response of wildlife to forest herbicide applications in northern coniferous ecosystems. *Can J For Res* 1993;**23**: 2286–2299.

Lautenschlager RA and Sullivan TP. Improving research into effects of forest herbicide use on biota in northern ecosystems. *Wildl Soc Bull* 2004;**32**:1061–1070.

Leffler AJ, Monaco TA and James JJ. Nitrogen acquisition by annual and perennial grass seedlings: testing the roles of performance and plasticity to explain plant invasion. *Plant Ecol* 2011;**212**: 1601–1611.

Loo J and Ives N. The Acadian Forest: historical condition and human impacts. *For Chron* 2003;**79**:462–474.

MacLean DA and Morgan MG. Long-term growth and yield response of young fir to manual and chemical release from shrub competition. *For Chron* 1983;**59**:177–183.

Noseworthy J and Beckley TM. Borealization of the New England – Acadian Forest: a review of the evidence. *Environ Rev* 2020;**28**: 284–293.

Oksanen J, Simpson G, Blanchet FG. *et al.* 2022. vegan: community ecology package. R Packag version 2.6–4, https://cran.r-project.org/package=vegan.

Olson MG, Wagner RG and Brissette JC. Forty years of spruce-fir stand development following herbicide application and precommercial thinning in Central Maine, USA. *Can J For Res* 2012;**42**:1–11.

Panebianco R and Willemsen RW. Seed germination of *Hieracium pratense*, a successional perennial. *Bot Gaz* 1976;**137**:255–261.

Pitt DG, Fleming RA, Thompson DG. *et al.* Glyphosate efficacy on eastern Canadian forest weeds. Part II: deposit-response relationships and crop tolerance. *Can J For Res* 1992;**22**: 1160–1171.

Poos MS and Jackson DA. Addressing the removal of rare species in multivariate bioassessments: the impact of methodological choices. *Ecol Indic* 2012;**18**:82–90.

Ristau TE, Stoleson SH, Horsley SB. *et al.* Ten-year response of the herbaceous layer to an operational herbicide-shelterwood treatment in a northern hardwood forest. *For Ecol Manage* 2011;**262**: 970–979.

Rolando C, Baillie B, Thompson D. *et al.* The risks associated with glyphosate-based herbicide use in planted forests. *Forests* 2017;**8**:208.

Santillo DJ, Leslie DM and Brown PW. Responses of small mammals and habitat to glyphosate application on clearcuts. *J Wildl Manage* 1989;**53**:164–172.

Seabloom EW, Harpole WS, Reichman OJ. *et al.* Invasion, competitive dominanace, and resource use by exotic and native California grassland species. *PNAS* 2003;**100**:13384–13389.

Spence JR. The new boreal forestry: adjusting timber management to accommodate biodiversity. *Trends Ecol Evol* 2001;**16**:591–593.

Stoleson SH, Ristau TE, DeCalesta DS. *et al.* Ten-year response of bird communities to an operational herbicide-shelterwood treatment in a northern hardwood forest. *For Ecol Manage* 2011;**262**: 1205–1214.

Sullivan TP, Lautenschlager RA and Wagner RG. Influence of glyphosate on vegetation dynamics in different successional stages of sub-boreal spruce forest. *Weed Technol* 1996;**10**: 439–446.

Sullivan TP, Nowotny C, Lautenschlager RA. *et al.* Silvicultural use of herbicide in sub-boreal spruce forest: implications for small mammal population dynamics. *J Wildl Manage* 1998;**62**: 1196–1206.

Sullivan TP and Sullivan DS. Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems. *Environ Rev* 2003;**11**:37–59.

Taylor AR, Boulanger Y, Price DT. *et al.* Rapid 21st century climate change projected to shift composition and growth of Canada's Acadian Forest region. *For Ecol Manage* 2017;**405**: 284–294.

Thiffault N and Roy V. Living without herbicides in Québec (Canada): historical context, current strategy, research and challenges in forest vegetation management. *Eur J For Res* 2011;**130**:117–133.

Thompson DG and Pitt DG. A review of Canadian forest vegetation management research and practice. *Ann For Sci* 2003;**60**: 559–572.

Ulappa AC, Shipley LA, Cook RC. *et al.* Silvicultural herbicides and forest succession influence understory vegetation and nutritional ecology of black-tailed deer in managed forests. *For Ecol Manage* 2020;**470**:118216.

Wagner RG, Newton M, Cole EC. *et al.* By Robert G. Wagner, Michael Newton, Elizabeth C. Cole, James H. Miller, and Barry D. Shiver. *Wildl Soc Bull* 2004;**32**:1028–1041.

Westhoff V and Van Der Maarel E. 1978. The Braun-Blanquet approach. In: RH, Whittaker (ed.), *Classification of Plant Communities*. The Hauge: Springer.

Wickham H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer Verlag.

Wickham H, François R, Henry, L. *et al.* 2023. Dplyr: a grammar of data manipulation. R package version 1.1.3, http://cran.r-project.org/package=dplyr.

Wiensczyk A, Swift K, Morneault A. *et al.* An overview of the efficacy of vegetation management alternatives for conifer regeneration in boreal forests. *For Chron* 2011;**87**:175–200.

Wood LJ. The presence of glyphosate in forest plants with different life strategies one-year after application. *Can J For Res* 2019;**49**: 586–594.

Zelazny VF. 2007. *Our Landscape Heritage: The Story of Ecological Land Classification in New Brunswick*. 2nd edn. Fredericton: New Brunswick Department of Natural Resources.

Zhang Y, Chen HYH and Taylor A. Multiple drivers of plant diversity in forest ecosystems. *Glob Ecol Biogeogr* 2014;**23**: 885–893.