Applied Vegetation Science **20** (2017) 446–458

Factors affecting soil seed banks of riparian communities in an agricultural ecosystem: potential for conservation of native plant diversity

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Keywords
Agriculture impact; *Bromus inermis*; Floristic quality; Nitrate; Non-native species; Nutrient enrichment, *Phalaris arundinacea*; Riparian plant; Soil seed bank; Species richness

Abbreviations
CC = coefficient of conservation; FQI = Floristic quality index.

Nomenclature
Gleason & Cronquist (1991); Crow & Hellquist (2000a,b); USDA Plants Database (http://plants.usda.gov, accessed 15 Mar 2016).

Co-ordinating Editor: Norbert Hölzel

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Abstract

Questions: Do agricultural land use and nitrogen (N) enrichment have negative effects on riparian soil seed banks? What is the potential of the soil seed bank for the conservation and restoration of native riparian plant diversity? Are non-native dominant grass species affected by agriculture and do they affect species richness?

Location: South Nation River watershed (an agricultural watershed), Ontario, Canada.

Methods: We examined the riparian above-ground vegetation at 24 sites located across a large (~4000 km^2) North American watershed and identified the corresponding soil seed bank composition from soil cores using the seedling emergence method. The above-ground vegetation and soil seed bank species compositions were compared in terms of species richness, percentage of non-native species and a floristic quality index. Factors affecting these descriptors of plants communities (concentration of in-stream nitrate and percentages of surrounding natural habitat and annual crop land) were assessed. The effects of agriculture on two dominant non-native grasses species and their effects on species richness were also assessed.

Results: In total, 274 plant taxa were identified, including 181 taxa in the soil seed bank and 231 taxa in the vegetation. Overall species richness was high in both the soil seed bank and above-ground vegetation and was unaffected by measures of agricultural intensity (surrounding annual crop land and N enrichment). Above-ground vegetation species richness was strongly negatively affected by the widespread and dominant non-native grasses, *Phalaris arundinacea* and *Bromus inermis,* whereas soil seed bank species richness was unaffected. The community compositions of both the soil seed bank and vegetation were negatively affected by the loss of natural habitat and by N enrichment. In fact, an increase in the percentage of non-native species and a decrease in floristic quality were observed along a gradient of agricultural intensity.

Conclusions: Species richness of the soil seed bank demonstrated resilience to invasions by *P. arundinacea* and *B. inermis,* and the soil seed bank showed good potential for conservation of taxonomic diversity. However, the loss of natural habitat and N enrichment had negative effects on the soil seed bank community composition that may lead to an eventual decline in above-ground species richness.

Introduction

Global biodiversity is critical in maintaining ecosystem services important to both ecosystem functioning and human well-being but is threatened by anthropogenic activities (Díaz et al. 2006). Land-use change is predicted to be the most important driver of global terrestrial biodiversity change by the year 2100, followed by climate change, nitrogen (N) deposition, species introductions and elevated CO_2 (Sala et al. 2000). In aquatic ecosystems, species

Doi: 10.1111/avsc.12313

introductions are predicted to be the biggest driver of biodiversity change (Sala et al. 2000). The conversion of land to agriculture is expected to lead to eutrophication and habitat destruction, and result in ecosystem simplification, species extinctions and a loss of ecosystem services (Tilman et al. 2001). While a decline in biodiversity with anthropogenic land use has been well established at the global scale, effects of land use on regional and local scales are less clear and may result in increases or decreases in biodiversity (Sax & Gaines 2003; Gerstner et al. 2014).

Within agroecosystems, the effects of agricultural land use on riparian habitats are particularly important (Williams et al. 2008). These areas form the critical interface between aquatic and terrestrial ecosystems and are essential for a number of ecosystem services. For example, riparian vegetation improves water quality (reviewed in Dosskey et al. 2010), moderates stream temperature, stabilizes banks and provides both food and movement corridors for various organisms (reviewed in Richardson et al. 2007). Riparian vegetation is generally diverse due to the dynamic nature of riparian zones, which experience frequent disturbance (e.g. flooding) and the movement of seeds and propagules downstream (Naiman et al. 1993; Wohl et al. 2005). Due to the proximity and connectivity of riparian zones to agricultural crop land, riparian vegetation may be negatively affected by intense agriculture. For example, Méndez-Toribio et al. (2014) observed lower riparian vegetation species richness in areas with adjacent agricultural land use compared to forested areas.

Species composition is also affected by agriculture. Adjacent agricultural land use is associated with an increase in non-native species in riparian habitats (Corbacho et al. 2003; Dalton et al. 2015; Chen et al. 2017). Agriculture, specifically N enrichment, has also been associated with a reduction in overall floristic quality of riparian and aquatic vegetation and with an increase in fast-growing, weedy species of low conservation value (Dalton et al. 2015). Two species fitting this description are the grasses *Bromus inermis* and *Phalaris arundinacea*, which both have the potential to be problematic in regions of North America, including Ontario, Canada. *B. inermis* is native to Southern Europe and was introduced and cultivated for erosion control and as a forage species (Dore & McNeill 1980). It is a moderately invasive upland species (White et al. 1993) and is well established in southeast Ontario roadsides and field margins (Dore & McNeill 1980). Populations of *P. arundinacea* can be composed of either a genotype native to North America or a more aggressive non-native genotype native to Eurasia (Lavergne & Molofsky 2004). The European genotype was widely introduced for forage (Dore & McNeill 1980) and is one of the principal invasive wetland species of Ontario (White et al. 1993). Distributions of the native and non-native genotypes are difficult to map due to a lack of distinguishing morphological features (Dore & McNeill 1980).

The soil seed bank contributes to the diversity of riparian plant communities (Williams et al. 2008). The soil seed bank reflects both past and potential future vegetation and serves as an important buffer against temporary unfavourable environmental conditions (Bossuyt & Honnay 2008). Habitats that experience frequent disturbance, such as riparian habitats, tend to have large numbers of long-lived seeds (reviewed in Bossuyt & Honnay 2008). These seeds are important in conserving the taxonomic variation of riparian plant communities and may be a seed source for the restoration of degraded habitats. However, the potential of the soil seed bank in restoration might be limited if it does not contain species of conservation interest or if stressors affecting above-ground vegetation also negatively affect the soil seed bank. A number of studies have concluded that restoration from soil seed banks alone is not feasible, particularly for species common to forests that rely upon seed dispersal rather than germination from soil seed banks (reviewed in Bossuyt & Honnay 2008; Greet et al. 2013; O'Donnell et al. 2015). For example, O'Donnell et al. (2016) found that soil seed banks of degraded river reaches were dominated by non-native species, limiting restoration potential. However, other studies, though fewer in number, have concluded that soil seed banks may have potential in initiating restoration, particularly of early successional species (Vosse et al. 2008; Plue & Cousins 2013; Metsoja et al. 2014).

In this study, we compared the riparian soil seed bank and above-ground vegetation across a large temperate watershed and along a gradient of agricultural land use. The main objective of our study was to determine if agriculture has negative effects on the soil seed bank. We assessed this objective using land use and nitrate enrichment to explain variation in species richness, the percentage of non-native species and the floristic quality of the soil seed bank. We examined the potential of the soil seed bank in conservation and restoration of native riparian plant diversity by comparing the composition and quality of the above-ground vegetation and soil seed bank. Our final objective was to examine the effects of agriculture on the non-native grass species *B. inermis* and *P. arundinacea*, and to determine the effects of these species on above-ground vegetation and soil seed bank species richness.

Methods

Study area and site characteristics

The South Nation River watershed is a large (3915 km^2) agricultural watershed located in eastern Ontario, Canada, within the Mixedwood Plains Ecozone. This ecozone is dominated by mixed forests, sugar maple (*Acer saccharum*),

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eastern white pine (*Pinus strobus*), sedges, mosses and meadow grasses (http://www.ecozones.ca/english/zone/MixedwoodPlains/plants.html; accessed 31 Jan 2017). The South Nation River watershed is characterized by a flat, poorly drained landscape, and agricultural fields are typically tile-drained and planted with crops of corn (*Zea mays*) and soybean (*Glycine max*). Flooding of riparian areas in this watershed occurs annually following the spring freshet (discharge and water levels typically peak at the beginning of Apr) and throughout the year following rain events (http://wateroffice.ec.gc.ca/search/statistics_e.html; accessed 31 Jan 2017). Average annual total precipitation in the nearby city of Ottawa, Canada, is 943.4 mm (1981–2010, http://climate.weather.gc.ca/climate_normals/index_e.html; accessed 1 Mar 2017).

Twenty-four field sites located throughout the South Nation River watershed were selected for study. Sites (20 m in length along both stream banks) were paired along a given tributary. Each pair contained one site that was surrounded by low levels of agriculture and one site surrounded by high levels of agriculture, with sites surrounded by low levels of agriculture located upstream of the sites surrounded by high levels of agriculture. Paired sites were an average of 8.8 ± 8.9 km apart, with two pairs of sites located along different tributaries due to a lack of suitable sites. Accessible, open canopy sites were selected in areas removed from rural road disturbance and were matched along tributaries as closely as possible in terms of visible features such as stream width and bank slope (details are described in Dalton et al. 2015).

The percentages of surrounding (500-m radius) annual crop land, perennial crop and pasture land, and natural habitat (including wetland, forest, shrub land, bare soil, rock and sediments) were calculated from raster data (30-m resolution) produced from Landsat satellite imagery and provided by Agriculture & Agri-Food Canada (Land cover for agricultural regions of Canada by UTM zone (circa 2000), http://open.canada.ca/data/en/dataset/16d2f828-96bb-468d-9b7d-1307c81e17b8, accessed 15 Mar 2016). In-stream nitrate concentrations were used as a proxy for nutrient enrichment since elevated nutrient concentrations represent agricultural contamination from synthetic fertilizers (Dubrovsky et al. 2010). Water samples (300 ml) were collected in Jun 2008 and 2010 during a period of expected run-off from fertilizers and analysed following established methods (Ontario Ministry of the Environment 2007). Aggregate soil samples were collected from both banks to characterize soil structure (% sand, silt and clay; Klute 1986). Bank slope was measured in triplicate along both banks and ranged from 0% for flat banks to 100% for banks cut-away at 90°. Field sites and sampling methods are described in detail in Dalton et al. (2015).

Above-ground vegetation survey

At each field site, the above-ground vegetation was surveyed along a 20-m open canopy stream length in four belt transects orientated perpendicular to the shore (Fig. 1). All vascular plants were identified in 1-m^2 quadrats spanning both stream banks. Each transect had two quadrats on each bank (16 quadrats in total at each field site), with the first quadrat positioned immediately upland from the water's edge and the second quadrat placed 2 m from the water's edge (Fig. 1). The waterline was marked in Apr 2010. Riparian vegetation was surveyed twice (3–8 June and 20–29 Jul 2010) so that the majority of species could be identified while in flower. Species were identified following Gleason & Cronquist (1991) and Crow & Hellquist (2000a,b), with nomenclature updated according to the USDA Plants Database (http://plants.usda.gov, accessed 15 Mar 2016). Data from each quadrat and time period were

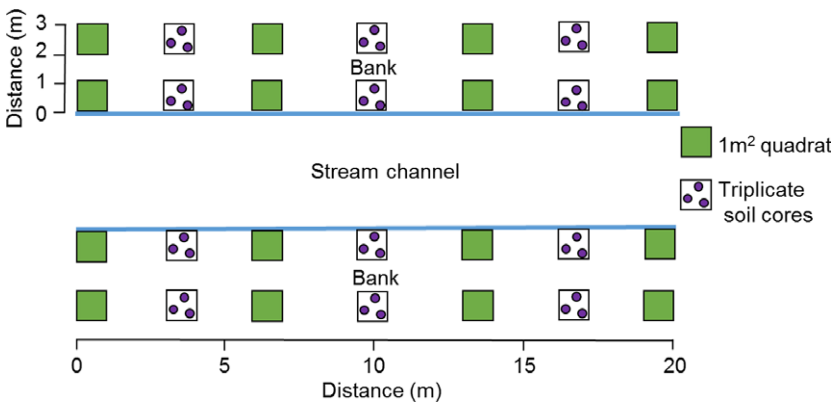

Fig. 1. Sampling design to survey riparian plant species in the above-ground vegetation (16 quadrats, each 1 m^2) and soil seed bank (36 soil cores) in transects spanning both banks across stream/rivers in the South Nation River watershed, Canada (n = 24 field sites). [Colour figure can be viewed at wileyonlinelibrary.com]

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later combined to give species presence/absence data for each site.

Soil seed bank study

The soil seed bank at each field site was sampled between 16–20 Apr 2010 when the soil was still bare with no vegetation. Soil samples (7.62-cm diameter × 15.24-cm depth) were taken using a cylindrical soil corer from three belt transects orientated perpendicular to the shore along both banks of each field site (Fig. 1). Three soil seed bank transects were evenly spaced between the transects of the vegetation survey. Triplicate soil cores were taken from within two quadrats placed along each transect and bank (36 cores per site in total; Fig. 1). Triplicate cores from both bank locations along a given transect were pooled in plastic bags, with cores from different transects and banks kept apart (six composite samples per site). Cores were stored (<2 d) at 4 °C in darkness until processing. Soil samples were homogenized and debris including stones and plant material was removed. Homogenized soil sub-samples (750 ml) were placed in 18 cm × 13 cm × 5 cm trays lined with cheesecloth to allow water drainage while preventing seed loss.

The soil seed bank composition was determined using the seedling emergence method over a 32-mo period (Apr 2010 to Dec 2012) in a greenhouse (National Wildlife Research Centre, Ottawa, ON, Canada). Controls of potting soil were randomly distributed between the six trays for each site to monitor cross-contamination. No seedlings emerged from these controls over the course of the experiment. Samples were exposed to natural sunlight with supplementary lighting for a 16-h photoperiod. The photosynthetically active radiation averaged 285 μmol photons $m^{-2}{\cdot}s^{-1}$ on cloudy and 1951 μmol photons $m^{-2}{\cdot}s^{-1}$ on sunny days. Supplemental heating or fans (as appropriate) moderated the greenhouse temperature. Average daily minimum (night) and maximum (day) temperatures (± SD) over the study period were 17 ± 3 and 39 ± 5 °C, respectively. Seedlings were counted and removed from the trays once they were large enough to be distinguished from other species. Representative seedlings were transplanted into 10-cm pots and grown until they were identified. The trays of soil were stirred regularly and were rotated within the greenhouse to maintain uniform growing conditions. After 8 mo and the onset of negligible germination, the soil seed bank was cold-stratified (4 °C) in darkness for 5 wk and then returned to the greenhouse for another 4 mo before the soil was discarded. Unidentified plants, primarily grasses (Poaceae) and sedges (Cyperaceae), were treated to multiple stratification periods and maintained for an additional 20 mo to induce flowering and aid in identification. Plants not in flower were identified by comparing physical features with flowering species present at the corresponding field site in 2010 and 2011. Forty-seven unknowns were also identified with DNA barcoding. Two regions (*matK* and *rbcL*) were sequenced (modified from Saarela et al. 2013) and species identified by comparing the sequences of the unknowns with those in several searchable databases (BLAST, Camacho et al. 2009; http://blast.ncbi.nlm.nih.gov/Blast.cgi; BOLD, Ratnasingham & Hebert 2007, http://www.boldsystems.org; and a *Carex* spp. database, Brianna Chouinard, unpublished data). Data were examined at the site level by pooling data from the six composite samples.

Data analysis

The species compositions of the above-ground vegetation and soil seed bank were characterized using several metrics that were used in subsequent data analysis. Species richness was calculated as the total number of species at each site for both the above-ground vegetation and the soil seed bank. Life span (annual, biennial/adaptive or perennial) and growth form (forb/herb, graminoid or shrub/tree) were determined from the USDA Plants Database (http://plants.usda.gov, accessed 15 Mar 2016). The native status and a coefficient of conservation (CC) were assigned to each species using a ranking system (values range from −3 for problematic invasive species to 10 for disturbance-intolerant native species; Oldham et al. 1995). To date, CC values have been assigned to 1615 native and 712 non-native southern Ontario plant species and can be used to calculate the overall floristic quality index (FQI) of plant communities (Oldham et al. 1995):

$$\mathrm{FQI} = \mathrm{aver.CC} \times \sqrt{N} \tag{1}$$

where aver.CC is the average CC value per site and N is the total number of species at that site. The calculation of FQI was modified from Oldham et al. (1995) to include non-native species. Species were also assigned a wetness category (ranging from −5 for obligate wetland species to 5 for obligate upland species; Oldham et al. 1995). The sum of the percentage cover of *P. arundinacea* and *B. inermis* in the above-ground vegetation at each site was also calculated.

The species compositions of the above-ground vegetation and soil seed bank were compared in several ways. The similarity in plant species composition between the above-ground vegetation and the soil seed bank was evaluated using Sørensen coefficients (S_s) (1948):

$$S_s = \frac{2a}{2a + b + c} \times 100 \tag{2}$$

where a is the number of species common to both locations, b is the number of species solely in the above-ground

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vegetation and c is the number of species solely in the soil seed bank. Paired t-tests were conducted to compare descriptors of plant communities (species richness, percentage of non-native species and FQI) between the soil seed bank and above-ground vegetation. The assumption of normality of differences was evaluated with a Shapiro-Wilk's test and data were transformed to meet these assumptions if necessary (square transformation for the paired t-test with FQI). Pearson's correlations were used to correlate descriptors of plant communities between the soil seed bank and above-ground vegetation. Correspondence analysis was used to compare the species composition (presence/absence) of the soil seed bank and above-ground vegetation using biplot scaling focused on inter-sample differences. Rare species were down-weighted. Species with frequencies 1/5 or less than that of the most common species were down-weighted in proportion to their frequency (Hill 1979).

Logistic regressions were used to further compare characteristics of the soil seed bank and above-ground vegetation. Two separate regressions were used to determine (1) the probability of a species being unique to the soil seed bank and (2) the probability of a species being more common to the soil seed bank compared to the vegetation. In both regressions, the following independent (explanatory) variables were included (categories are in brackets): life span (annual, biennial/adaptive or perennial), growth form (forb/herb, graminoid or shrub/tree), native status (native or non-native), coefficient of conservation (−3 to 3 or 4 to 10) and wetness category (−5 to −2, −1 to 1 or 2 to 5). Dichotomous dependent (response) variables were created using subsets of the species data with values defined as 0 or 1. In the first regression, the species included in the model were either unique to the soil seed bank or unique to the vegetation. In the second regression, the species included in the model were either more common in the soil seed bank or more common in the vegetation. Species were defined as being more commonly found in a particular location if the difference in occurrence between the two locations was ≥50% and the species occurred in ≥25% of the sites. The significance of the independent variables was evaluated with a Wald statistic and the overall model evaluated from the −2 Log likelihood value, Nagelkerke pseudo R^2 and the percentage of cases correctly classified.

The effects of measures of agriculture (in-stream Jun nitrate concentrations and percentages of surrounding

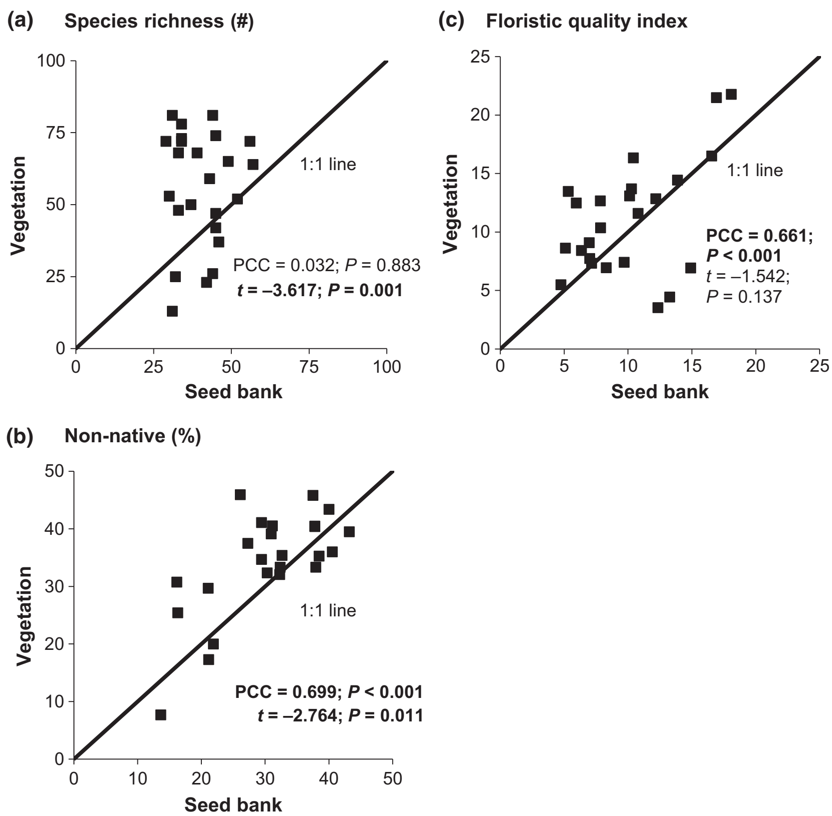

Fig. 2. Comparison of riparian plant community (**a**) species richness, (**b**) percentage of non-native species and (**c**) floristic quality between the above-ground vegetation and soil seed bank in the South Nation River watershed, Canada (n = 24 field sites). Bold text: Significant ($P \leq 0.05$) Pearson's correlation coefficients (PCC) or paired t-tests for the vegetation and soil seed bank.

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annual crop land and natural habitat) and the effects of the dominant non-native grasses *P. arundinacea* and *B. inermis* on descriptors of plant communities were assessed using simple linear regression. The effects of measures of agriculture on the summed percentage cover of *P. arundinacea* and *B. inermis* were evaluated using simple linear regression. The percentage cover of these two species was also used as an independent variable to assess the effects of these species on the species richness of both the above-ground vegetation and soil seed bank using simple linear regression. The assumption of normality of residuals (regression) was evaluated with a Shapiro-Wilk's test.

Canonical correspondence analysis was subsequently used to examine the influence of bank characteristics, land use and in-stream nitrate on the species composition (presence/absence) of the soil seed bank. Variables were normalized if necessary following an assessment of normality using Shapiro-Wilk's tests. Variables were standardized to a mean of 0 and SD of 1 (z-score transformation). Biplot scaling focused on inter-species differences and rare species were down-weighted. Step-wise regression and Monte Carlo permutations were used to test the significance of the environmental variables. Univariate analyses were conducted using SPSS v 21 (IBM, Armonk, NY, US) and multivariate analyses were conducted using CANOCO v 4.5 (Plant Research International, Wageningen, NL).

Results

Comparison of the riparian soil seed bank and above-ground vegetation

In total, 274 plant taxa were identified, including 181 taxa in the soil seed bank and 231 taxa in the riparian above-ground vegetation of the South Nation River watershed. The most common species, occurring in at least 80% of the 48 possible locations (24 soil seed banks and 24 above-ground vegetation sites), were *P. arundinacea*, *Urtica dioica* ssp. *gracilis*, *Pilea pumila*, *Lythrum salicaria*, *Erysimum cheiranthoides* and *Plantago major*. The complete list of species and their characteristics is provided in Appendix S1. A total of 11 436 seedlings germinated and were identified from the soil seed bank. Average (± SD) seed density per site was 16 137 ± 10 003 seedlings·m^{-2} (ranging from 7518 to 43 451 seedlings·m^{-2}). Species richness in the vegetation and soil seed bank were not correlated, with higher species richness observed in the vegetation compared to the soil seed bank (Fig. 2a).

Species composition clearly differed between the above-ground vegetation and soil seed bank. Sørensen coefficients indicated that the compositions of the soil seed bank and vegetation were dissimilar (defined as <50%). The average Sørensen coefficient (± SD) across 24 field sites was 29.0 ± 4.3, and ranged from 15.4 to 34.3. Correspondence analysis also illustrated a clear divide in the vegetation and soil seed bank species composition in terms of the presence or absence of species (Fig. 3). The soil seed bank had higher Axis 1 scores ($F = 269.462$, $P \leq 0.001$, $R^2 = 0.862$) compared to the vegetation, confirming that there was a statistically significant difference in the soil seed bank and vegetation species compositions.

The percentage of non-native species was higher in the vegetation compared to the soil seed bank (Fig. 2b). Floristic quality also tended to be higher in the vegetation but this trend was not significant due to high variation (Fig. 2c). Logistic regressions indicated that growth form had a significant effect on the likelihood of a species being found both uniquely and more commonly in the soil seed bank compared to the vegetation (Table 1). Species unique and common to the soil seed bank were more likely to be graminoids (e.g. *Juncus tenuis* and *Carex vulpinoidea*, respectively), whereas species unique and common to the vegetation were more likely to be forbs/herbs (e.g. *Bidens frondosa* and *Vicia cracca*, respectively; Table 1). Life span, native status, coefficient of conservation and wetness category all had no influence on the likelihood of a species

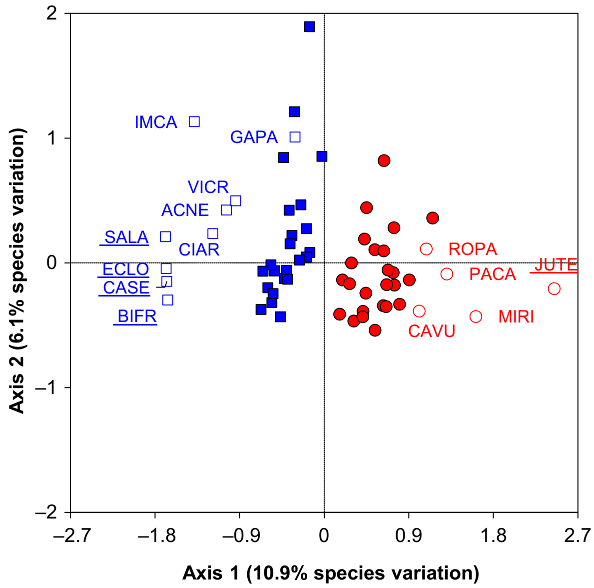

Fig. 3. Correspondence analysis ordinating 24 field sites in the South Nation River watershed, Canada, by above-ground vegetation (■) and soil seed bank (●) riparian species presence/absence data. The five most commonly found species in the above-ground vegetation (□) and seed bank (○) are shown, along with species found uniquely in the above-ground vegetation or seed bank in 12 or more of the field sites (underlined). *Acer negundo* (ACNE), *Bidens frondosa* (BIFR), *Calystegia sepium* (CASE), *Carex vulpinoidea* (CAVU), *Cirsium arvense* (CIAR), *Echinocystis lobata* (ECLO), *Galium palustre* (GAPA), *Impatiens capensis* (IMCA), *Juncus tenuis* (JUTE), *Mimulus ringens* (MIRI), *Panicum capillare* (PACA), *Rorippa palustris* (ROPA), *Sagittaria latifolia* (SALA), *Vicia cracca* (VICR). [Colour figure can be viewed at wileyonlinelibrary.com]

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Table 1. Characteristics of riparian plant species identified solely in the soil seed bank or in the above-ground vegetation or species common to both habitats as determined by logistic regression. Significant independent variables are shown in bold.

	1st Logistic Regression		2nd Logistic Regression	
	Unique to Soil Seed Bank (n = 43)	Unique to Vegetation (n = 93)	More Common[a] in Soil Seed Bank (n = 21)	More common in Vegetation (n = 52)
Life Span	Wald[d] = 0.77; P = 0.828		**Wald = 8.124; P = 0.017**	
Annual (%)	18.6	15.1	9.5	25.0
Biennial/Adaptive[b] (%)	14.0	17.2	38.1	15.4
Perennial (%)	67.4	67.7	52.4	59.6
Growth Form	**Wald = 20.252; P < 0.001**		**Wald = 3.839; P = 0.050**	
Forb/Herb[c] (%)	34.9	75.3	66.7	88.5
Graminoid (%)	53.5	12.9	33.3	9.6
Shrub/Tree (%)	11.6	11.8	0.0	1.9
Native Status	Wald = 0.097; P = 0.756		**Wald = 5.506; P = 0.019**	
Native (%)	76.7	72.0	85.7	63.5
Non-native (%)	23.3	28.0	14.3	36.5
Coefficient of Conservation	Wald = 0.612; P = 0.434		Wald = 2.512; P = 0.113	
Average ± SD	3.7 ± 3.4	3.1 ± 3.5	2.1 ± 2.6	1.5 ± 2.9
−3 to 3 (%)	37.2	48.4	66.7	71.2
4 to 10 (%)	62.8	51.6	33.3	28.8
Wetness Category	Wald = 1.642; P = 0.200		Wald = 0.522; P = 0.470	
Average ± SD	−2.2 ± 3.3	−0.9 ± 3.7	−2.0 ± 3.5	−0.8 ± 3.7
−5 to −2 (%)	67.4	50.5	57.1	50.0
−1 to 1 (%)	14.0	21.5	23.8	19.2
2 to 5 (%)	18.6	28.0	19.1	30.8
Model Summary				
−2 Log Likelihood	142.0		67.5	
Nagelkerke R^2	0.258		0.337	
Cases Correctly Classified (%)	76.5		76.4	

[a]Present at more than six sites and with a difference in occurrence in the soil seed bank and vegetation of ≥50%.

[b]Biennial/Adaptive includes biennial species and those that may complete their life cycle as annuals/biennials/perennials.

[c]Forb/Herb includes sub-shrubs and vines.

[d]Used to evaluate the significance of the independent variables.

being unique to the soil seed bank. The coefficient of conservation and wetness category had no effect on the likelihood of a species being common to the vegetation or soil seed bank. Both life span and native status had an effect. Annual species were more likely to be common to the vegetation (e.g. *Impatiens capensis*) and biennial/adaptive species were more likely to be common to the soil seed bank (e.g. *Rorippa palustris*; Table 1). Native species were more likely to be common to the soil seed bank (e.g. *Mimulus ringens*), whereas non-native species were more likely to be common to the vegetation (e.g. *Cirsium arvense*; Table 1).

Effects of agriculture on vegetation and soil seed bank community composition

Land use varied from 6.7% to 97.4% annual crops and from 1.1% to 77.8% natural habitat at 24 sites located across this large North American watershed. In-stream nitrate concentrations ranged from 3 to 3981 $\mu g \cdot l^{-1}$, with the majority (20 of 24) exceeding the expected background concentration due to natural processes (240 $\mu g \cdot l^{-1}$) estimated for US streams (Dubrovsky et al. 2010). At the watershed scale, land use and in-stream nitrate gradients had no effect on species richness (Fig. 4a–c). The percentage of non-native species significantly decreased with increasing natural habitat (Fig. 4e) and increased with increasing nitrate (Fig. 4f) for both the soil seed bank and vegetation. For both plant communities, floristic quality increased with increasing natural habitat (Fig. 4h) and decreased with increasing nitrate (Fig. 4i). In general, the percentage of surrounding annual crops was less predictive of changes in the percentage of non-native species (Fig. 4d) and FQI (Fig. 4g) than were the percentages of natural habitat (Fig. 4e,h) and in-stream nitrate concentrations (Fig. 4f,i).

When the entire species composition of the soil seed bank was considered in a CCA, the composition was influenced primarily by soil structure and by nitrate concentrations (Fig. 5). Low agriculture sites with high floristic quality tended to have higher percentages of sand, whereas high agriculture sites with low floristic quality tended to have higher percentages of clay. Sites with low

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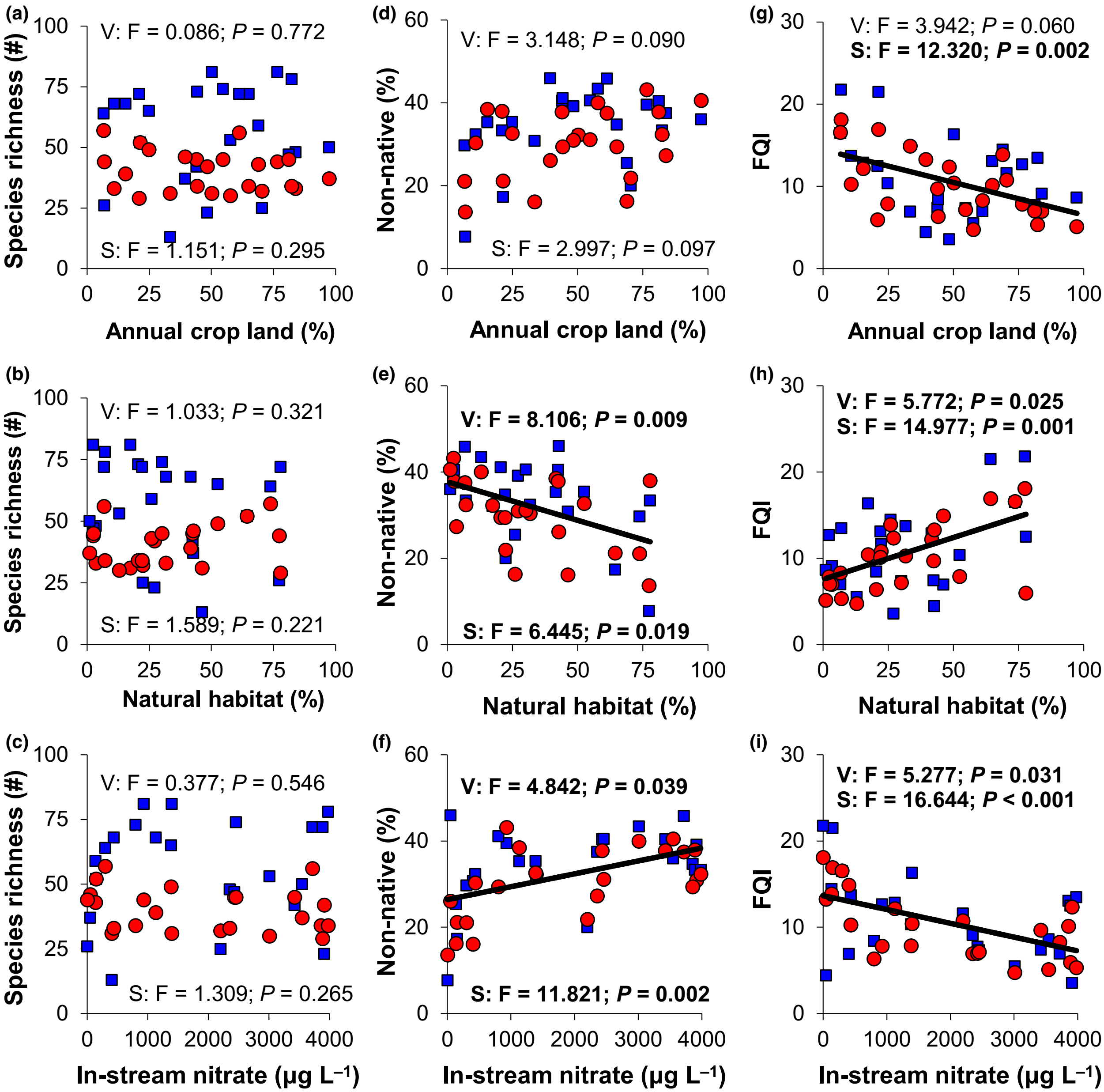

Fig. 4. Comparison of above-ground vegetation and soil seed bank characteristics along gradients of surrounding agriculture in the South Nation River watershed, Canada (n = 24 field sites). (■) Above-ground vegetation (V); (●) Soil seed bank (S). Bold text: Significant linear regressions for the vegetation (– -) and soil seed bank (—) ($P \leq 0.05$). Trend lines overlap. FQI: floristic quality index. [Colour figure can be viewed at wileyonlinelibrary.com]

in-stream nitrate concentrations tended to have soil seed bank compositions with higher floristic quality compared to sites with high in-stream nitrate concentrations.

Effect of dominant non-native grasses on species richness

The grasses *P. arundinacea* and *B. inermis* were both widespread and dominant at a number of field sites. *P. arundinacea* occurred in the vegetation and soil seed bank at 100% and 95.8% of the sites, respectively. *B. inermis* was less widespread and occurred in the vegetation and soil seed bank at 70.8% and 45.8% of the sites, respectively. The average (± SD) percentage cover of these two species was 33.1 ± 22.2% (ranging from 4.4 to 86.9%) for *P. arundinacea* and 14.5 ± 15.4% (ranging from 0.0 to 53.8%) for *B. inermis*, with an average percentage cover for both species combined of almost 50% (average 47.6 ± 16.6%, ranging

Doi: 10.1111/avsc.12313

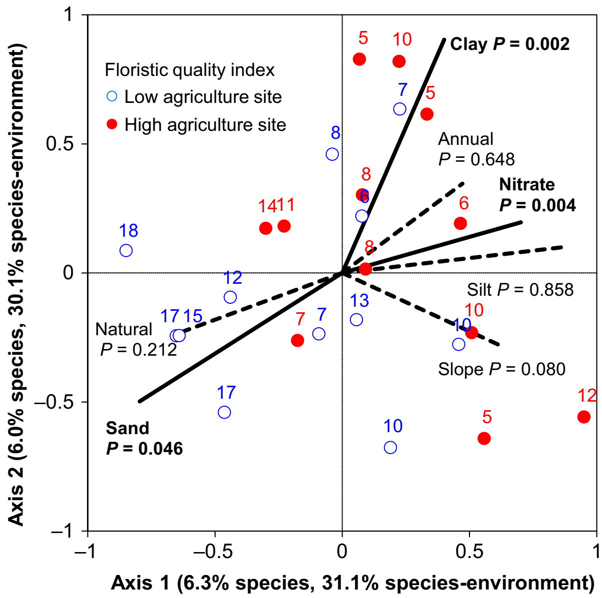

Fig. 5. Canonical correspondence analysis ordinating 24 field sites in the South Nation River watershed, Canada, by variation in the riparian soil seed bank species composition (presence/absence), constrained to the variation explained by physical and land-use variables. Significant ($P \leq 0.05$) variables (shown in solid bold lines) were determined with stepwise regressions and Monte Carlo permutations. [Colour figure can be viewed at wileyonlinelibrary.com]

from 27.9 to 86.9%). Across the watershed, the percentage of annual crop land, percentage of natural habitat and in-stream nitrate concentrations had no effect on the percentage of *P. arundinacea* and *B. inermis* (Fig. 6a–c). Vegetation species richness decreased significantly with increasing cover of *P. arundinacea* and *B. inermis* (Fig. 6d). In contrast, *P. arundinacea* and *B. inermis* had no apparent effect on soil seed bank species richness (Fig. 6e).

Discussion

Species richness of both the above-ground vegetation and soil seed bank was unaffected by agricultural land use or N enrichment, suggesting that species richness is not a sensitive indicator of these anthropogenic stressors. A similar conclusion was reached by Bowers & Boutin (2008) who examined the effects of agricultural disturbance on above-ground riparian vegetation in southeast Ontario, Canada. Across a gradient of agricultural intensity, overall species richness of both the soil seed bank and above-ground vegetation was high in the South Nation River watershed. For example, riparian soil seed banks in the South Nation River watershed had higher species richness (average 40 species per field site, n = 24; Fig. 2a) compared to the average species richness reported for three habitats in 102 European soil seed bank studies (approximately 32, 26 and 24 species for grasslands, forests and marshes, respectively; reviewed in Bossuyt & Honnay 2008). Soil seed bank species richness in the South Nation River watershed was similar to the average species richness reported for a number of different wetland types (average of 42 species calculated from 42 studies representing ten countries, including Canada and USA; Hopfensperger 2007). Our results illustrate that agricultural watersheds can be an important source of regional biodiversity. Although species richness was generally high in the South Nation River watershed, agricultural land use and N enrichment both had several negative effects on the community composition of the vegetation and soil seed bank.

At the watershed scale, the loss of natural habitat and N enrichment led to an increase in the percentage of non-native species and a reduction in the FQI in both the vegetation and soil seed bank, similar to the trend observed for above-ground riparian and aquatic plant community composition (Dalton et al. 2015). This change did not result in a loss of species richness. In contrast, European aquatic and terrestrial ecosystems exhibit an inverse relationship between diversity and N enrichment (reviewed in Moss et al. 2013). The South Nation River watershed has a comparatively shorter history of agriculture and nutrient enrichment compared to European ecosystems. However, the observed degradation of the soil seed bank, particularly with N enrichment, may eventually lead to a loss of species richness in the riparian plant community.

The soil seed bank and above-ground vegetation communities clearly differed. Soil seed bank and vegetation communities are expected to diverge most under either stable conditions with low disturbance or under stressful conditions (e.g. frequent water level changes; Bossuyt & Honnay 2008), such as those we observed. The vegetation was more likely to contain forb/herb species, whereas the soil seed bank was more likely to contain graminoid species. Similarly, Williams et al. (2008) found that riparian soil seed banks in temperate southeast Australia had high species richness and were comprised mainly of graminoids. These observations may have important implications for both conservation and restoration of riparian plant communities. The soil seed bank may be an important reservoir and source of taxonomic diversity for graminoids, but not necessarily for forbs/herbs because the latter are not as well represented in the soil seed bank. Biennial and adaptive species with variable life spans were more likely to appear in the soil seed bank. Wetland soil seed banks typically contain long-lived seeds (Bossuyt & Honnay 2008) that are able to delay germination until favourable moisture conditions are attained (reviewed in Faist et al. 2013). Previous studies have also concluded that a main constraint for soil seed bank-initiated

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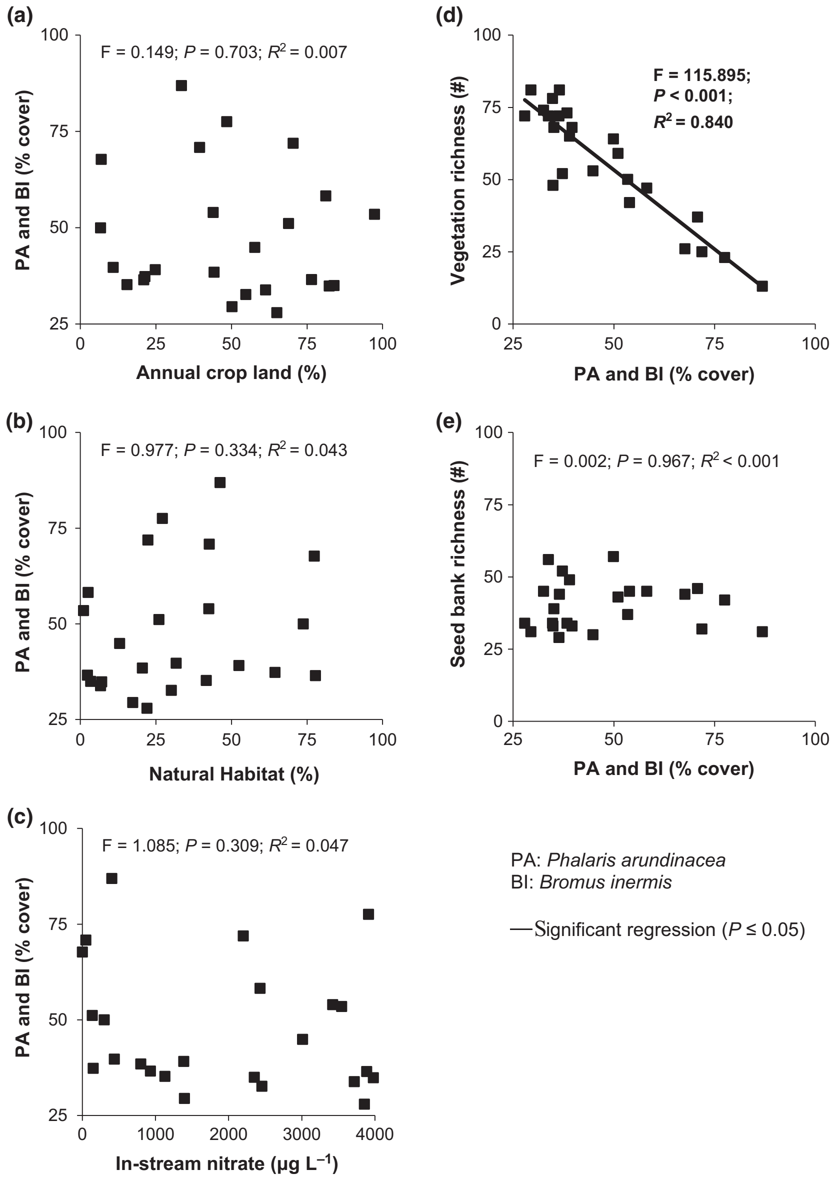

Fig. 6. Relationships between the sum of percentage cover of the non-native species *P. arundinacea* and *B. inermis* and (**a**) surrounding annual crop land, (**b**) surrounding natural habit, (**c**) in-stream nitrate, (**d**) vegetation species richness and (**e**) soil seed bank species richness at 24 sites in the South Nation River watershed, Canada.

restoration is the particular suite of species found in the soil seed bank (reviewed in Bossuyt & Honnay 2008; Greet et al. 2013; O'Donnell et al. 2015).

Compared to the vegetation, native species were more likely to be common to the soil seed bank, suggesting that the soil seed bank is an important reservoir of native riparian species. This finding is in contrast to that of O'Donnell et al. (2016) who found that non-native species became more dominant as degradation of southeast Australian river reaches increased. These conflicting results could be due to differences in the degree to which the watersheds are degraded, as well as factors such as the length and intensity of invasion. In the South Nation River watershed, aggressive non-native grass species had strong negative effects on above-ground vegetation but not on the soil seed bank. Above-ground vegetation species richness declined sharply with increasing cover of the non-native grasses *P. arundinacea* and *B. inermis*. *P. arundinacea* in particular has become dominant in many North American wetlands and is capable of reducing diversity (Schooler et al. 2006). Extensive underground networks of rhizomes allow *P. arundinacea* to spread aggressively, to replace native species and to form monospecific stands by suppressing the germination and colonization of native species (Lavergne & Molofsky 2004). Species richness of the soil seed bank was unaffected by *P. arundinacea* and *B. inermis*, suggesting that the soil seed bank would be a good source for regeneration of native species following efforts to remove these invasive plants. Considerable management effort would be needed because the control of *P. arundinacea* is challenging and the restoration of native species would require multiple years of effort and several approaches (Adams & Galatowitsch 2006; Healy & Zedler 2010). Despite these challenges, the soil seed bank has good potential for restoration efforts. Soil seed banks have been shown to have some resilience to invasions and a different temporal rate of invasion compared to above-ground vegetation (Faist et al. 2013). Even after heavy invasions of non-native species, the soil seed bank has potential to initiate restoration efforts (Vosse et al. 2008). However, time is of the essence. *P. arundinacea* has completed excluded native species in some nearby riparian areas of the Ottawa and St. Lawrence Rivers, Canada (Dore & McNeill 1980), and soil seed banks may eventually become devoid of other species after prolonged periods of *P. arundinacea* monocultures (Apfelbaum & Sams 1987).

Conclusions

Results from our study in a large North American watershed provide evidence that agricultural watersheds face considerable pressure from stressors known to negatively impact diversity on a global scale: conversion of land to agriculture, N enrichment and biological invasions (Sala et al. 2000). The loss of natural habitat and N enrichment led to increases in the percentage of non-native species in both the soil seed bank and vegetation. However, once aggressive non-native grass species were well established in the watershed, their negative impacts on vegetation species diversity were independent of agricultural land and fertilizer use. Conservation of native plant diversity within agricultural watersheds will involve several challenges, including the preservation of natural habitat, a reduction in the use of N-based fertilizers, a reduction in the spread of aggressive non-native species and restoration of heavily invaded areas. The riparian soil seed bank represents a valuable source of taxonomic diversity and a source of native plant species, particularly graminoid species, for restoration.

Acknowledgements

This research was funded by grants to C. Boutin from the Natural Sciences and Engineering Research Council of Canada (249771) and from Environment and Climate Change Canada. DNA barcoding was conducted at the Canadian Museum of Nature (Ottawa, ON, Canada). We thank Philippe Thomas for field assistance and analysis of GIS land-use data. Thanks to Dr. Montse Bassa and Hélène Goossens for assistance in the field and greenhouse.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Plant taxa identified in the soil seed bank and above-ground vegetation.

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Doi: 10.1111/avsc.12313