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Rhododendron maximum impacts seed bank composition and richness following *Tsuga canadensis* loss in riparian forests

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Abstract. Southern Appalachian riparian forests have undergone changes in composition and function from invasive pathogens and pests. *Castanea dentata* mortality in the 1930s from chestnut blight (*Cryphonectria parasitica*) and *Tsuga canadensis* mortality in the 2000s from the hemlock woolly adelgid (*Adelges tsugae*) have led to the expansion and increased growth of *Rhododendron maximum*, an evergreen subcanopy shrub. A better understanding of seed bank characteristics and the various abiotic and biotic factors that affect the seed bank may be useful in determining the restoration potential of forest communities following invasion-related disturbances. We compared the seed bank of two deciduous forest types: hardwood forests with a dense *R. maximum* subcanopy (hereafter, RR) and hardwood forests without *R. maximum* (hereafter, HWD). We evaluated numerous microenvironmental variables through principal component analysis (PCA) and correlated the derived PCA axes scores to seed bank density and richness across forest types. We found that seed bank density was comparable between the forests types; however, seed bank richness was much lower in RR than HWD and the species composition was dissimilar between forest types. Twenty-eight of 64 (44%) species in the seed bank of HWD were not found in the seed bank of RR. Species that were represented in both forest types were often found in contrasting densities. Most notably, seed bank densities of several woody species were considerably higher in RR (85%) than HWD (45%), while herbaceous seed bank density was lower in RR (11%) than HWD (50%). Mineral soil pH, soil nutrient availability, and soil moisture were lower, and organic soil (Oi + Oe + Oa) depth and mass were greater in the RR than HWD forest type. PCA correlations revealed that PCA4 (represented by understory density and Oe + Oa phosphorus and carbon/nitrogen ratio) was negatively correlated with total seed bank density. PCA1 (represented by Oe + Oa cations and phosphorus, understory richness, ground-layer cover, and mineral soil pH) and PCA4 were positively correlated with total seed bank richness. These results suggest that the soil seed bank will not be the primary mode of recruitment to establish a diverse and herbaceous-rich community if a RR is present.

Key words: environmental variables; evergreen shrub; invasive species; principal component analysis; restoration.

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INTRODUCTION

Human activities over the past century have strongly impacted forest disturbance regimes in many parts of the world. Repeated outbreaks of native insect pests and exotic invasive species have caused widespread tree mortality in North America (Orwig et al. 2002, Poland and

McCullough 2006, Kurz et al. 2008) and Europe (Lausch et al. 2013, Maclean et al. 2018*a*), where introduced species continue to invade new territory despite efforts to contain their spread. Examples of non-native forest insects and pathogens that have resulted in tree mortality include gypsy moth (*Lymantria dispar* L.), emerald ash borer (*Agrilus planipennis* Fairmaire), chestnut blight (*Cryphonectria parasitica* (Murr.) Barr), Dutch elm disease (*Ophiostoma novo-ulmi*), and hemlock woolly adelgid (*Adelges tsugae* Annand), and these disturbances have inflicted ecological and economic damage (Lovett et al. 2016). Invasive species introductions are expected to continue, and even intensify, with potentially far-reaching consequences for forest communities and their associated ecosystem services (e.g., carbon storage, nutrient cycling; Ellison et al. 2005, Lovett et al. 2016, Dukes et al. 2009).

Changes in forest disturbance regimes may in some cases result in increased abundance of one or more understory plant species (Mallik 2003, Royo and Carson 2006). In the southern Appalachian Mountains, *Castanea dentata* (Marsh.) Borkh. tree mortality in the mid-1930s due to the chestnut blight resulted in expansion of *Rhododendron maximum* L. shrubs (Elliott and Vose 2012), and the more recent *Tsuga canadensis* (L.) Carrière tree mortality from infestation by the hemlock woolly adelgid has led to further increased growth of *R. maximum* (Ford et al. 2012). *Rhododendron maximum* is an ericaceous evergreen shrub that, at high densities, inhibits tree seedling recruitment and limits overstory regeneration (Clinton 1995, Lei et al. 2002, 2006).

Rhododendron maximum inhibits seedling recruitment by substantially altering resource availability through physical and chemical pathways, suggesting active management of this species in post-*Tsuga canadensis* forests may be required for restoration. Where thickets are present, incident light might be reduced to <2% full sun during the growing season due to *R. maximum*'s thick canopy (Clinton 1995, Nilsen et al. 2001, Lei et al. 2006). Under such conditions, incident light is often below the compensation point for many deciduous species and net photosynthetic carbon gain may be insufficient to support seedling development (Lei et al. 2006). The impact of low light levels on seedling recruitment under *R. maximum* thickets is compounded by competition for water and nutrients (Horton et al. 2009). Within *R. maximum* thickets, available water, soil-extractable cation concentration, nitrogen mineralization rates, and pH can all be markedly lower compared to open understories (Nilsen et al. 2001). Differences in belowground resources may be explained by the poor quality (relatively low nutrient concentrations, high lignin concentration, and low decomposition rate) of *R. maximum* leaf litter and the development of a thick recalcitrant soil organic layer (Wurzburger and Hendrick 2007, Horton et al. 2009).

Similar to other ericaceous species, *R. maximum* has low nutrient requirements, yet a high nutrient retention capacity. Although *R. maximum* leaves represent <20% of their average total biomass, leaf longevity (6–8 yr) makes them an important nutrient pool (Monk et al. 1985). Most of these resources are resorbed following senescence, and only a small fraction remain in leaves prior to leaf fall. In addition, both the leaves and roots of *R. maximum* are rich in phenolic compounds capable of forming recalcitrant polyphenolic–organic complexes. These complexes reduce nitrogen mineralization, which in turn decreases inorganic nitrogen availability in soil beneath *R. maximum* (Wurzburger and Hendrick 2007, 2009, Horton et al. 2009). Because *R. maximum* frequently occurs in close association with *T. canadensis* trees, continued growth and expansion of *R. maximum* shrubs is expected as they replace declining *T. canadensis* stands (Roberts et al. 2009, Ford et al. 2012). Thus, at least the partial removal of *R. maximum* may be needed to promote recovery of ecosystem structure and function. In addition, successful restoration may require the replacement of plant communities that have been locally extirpated or are severely depressed. Under these conditions, the seed bank may represent a potential source of propagules for recruitment of some target species (Saatkamp et al. 2014) and therefore should be considered when planning and implementing restoration activities. For example, Hille Ris Lambers et al. (2005) found that more than 40% of the seeds of *Liriodendron tulipifera* and *Betula* spp. available for germination come from a persistent seed bank. If other plant life-forms (e.g., forbs, graminoids, and shrubs) are also found in the seed bank, this could provide propagules for a diverse

plant community (Schuler et al. 2010, Small and McCarthy 2010, Saatkamp et al. 2014).

A better understanding of seed bank characteristics and the various abiotic and biotic factors that affect seed bank density and composition may be useful in determining the restoration potential of forest communities following invasion-related disturbances (see review, Gioria and Pyšek 2016). To investigate the contribution of the soil seed bank as a recruitment source, we compared the seed bank of two deciduous forest types: hardwood forests with a dense *R. maximum* subcanopy (hereafter, RR) and hardwood forests without *R. maximum* (hereafter, HWD). We hypothesized that (1) seed bank density would be lower in RR than HWD; (2) if a viable seed bank was present, then seed bank richness would be lower in RR than HWD; (3) light transmittance, soil moisture, mineral soil pH, and nutrient availability would be lower, and organic soil depth and mass would be greater in RR than HWD; and 4) variation in the seed bank characteristics would relate to variation in microenvironment and edaphic conditions across the two forest types. We also considered whether the seed bank could contribute to the restoration of plant communities in southern Appalachian riparian forests affected by adelgid-induced *T. canadensis* mortality.

Materials and Methods

Study sites

We conducted our study at two sites in the southern Appalachian Mountains of western North Carolina, USA: the Coweeta Basin (CWT; 35°03′ N, 83°25′ W) and White Oak Creek Basin (WOC; 35°15′ N, 83°35′ W). Mean annual temperature is 12.6°C at CWT and 10.8°C at WOC (Elliott and Knoepp 2005, Laseter et al. 2012). Mean annual precipitation is ~200 cm at both sites, with little seasonal variation; however, dry years are increasingly common. Soils are deep sandy loams underlain by folded schist and gneiss (Thomas 1996). Vegetation is characterized as southern mixed deciduous forest with overstory codominance by *Quercus*, *Acer*, *Carya*, and *Liriodendron* species and understory dominance by *Rhododendron maximum* (Day et al. 1988). Forests at both sites were 70–90 yr old and were infested with hemlock woolly adelgid between 2003 and 2005. Substantial *Tsuga canadensis* decline (80% crown loss, 33% mortality) was documented by 2007 (Elliott and Vose 2011), and complete hemlock mortality was observed by 2012. At the time of this study (2014), overstory *T. canadensis* trees had been dead for at least two years.

We established 32 plots at CWT and 24 plots at WOC in mesic riparian areas with similar elevation range, slope, topography, tree species composition, and abundances of dead *T. canadensis* (>40% basal area prior to mortality). Plots were located at low-to-moderate slopes (<30%), and elevation ranges from 760 to 1060 m at CWT and 1160 to 1390 m at WOC. First, we installed and delineated the 20 × 20 m RR forest type plots with a dense RR, and then, we located nearby 10 × 10 m HWD forest type plots without a *R. maximum* subcanopy. We established HWD plots close to RR plots (within 100 m of at least one RR plot), so that we had equal numbers of RR and HWD plots and covered the same geographic and physiographic distribution of RR plots at each location. To avoid edge effects and any influence of *R. maximum* subcanopies on HWD plots, all plots were placed well within their respective forest type with at least a 15-m buffer on all sides. This sampling design resulted in a total of 56 plots (28 RR, 28 HWD). In the RR forest type, *R. maximum* mean density was 10,000 ± 918 stems/ha, and ranged from 2,300 to 21,000 stems/ha; and *R. maximum* mean basal area was 6.43 ± 0.50 m^2/ha, and ranged from 2.84 to 11.93 m^2/ha. Even though RR and HWD plots sizes were dissimilar, the sampling was conducted similarly across plots (i.e., soil seed bank, ground-layer vegetation, microclimate, and soils). We adjusted for plot size in the overstory layer calculations of density, basal area, and leaf area index (LAI), and understory density (see *Vegetation sampling* below).

Soil seed bank sampling

In late July 2014, we divided each plot into four equal quadrants and extracted a soil core from the center of each quadrant using a PVC collar (7.62 cm inside diameter × 10 cm depth). To prevent premature germination and minimize disturbance, soil cores were left in collars and capped in order to safely transport to the climate-controlled greenhouse at University of

Texas at San Antonio. We assessed the seed bank using the seedling emergence approach (Thompson and Grime 1979, Thompson 1987, Keyser et al. 2012, Maclean et al. 2018*b*). Soil samples were composited by plot and cold–wet stratified at 4°C for two months without light to break seed dormancy (Milberg and Andersson 1998, Baskin and Baskin 2001) and to assure the largest number of species break dormancy and germinate. Following stratification, we sorted samples by hand to remove pieces of root, rocks, and coarse woody debris and then spread each sample evenly over a 3-cm layer of potting soil and vermiculate (1:1) in a 52.7 × 32.7 × 8.0 cm germination tray. Trays were arranged randomly throughout the greenhouse, and 15 trays containing only potting soil and vermiculate were interspersed between sample trays to detect contamination from outside seed sources. All trays were monitored daily, watered as needed, and rearranged monthly for 11 months. Newly emerged seedlings were identified to species and counted, except for *Rubus* and *Betula* species, which were identified to genus. Following identification, we clipped seedlings at their base to prevent competition with seeds that had not yet emerged. Unidentified specimens were transferred to separate pots and grown until identification was possible. When no new germination was observed, we removed all remaining seedlings, mixed each soil sample by hand, and monitored emergence for one subsequent month. Other studies have monitored seed bank trays for a much shorter period (e.g., Keyser et al. 2012, Maclean et al. 2018*b*), and this extra step, mixing and observing for one or more months, is often not taken (e.g., Augusto et al. 2001, Small and McCarthy 2010, Keyser et al. 2012). In our study, seed bank samples from both forest types (RR, HWD) across sites (CWT, WOC) experienced the same greenhouse emergence method described above.

Vegetation sampling

To characterize the vegetation composition of HWD and RR forest types, we sampled the existing vegetation in each plot by layer: overstory, understory, and ground-layer. In the overstory layer, we measured all trees and shrubs ≥2.5 cm at diameter at breast height (DBH, 1.37 m above ground) to the nearest 0.1 cm. Leaf area index (m^2 projected leaf/m^2 ground area) was estimated using DBH and allometric equations developed for woody species in the southern Appalachians (Boring and Swank 1986, Martin et al. 1998, Elliott et al. 2002). In the understory layer, we counted all trees and shrubs (<2.5 cm DBH and ≥0.5 m height) in a 4.0 m wide belt nested within each plot. The ground-layer was sampled in mid-to-late June 2014, the time of peak biomass accumulation; however, some spring ephemerals could have been missed. We placed two 1.0 × 1.0 m quadrats in opposite plot corners and recorded the percent cover of all plants (woody stems <0.5 m height and all herbaceous plants) using a scale that emphasizes intermediate accuracy (Gauch 1982): in 1% intervals from 1% to 5%, in 5% intervals from 5% to 20%, and in 10% intervals above 20%. All species nomenclature follows Gleason and Cronquist (1991).

Microenvironment measurements

We measured soil water content (%), soil temperature (°C), organic soil (defined below) depth (cm), and photosynthetically active photon flux density (PPFD; μmol photons·m^{-2}·s^{-1}) at four equidistant points along a diagonal transect within each plot. Soil water content was measured at 20 cm depth using a handheld HydroSense Soil Water Measurement System (Campbell Scientific, Logan, Utah, USA), and soil temperature was measured at 10 cm depth with a Type T Thermocouple (Barnant Instruments, Barrington, Illinois, USA). Organic soil depth was measured to the nearest 0.1 cm. $PPFD_{incident}$ was measured with a portable light meter (Sunfleck Ceptometer, Decagon Devices, Pullman, Washington, USA) at 1.0 m above the forest floor and within ± 2 h of solar noon under clear, sunny conditions. Additional $PPFD_{incident}$ measurements were taken over each ground-layer quadrat to capture the light transmittance across the plot ($n = 6$ per plot). $PPFD_{open}$ was measured in open conditions within 30 min of the $PPFD_{incident}$ measurements. Light transmittance was calculated as $PPFD_{incident} \div PPFD_{open}$ and expressed as percent. Microenvironment measurements were taken three times in 2014 over the summer months (June, July, and August), and monthly values were averaged as a growing season estimate per plot.

Organic and mineral soil sampling and nutrient concentrations

We sampled organic and mineral soil because plant nutrients and viable seeds are found in both, and fine roots and mycorrhizae can acquire nutrients directly from organic layers, particularly the Oa (humus layer). We collected two organic soil samples from each plot using a 0.09-m^2 sampling frame. Samples were separated into two organic soil layers: Oi (litter, where senesced leaves and twigs are deposited in the fall) and Oe + Oa (Oe = fermentation, where leaves have fractured and are partially decomposed; Oa = humus, dark, and decomposed, no longer recognizable as leaves or twigs). Each layer was placed in a paper bag, oven-dried at 60°C to a constant weight, and weighed. Samples were then composited by plot and layer, ground to <1 mm, and analyzed for total C, N, P, Ca, Mg, K, and Al concentrations. We determined total C and N by combustion on a Flash EA 1112 NC Elemental Analyzer (Thermo Scientific, Waltham, Massachusetts, USA) and total Ca, Mg, K, P, and Al by dry-ashing a subsample at 480°C, digesting it in HNO_3 acid, and analyzing it on an inductively coupled plasma spectrophotometer (Horiba, Edison, New Jersey, USA; Brown et al. 2015).

We collected mineral soil samples in each plot to a depth of 10 cm using an Oakfield soil probe. Each sample was a composite of 15–20 individual samples distributed systematically across the plot to provide a representative plot sample. All soil samples were air-dried and sieved to <2 mm before analysis. We determined total soil C and N by combustion as above, and soil pH in a 1:1 soil to 0.01 mol/L $CaCl_2$ slurry using an Orion portable pH meter (model 250A) with a Thermo Scientific Orion pH probe (Brown et al. 2015).

Statistical analyses

We conducted all statistical analyses using SAS computer software (v9.4; SAS Institute, Cary, North Carolina, USA). We used mixed linear models (PROC MIXED) to evaluate the main effects of forest type (RR, HWD) and site (CWT, WOC) and forest type × site interaction on seed bank and environmental variables. If overall *F*-tests for the interaction effect were significant ($P \leq 0.05$), we used least square means (LS means, Tukey-Kramer-adjusted *t*-statistic) tests to evaluate significance. Degrees of freedom were approximated using Satterthwaite's formula (Littell et al. 2004).

We used principal component analysis (PCA, PROC FACTOR) to reduce the dimensionality of environmental variables across forest types (RR, HWD) and sites (CWT, WOC) into a set of uncorrelated principal components (Graham 2003) and to examine the combined influence of multiple environmental variables on seed bank density and richness. All environmental variables (vegetation, microenvironment, and soils) were evaluated in the PCA. Principal components with eigenvalues >1.0 were retained for further analysis (Kaiser 1960), and environmental variables with loadings >|0.50| were considered significant (Tabachnick and Fidell 2001) and used to define components. Extracted principal component axes scores were then related to seed bank variables using Spearman's rank correlation analysis (Wagner 2013, Chatterjee and Hadi 2015).

Results

Soil seed bank characteristics

Total seed bank density was not different between forest types or sites; however, the composition of the seed bank differed (Table 1). Herbaceous and graminoid seed densities were consistently lower in the RR than HWD forest type. In contrast, tree seed density was consistently greater in RR than HWD at both sites, and shrub seed densities were significantly greater in RR than HWD, but only at WOC (Tables 1, 2). A significant interaction effect (Table 1) revealed that shrub seed density was similar at CWT between forest types ($t_{1,35.2} = -0.62$, $P = 0.924$); however, at WOC, shrub seed density was much greater in RR than HWD ($t_{1,35.2} = -3.51$, $P = 0.007$; Table 2). *Rhododendron maximum* was a common associated species in the RR seed bank, accounting for 37%, but was absent in the HWD seed bank (Appendix S1: Table S1). *Rubus* sp. was the most abundant shrub species across sites, accounting for 71% and 89% of the shrub seed bank density in the RR and HWD, respectively (Appendix S1: Table S1). In the RR forest type, total seed bank density and plant life-form were not significantly related to *R. maximum* density (Appendix S1: Fig. S1). Only tree seed density was positively related to *R. maximum*

Table 1. Mixed-model analysis for effects of forest type (RR, hardwood forest with dense *Rhododendron maximum* subcanopy; HWD, hardwood forest without *R. maximum*), site (CWT, Coweeta Basin; and WOC, White Oak Creek), and their interaction.

Parameters	Forest type			Site			Forest type × site		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Seed bank density									
Total	39.6	0.91	0.345	44.9	0.92	0.342	39.6	1.19	0.283
Forb	36.9	**15.97**	**<0.001**	43.0	0.07	0.793	36.9	0.35	0.556
Graminoid	38.2	**5.44**	**0.025**	44.0	**4.55**	**0.038**	38.2	0.97	0.332
Tree	30.0	**9.51**	**0.004**	30.1	3.31	0.079	13.6	1.30	0.273
Shrub	35.2	**9.35**	**0.004**	41.8	0.68	0.415	35.2	**5.03**	**0.031**
Vine	38.8	0.45	0.507	44.3	3.77	0.059	38.8	1.21	0.279
Seed bank richness									
Total	34.5	**8.93**	**0.005**	40.1	**8.14**	**0.007**	34.5	0.01	0.928
Forb	35.8	**17.52**	**<0.001**	40.8	0.55	0.462	35.8	0.22	0.642
Graminoid	39.3	3.69	0.062	44.8	**4.57**	**0.038**	39.3	0.09	0.763
Tree	36.6	1.84	0.183	41.6	**11.18**	**0.002**	36.6	1.84	0.183
Shrub	30.8	**19.06**	**<0.001**	30.9	0.35	0.550	14.4	0.02	0.550
Vine	35.4	0.01	0.936	41.6	**5.30**	**0.026**	35.4	0.32	0.577
Vegetation									
Overstory LAI	39.5	6.24	0.017	44.8	2.81	0.100	39.5	0.06	0.809
Overstory basal area	39.3	3.44	0.071	44.7	1.17	0.285	39.3	0.63	0.432
Overstory density	51.2	**72.10**	**<0.001**	52.0	**8.17**	**0.006**	51.2	0.35	0.559
Understory density	30.2	4.01	0.053	30.1	0.01	0.916	14.9	2.42	0.141
Understory richness	23.8	**24.69**	**<0.001**	25.8	0.53	0.404	14.9	3.11	0.098
Ground-layer cover	31.5	**35.31**	**<0.001**	31.4	1.16	0.290	14.7	0.67	0.427
Ground-layer richness	38.3	**39.20**	**<0.001**	44.1	3.03	0.089	38.3	0.59	0.447
Microenvironment									
Light transmittance	31.0	1.01	0.323	31.1	0.52	0.475	15.6	3.97	0.064
Soil temperature	11.5	**42.8**	**<0.001**	11.1	**69.0**	**<0.001**	10.9	1.34	0.272
Soil water content	30.4	**6.95**	**0.013**	30.4	0.01	0.957	15.5	0.16	0.695
Organic soil									
Oi + Oe + Oa depth	39.6	**92.80**	**<0.001**	44.7	**16.73**	**<0.001**	39.6	**4.25**	**0.046**
Oi + Oe + Oa mass	38.6	**57.71**	**<0.001**	44.2	0.36	0.554	38.6	0.96	0.332
Oi N	26.7	0.47	0.500	27.6	**7.79**	**0.009**	14.3	0.81	0.382
Oi C	27.9	**13.14**	**0.001**	27.1	2.50	0.125	12.0	2.73	0.124
Oi K	38.8	**41.08**	**<0.001**	44.1	**4.96**	**0.031**	38.8	1.22	0.276
Oi Ca	22.9	0.99	0.329	24.0	**4.28**	**0.049**	13.6	**4.87**	**0.045**
Oi Mg	23.3	1.72	0.203	21.8	**10.54**	**0.004**	10.7	**12.56**	**0.005**
Oi P	20.9	3.26	0.085	20.9	0.57	0.458	9.6	0.80	0.392
Oi Al	39.9	**4.88**	**0.033**	45.2	3.44	0.070	39.9	0.11	0.739
Oe + Oa N	36.6	**21.91**	**<0.001**	41.9	**14.97**	**<0.001**	36.6	**9.19**	**0.004**
Oe + Oa C	25.4	0.05	0.834	24.8	1.65	0.210	10.6	3.40	0.093
Oe + Oa K	22.7	**81.35**	**<0.001**	24.3	**7.46**	**0.012**	14.2	**9.67**	**0.008**
Oe + Oa Ca	24.3	**58.24**	**<0.001**	24.8	**14.35**	**<0.001**	13.1	**5.24**	**0.026**
Oe + Oa Mg	23.0	**20.75**	**<0.001**	22.8	2.77	0.110	11.6	4.23	0.063
Oe + Oa P	33.4	**5.88**	**0.021**	38.9	**5.26**	**0.027**	33.4	0.73	0.399
Oe + Oa Al	27.6	0.00	0.975	26.7	0.24	0.631	10.7	0.00	0.955
Mineral soil									
N	30.5	0.67	0.419	30.8	**63.69**	**<0.001**	15.9	0.23	0.641
C	36.8	0.48	0.494	42.9	**127.2**	**<0.001**	36.8	0.07	0.794
pH	14.8	**28.71**	**<0.001**	11.0	**18.97**	**0.001**	11.7	**19.86**	**0.001**

Notes: *F* and *P* values are for soil seed bank (density and richness by life-form); vegetation (overstory leaf area index [LAI], basal area, and density; understory density and richness; and ground-layer cover and richness); microenvironment (light transmittance, soil temperature, and soil water content); organic soil (Oi + Oe + Oa depth and mass, Oi N, C, K, Ca, Mg, P, and Al concentrations, and Oe + Oa N, C, K, Ca, Mg, P, and Al concentrations); and mineral soil (N and C concentrations and pH). Values in bold type indicate a significant forest type, site, or forest type × site interaction effect. Numerator degrees of freedom = 1 and denominator degrees of freedom (df) are provided in the table.

Table 2. Mean (± SE) soil seed bank density and richness by life-form and environmental variables.

Parameters	CWT		WOC	
	RR	HWD	RR	HWD
Seed bank density (seeds/m^2)				
Total	1408 ± 248	1439 ± 194	1453 ± 221	978 ± 195
Forb	147 ± 27	425 ± 96	78 ± 27	452 ± 131
Graminoid	69 ± 26	271 ± 96	5 ± 5	87 ± 27
Tree	630 ± 118	308 ± 67	411 ± 73	224 ± 59
Shrub	490 ± 114	373 ± 125	937 ± 200	178 ± 77
Vine	65 ± 34	27 ± 12	<1	9 ± 6
Seed bank richness (species/plot)				
Total	11 ± 0.6	13 ± 0.9	9 ± 0.4	11 ± 0.9
Forb	3 ± 0.4	5 ± 0.8	2 ± 0.3	4 ± 0.9
Graminoid	1 ± 0.3	2 ± 0.5	1 ± 0.1	1 ± 0.3
Tree	2 ± 0.2	2 ± 0.3	2 ± 0.2	1 ± 0.2
Shrub	2 ± 0.2	1 ± 0.1	2 ± 0.2	1 ± 0.2
Vine	1 ± 0.3	1 ± 0.2	0	0.2 ± 0.2
Vegetation				
Overstory LAI (m^2/m^2)	4.69 ± 0.44	7.13 ± 1.08	6.25 ± 0.73	9.18 ± 1.78
Overstory basal area (m^2/ha)	28.40 ± 2.15	33.44 ± 4.57	29.76 ± 8.22	42.34 ± 8.22
Overstory density (trees/ha)	2750 ± 174	844 ± 97	3581 ± 419	1392 ± 258
Understory density (stems/ha)	0.64 ± 0.07	2.28 ± 0.72	1.37 ± 0.16	1.69 ± 0.54
Understory richness (species/plot)	3 ± 0	7 ± 1	4 ± 1	7 ± 1
Ground-layer cover (%)	14.96 ± 2.79	44.42 ± 5.24	17.38 ± 3.57	54.05 ± 8.63
Ground-layer richness (species/m^2)	7 ± 1	15 ± 1	6 ± 1	13 ± 2
Microenvironment				
Light transmittance (%)	4.57 ± 0.99	3.54 ± 0.63	1.51 ± 0.40	4.89 ± 2.06
Soil temperature (°C)	17.30 ± 0.09	17.87 ± 0.09	15.28 ± 0.22	16.11 ± 0.20
Soil water content (%)	29.67 ± 0.65	33.09 ± 0.95	30.02 ± 1.51	32.56 ± 1.55
Forest Floor				
Oi + Oe + Oa depth (cm)	6.1 ± 0.3	1.7 ± 0.2	9.6 ± 1.2	2.9 ± 0.4
Oi + Oe + Oa mass (g/m^2)	2985 ± 454	689 ± 39	3536 ± 527	558 ± 48
Oi N (%)	0.70 ± 0.03	0.76 ± 0.04	0.85 ± 0.05	0.85 ± 0.05
Oi C (%)	48.22 ± 0.33	46.87 ± 0.20	47.42 ± 0.13	46.75 ± 0.15
Oi K (mg/g)	2.05 ± 0.06	3.03 ± 0.15	1.92 ± 0.14	2.61 ± 0.11
Oi Ca (mg/g)	11.14 ± 0.50	11.76 ± 0.86	11.00 ± 0.52	9.15 ± 0.39
Oi Mg (mg/g)	1.74 ± 0.10	1.95 ± 0.11	1.73 ± 0.11	1.29 ± 0.09
Oi P (mg/g)	0.45 ± 0.03	0.55 ± 0.05	0.51 ± 0.02	0.56 ± 0.03
Oi Al (mg/g)	0.45 ± 0.08	0.75 ± 0.17	0.09 ± 0.01	0.50 ± 0.26
Oe + Oa N (%)	1.30 ± 0.04	1.22 ± 0.05	1.63 ± 0.06	1.27 ± 0.06
Oe + Oa C (%)	43.46 ± 0.46	44.40 ± 0.70	45.34 ± 0.76	44.33 ± 0.84
Oe + Oa K (mg/g)	1.00 ± 0.08	1.58 ± 0.04	0.99 ± 0.04	2.10 ± 0.16
Oe + Oa Ca (mg/g)	5.97 ± 0.55	12.60 ± 0.88	4.97 ± 0.46	8.54 ± 0.51
Oe + Oa Mg (mg/g)	1.01 ± 0.09	1.51 ± 0.09	0.95 ± 0.06	1.22 ± 0.11
Oe + Oa P (mg/g)	0.68 ± 0.02	0.78 ± 0.03	0.78 ± 0.02	0.82 ± 0.05
Oe + Oa Al (mg/g)	2.69 ± 0.37	2.68 ± 0.32	2.58 ± 0.57	2.46 ± 0.61
Mineral soil				
Total N (%)	0.23 ± 0.01	0.27 ± 0.02	0.49 ± 0.04	0.50 ± 0.04
Total C (%)	5.30 ± 0.30	4.93 ± 0.32	9.47 ± 0.50	9.31 ± 0.41
pH	3.95 ± 0.04	4.31 ± 0.06	3.82 ± 0.06	3.98 ± 0.04

Notes: Vegetation (overstory leaf area index [LAI], basal area, and density; understory density and richness; ground-layer cover and richness); microenvironment (light transmittance, soil temperature, and soil water content); organic soil (Oi + Oe + Oa depth and mass, and Oi N, C, K, Ca, Mg, P, and Al concentrations; Oe + Oa N, C, K, Ca, Mg, P, and Al concentrations); and mineral soil (N and C concentrations, and pH) in two deciduous forest types (RR, hardwood forest with a dense *Rhododendron maximum* subcanopy; and HWD, hardwood forest without *R. maximum*) within the Coweeta Basin (CWT) and White Oak Creek (WOC) sites.

basal area (R = 0.44, P = 0.019, n = 28), whereas total seed bank and other plant life-forms were not related to *R. maximum* basal area (Appendix S1: Fig. S2).

Total seed bank richness was lower under RR than HWD at both sites (Tables 1, 2). The seed bank contained a much smaller proportion of herbaceous species (forbs and graminoids) and a greater proportion of woody species (trees and shrubs) in RR than HWD (Fig. 1). Herbaceous seed bank richness was significantly lower in the RR than HWD, but there was no difference between sites (Tables 1, 2). There was a greater number of graminoids at WOC than CWT, but no difference between forest types (Tables 1, 2). Common herbaceous species found in the HWD seed bank included *Lobelia inflata*, *Viola blanda*, *Oxalis stricta*, and *Ageratina altissima*; and *Cardamine hirsuta* and *Oxalis stricta* in the RR seed bank (Appendix S1: Table S1). Common graminoid species in both the RR and HWD seed banks included *Juncus tenuis*, *Danthonia compressa*, and *Cyperus strigosus* (Appendix S1: Table S1). We found a total of seven tree species in the seed bank (Appendix S1: Table S1). There was no significant difference in tree seed richness between RR and HWD (Table 1); however, tree seed richness in HWD was greater at CWT than WOC ($t_{1,39.2}$ = 3.34, P = 0.010), whereas there was no difference in tree seed richness in RR between sites ($t_{1,39.2}$ = 1.44, P = 0.481; Table 2). *Betula* spp. was the most abundant tree species in the soil seed bank across forest types and sites; however, its seed numbers were much greater in RR than HWD. Associated tree species included *Liriodendron tulipifera*, *Oxydendrum arboreum*, and *Robinia pseudoacacia*, which were found in both forest types (Appendix S1: Table S1).

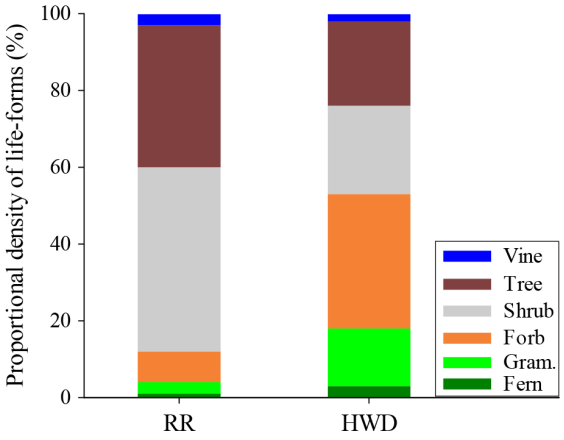

Fig. 1. Seed bank species composition by proportional density of life-forms (fern, graminoid, forb, shrub, tree, and vine) for deciduous forests with a dense *Rhododendron maximum* subcanopy (RR) and without *R. maximum* (HWD). Proportions are shown across sites (CWT, Coweeta Basin; and WOC, White Oak Creek).

Environmental variables: Vegetation, microenvironment, and soils

Overstory basal area was similar across forest types and sites; however, overstory LAI was lower under RR than HWD (Tables 1, 2). Overstory density was greater in RR than HWD, due to the high numbers of *R. maximum* stems >2.5 cm dbh (Appendix S1: Table S2), and it was greater at WOC than CWT (Tables 1, 2). *Acer rubrum* was the dominant overstory species in HWD at WOC and was codominant with *L. tulipifera* and *Betula lenta* in HWD at CWT. Associated tree species at both sites were *Fagus grandifolia*, *Quercus rubra*, *Quercus montana*, and members of the genus *Carya*. Many of the tree species present in HWD occurred in RR, albeit in lower densities (Appendix S1: Table S2).

Understory density and richness were consistently lower under RR than HWD (Tables 1, 2). The understory layer in the HWD forest type at both sites contained high densities of *Gaylussacia ursina* and the woody vine, *Smilax rotundifolia*. Other common understory species included *Tsuga canadensis*, *A. rubrum*, and *Quercus coccinea* at CWT; and *F. grandifolia*, *Hamamelis virginiana*, *Pyrularia pubera*, *Halesia carolina*, and *Rubus* sp. at WOC (Appendix S1: Table S3). *Rhododendron maximum* was the most abundant understory species in the RR forest type, accounting for approximately 80% of the total stem density at both sites. All other understory species were in low numbers in the RR forest type (Appendix S1: Table S3). In the RR forest type, density of understory species other than *R. maximum* was not significantly related to *R. maximum* density (R = 0.012, P = 0.952, n = 28) or *R. maximum* basal area (R = 0.089, P = 0.651, n = 28).

Both ground-layer cover and richness were consistently lower under RR than HWD

(Tables 1, 2). The most abundant species in the ground-layer in RR were *Galax aphylla*, *G. ursina*, and *R. maximum;* abundant species in HWD included *A. rubrum*, *Aster divaricatus*, and *Thelypteris noveboracensis* (Appendix S1: Table S4). In the RR forest type, similar to the understory layer, *R. maximum* basal area was not related to ground-layer cover ($R = -0.110$, $P = 0.577$, $n = 28$) or richness ($R = -0.067$, $P = 0.734$, $n = 28$), likely because we chose RR plots with a dense *R. maximum* subcanopy.

Soil moisture, pH, and nutrient availability were lower, and organic soil depth and mass were greater in the RR than HWD forest type. Light transmittance below the canopy was low and similar under RR and HWD forest types (RR, 3.26 ± 0.65%; HWD, 4.12 ± 0.94%). Soil water content and soil temperature were lower in RR than HWD across sites. There was no difference in soil water content between sites, but soil temperature was significantly higher at CWT than WOC (Tables 1, 2). Organic soil mass and nutrient concentrations varied considerably by forest type and between sites (Table 2). Organic soil depth was greater in the RR than HWD forest type at both sites; and organic soil depth in RR was greater at WOC than CWT ($t_{1,42.2} = 4.35$, $P < 0.001$). Total organic soil mass (Oi + Oe + Oa layer) was also consistently greater in RR than HWD (Tables 1, 2).

Oi N did not differ between forest types, whereas Oi C was consistently greater in RR than HWD (Tables 1, 2). Oi K was consistently lower in RR than HWD, and it was greater at CWT than WOC. Oi Ca and Oi Mg were not different between forest types; however, Oi Mg was greater at CWT than WOC in HWD ($t_{1,35.9} = 4.80$, $P = 0.003$), while there was no difference between sites in RR ($t_{1,35.9} = -0.890$, $P = 0.999$). Oi P was similar between forest types and sites; and Oi Al was greater in RR than HWD (Tables 1, 2).

Oe + Oa N was greater in the RR than HWD at WOC ($t_{1,36.6} = 5.10$, $P < 0.001$), but did not differ between forest types at CWT ($t_{1,36.6} = 1.26$, $P = 0.594$); and in RR, it was greater at WOC than CWT ($t_{1,39.3} = -4.59$, $P < 0.001$), while in HWD, it was similar between sites ($t_{1,39.3} = -0.64$, $P = 0.918$). There was no difference in the Oe + Oa C between forest types and sites. Oe + Oa K and Oe + Oa Ca were lower in the RR than HWD (Tables 1, 2). In HWD, Oe + Oa K and Oe + Oa Ca were greater at WOC than CWT ($t_{1,36.5} = -4.11$, $P = <0.001$ for K; $t_{1,36.6} = 4.29$, $P < 0.001$ for Ca), but they were similar in RR at the two sites ($t_{1,36.5} = 0.02$, $P = 1.000$ for K; $t_{1,36.6} = 1.09$, $P = 0.703$ for Ca). Both Oe + Oa Mg and Oe + Oa P were lower in RR than HWD. No significant effects were found for Oe + Oa Al concentration (Tables 1, 2).

Mineral soil pH was consistently lower in RR than HWD (Tables 1, 2). In HWD, soil pH was higher at WOC than CWT ($t_{1,12.1} = 5.86$, $P < 0.001$); in RR, soil pH was similar between sites ($t_{1,10.8} = -1.92$, $P = 0.270$). Mineral soil N and C were higher at WOC than CWT (Table 2), but there were no differences between forest types (Table 1).

Relationships among environmental variables and seed bank characteristics

To combine environmental variables and reduce dimensionality, we used PCA and then correlated the PCA axes scores with seed bank density and richness. The first four PCA axes explained a large proportion (64%) of the variation in environmental variables across forest types and sites (Table 3), compared to others using PCA and environmental data (e.g., Eide et al. 2017, Lévesque et al. 2017). PCA4 was negatively correlated with total seed bank density (Fig. 2). PCA1 and PCA4 were positively correlated with total seed bank richness (Fig. 2). PCA1 was positively correlated with herbaceous seed density and richness, and graminoid seed density and richness; and negatively correlated with tree seed bank density (Appendix S1: Table S5). PCA4 was negatively correlated with herbaceous seed bank density and richness (Appendix S1: Table S5). Positive loadings for PCA1 were Oe + Oa cations (K + Ca + Mg), Oi cations, Oi P, understory richness, ground-layer cover and richness, and soil pH; and negative loadings were organic soil depth and mineral soil C:N ratio. Positive loadings for PCA4 were understory density and Oe + Oa P, and a negative loading was Oe + Oa C:N ratio (Table 3).

Table 3. Principal component analysis (PCA) axes scores and loadings generated from environmental variables (vegetation, microenvironment, and soils) measured across forest types.

Parameters	PCA1	PCA2	PCA3	PCA4
Eigenvalue	6.09	2.68	2.43	1.67
Variance explained (%)	30.43	13.39	12.17	8.37
Cumulative variance explained (%)	30.43	43.83	55.99	64.37
Overstory LAI	0.2345	−0.1821	**0.8823**	−0.1064
Overstory basal area	0.2989	−0.1398	**0.8608**	−0.1465
Understory density	−0.0472	0.3180	0.2054	**0.7031**
Understory richness	**0.6869**	0.0208	0.0436	0.2498
Ground-layer cover	**0.7485**	0.0689	0.2568	0.2081
Ground-layer richness	**0.8624**	0.1467	0.0750	−0.0126
Light transmittance	0.1884	0.3860	−0.0376	−0.1736
Soil temperature	0.4117	**0.7110**	−0.1769	−0.2164
Soil water content	0.4596	−0.2341	−0.2140	0.1082
Organic soil (Oi + Oe + Oa) depth	**−0.7216**	−0.3771	−0.1504	−0.0800
Oi cations	**0.4996**	−0.2789	−0.4669	−0.2727
Oi P	**0.6190**	**−0.5196**	−0.2755	−0.1392
Oi Al	0.4524	0.3955	−0.1114	0.3164
Oi C:N	−0.4395	**0.7293**	0.0237	0.1458
Oe + Oa cations	**0.8979**	0.0671	−0.0203	0.0351
Oe + Oa P	0.4549	−0.3237	−0.1062	**0.6095**
Oe + Oa Al	0.1604	0.2111	−0.4581	−0.1280
Oe + Oa C:N	0.2343	0.4532	0.3128	**−0.5473**
Mineral soil C:N	**−0.7062**	0.4146	−0.0807	0.1548
Mineral soil pH	**0.7774**	0.2822	−0.2536	−0.1385

Notes: LAI, leaf area index. RR is deciduous forest with a dense *Rhododendron maximum* subcanopy; and HWD is without *R. maximum*, and sites (CWT, Coweeta Basin; and WOC, White Oak Creek). Variables with significant loadings (≥| 0.50|) are set in bold type.

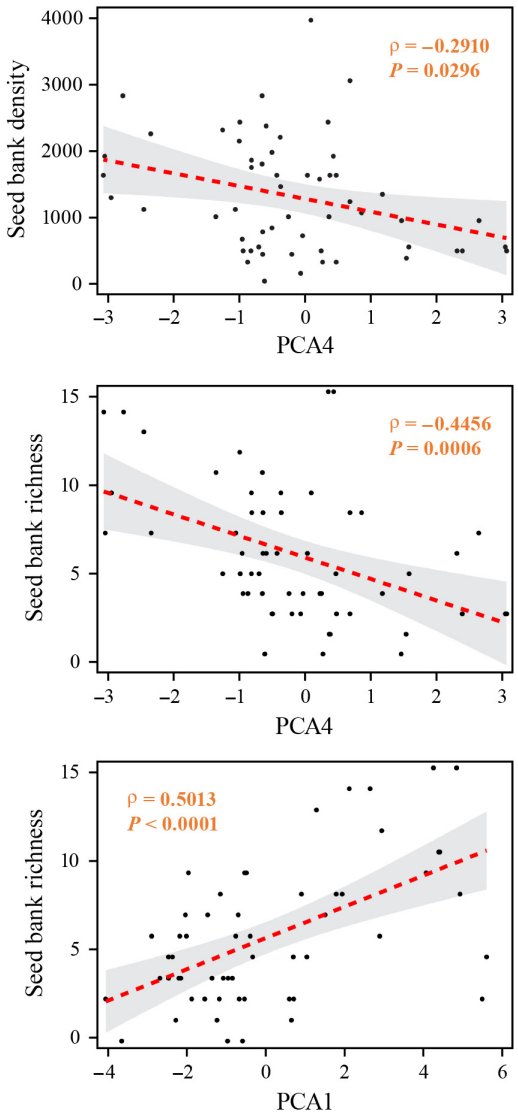

Fig. 2. Spearman's rank-order correlation analysis of total seed bank density (seeds/m^2) and richness (species/plot) with principal component axes (PCA1, PCA4) generated from environmental variables across forest types (RR, deciduous forests with a dense *Rhododendron maximum* subcanopy; and HWD, deciduous forest without *R. maximum*) and sites (CWT, Coweeta Basin; and WOC, White Oak Creek). Significant loadings describing PCA1 were understory richness, ground-layer cover and richness, organic soil depth, Oi cations and P, Oe + Oa cations, and mineral soil C:N and pH. Significant loadings describing PCA4 were understory density and Oe + Oa P (see Table 3). PCA2 and PCA3 were not significantly related to total seed bank density and richness.

Discussion

Effects of forest type on seed bank characteristics

Despite having similar seed bank densities, RR and HWD shared few other seed bank characteristics. Those species only present in the seed bank of HWD were predominantly graminoids and perennial forbs that are dispersed over short distances, and these species contribute to a diverse understory and typically do not impede tree regeneration (Elliott et al. 2014, 2015). By contrast, species only found in the seed bank of the RR forest type shared few distinguishing

characteristics. Four of these species produce seeds that are self-dispersed, one produces wind-dispersed seeds and another produces animal-dispersed seeds. None of these species were found in the standing vegetation of the RR forest type. Given their degree of dissimilarity in mode of dispersal, it is unclear why these species were found in the seed bank of RR but not HWD. The same cannot be said for *Rhododendron maximum* seeds, however, which were found in high densities in the seed bank of RR, but were entirely absent from the seed bank of HWD. Seeds from *R. maximum* were also reported in the seed banks of temperate forests described by Hille Ris Lambers et al. (2005), although no claim was made regarding the persistence of *R. maximum* seeds in the seed bank, given that seeds were too small to be collected in seed traps, and the relationship between yearly seed rain and seed bank density was unknown.

Seed bank densities of several woody species were considerably higher in RR than HWD, even of species that were represented in both the RR and HWD forest types. A comparison of these results with other studies of temperate forest seed banks in this region reveals that the seed densities of woody taxa in the RR forest type exceeded densities that had been previously reported for many species (Lei et al. 2002, Hille Ris Lambers et al. 2005, Keyser et al. 2012). For example, the seed bank density of *Betula* spp. in the RR forest type was nearly two times greater than in comparable temperate forests in the Coweeta Basin described by Hille Ris Lambers et al. (2005) and Lei et al. (2002). Likewise, seed bank densities of *Rubus* sp. and *Liriodendron tulipifera* in the RR forest type exceeded published values by threefold (Lei et al. 2002, Hille Ris Lambers et al. 2005). By contrast, seed bank density of tree species in the HWD forest type was within the range reported by Hille Ris Lambers et al. (2005) and Lei et al. (2002).

Seed bank relationships with environmental variables

Variation in seed bank richness and in plant life-form-specific seed bank density between forest types may be partially explained by differences in environmental conditions. For instance, mineral soil pH, soil nutrient availability, and soil moisture were lower, and organic soil depth and mass were greater in the RR than HWD forest type. These microclimate and edaphic conditions can affect the seed bank directly, by influencing seed losses from the seed bank through germination and decay, and indirectly, by influencing seed inputs to the seed bank from the standing vegetation.

Our research supports a general pattern of increasing seed bank richness with increasing soil fertility and decreasing soil acidity (Staaf et al. 1987, Thompson 1987, Leckie et al. 2000, Maclean et al. 2018*b*). Principal component analysis correlations revealed that multiple environmental variables influenced seed bank density and richness. PCA4 (represented by Oe + Oa phosphorus and carbon/nitrogen ratio, and understory richness) was negatively correlated with total seed bank density. PCA1 (represented by Oe + Oa cations and phosphorus, mineral soil pH, understory richness, and ground-layer cover) and PCA4 were positively correlated with total seed bank richness.

Lower soil nutrient availability and greater soil acidity, such as in RR, may restrict the reproductive capacity of some species in the standing vegetation, potentially reducing the quantity and richness of seed inputs from local seed rain. Lei et al. (2002) reported no significant effect of *R. maximum* subcanopies on the quantity of seed rain from several tree species (*Acer rubrum*, *L. tulipifera*, *Betula lenta*, and *Quercus* spp.), but because some of these species are wind-dispersed, it was unclear whether seeds collected in seed traps were produced locally by maternal trees within the *R. maximum* thickets or were introduced from nearby forest stands. Many tree species have long-distance dispersal (>100 m), particularly those that are wind dispersed (Clark et al. 1999), whereas most forest herbs have short-distance or local seed dispersal (Bakker et al. 1996). In our study, both the HWD and RR forest types had an intact overstory tree canopy that could provide a local seed source, and long-distance dispersal is possible for many tree species. We did not quantify seed rain and do not know the origin of seeds (i.e., local or long-distance dispersal); however, we did find greater seed numbers of *L. tulipifera* and *Betula* spp. in the seed bank of RR than HWD (Appendix S1: Table S1).

Increasing soil nutrients and decreasing soil acidity, as shown in the PCA, were positively correlated with seed bank richness and negatively correlated with tree seed density (Appendix S1: Table S5). We also found that tree seed density was positively related to *R. maximum* basal area (Appendix S1: Fig. S2). While it is unclear whether soil fertility has a direct effect on seed viability and germination (Bekker et al. 1998), lower nutrient availability and greater soil acidity could inhibit microbial activity, slowing decomposition and increasing seed longevity for some species (Champness and Morris 1948, Leck et al. 1989). Hence, while edaphic factors may limit local seed production from some species in the standing vegetation, they could also preserve seed longevity for species that are capable of dispersing into the RR forest type. Taken together, these results may explain the scarcity of herbaceous seeds and the abundance of tree seeds in the seed bank of the RR forest type, as the latter are more likely to be dispersed over greater distances and seed longevity, particularly *Betula* spp., is much greater than the former.

Leaf litter input in the RR forest type may also influence the reproductive capacity of seeds in the seed bank. *Rhododendron maximum* can produce as much as 125 kg/ha of leaf litter per year (Monk et al. 1985) resulting in the formation of thick recalcitrant organic soil layer. Under these circumstances, the steady build-up of organic soil beneath *R. maximum*, as seen in our study, could bury seeds too deeply for germination.

Implications for restoration

Restoration of southern Appalachian riparian forests affected by *Tsuga canadensis* mortality may involve at least the partial removal of *R. maximum* in order to promote recovery of ecosystem structure and function. Because there is often little to no herbaceous and tree seedling cover beneath *R. maximum* (Clinton and Boring 1994, Beckage et al. 2000), successful restoration will require the replacement of plant communities that have been locally extirpated or are severely depressed. Under these conditions, the seed bank may represent a potential source of propagules for recruitment of some target species, and therefore should be considered when planning and implementing restoration activities.

Our results indicate that seed bank communities under *R. maximum* are dominated by the tree and woody shrub life-forms, and by a low number of species. Although seed banks in the RR forest type contained a high density of potentially desirable tree species (e.g., *A. rubrum*, *L. tulipifera*, and *Betula* spp.), other common woody shrubs, such as *Rubus* sp. and *R. maximum*, in the seed bank could suppress the regeneration of some target species and reduce the vigor of others (Meilleur et al. 1994, Royo and Carson 2006). Competition from *Rubus*, however, may be short term due to its high-light requirements (Elliott et al. 2002, Elliott and Knoepp 2005). *Rhododendron maximum* seeds require high light for germination (Blazich et al. 1991) but are shade-tolerant and therefore will presumably germinate in mass if the canopy or subcanopy is removed as long as the substrate remains conducive for seed germination (e.g., moist soils, moss cover). Consequently, if the subcanopy of *R. maximum* is removed from riparian forest communities, a *R. maximum* seed bank could ensure its recolonization, particularly in the presence of ericoid mycorrhizae (Wurzburger and Hendrick 2009), without continued manipulation or active management (e.g., repeated fires). In contrast to the high numbers of tree and woody shrub seeds (85%) in the RR forest type, relatively fewer herbaceous species (14%) were found. Thus, the probability of a diverse herbaceous layer recruiting from the seed bank will likely be low, which could have long-term effects on nutrient cycling and overall forest plant diversity. For example, herbaceous foliage is substantially more nutrient dense than that in trees, can contribute ~20% of the forest foliar litter and 70–90% of the forest plant species diversity (Muller 1978, Gilliam 2007, Welch et al. 2007).

Our results suggest that the soil seed bank may not be the primary mode of recruitment to establish a diverse herbaceous community even if *R. maximum* is removed from these forests, but it could likely facilitate forest canopy recruitment. The high proportion of aggressive ruderal species (i.e., *Rubus* sp.) in the seed bank could limit the growth and regeneration of some target tree species, however, if *R. maximum* is removed without sustained management. Lessons from studies comparing the effects of *Rhododendron ponticum* (evergreen similar to *R. maximum*), a

non-native invasive in Scotland, on native community species may be relevant to seed bank implications here. Similar to our system, as *R. ponticum* density increases, native community species decline (Maclean et al. 2018*b*), and where *R. ponticum* had once been, even after clearing, the native community did not return even after 30 yr. Thus, after an initial *R. maximum* clearing, successive restoration efforts such as prescribed fire, herbicide, and cutting may be required to remove *R. maximum*; and soil amendments and seed introduction (Maclean et al. 2018*a, b*) may improve ecosystem function and promote diversity.

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Literature Cited

Augusto, L., J.-L. Dupouey, J.-F. Picard, and J. Ranger. 2001. Potential contribution of the seed bank in coniferous plantations to the restoration of native deciduous forest vegetation. Acta Oecologica 22:87–98.

Bakker, J. P., P. Poschlod, R. J. Strykstra, R. M. Bekker, and K. Thompson. 1996. Seed banks and seed dispersal: important topics in restoration ecology. Acta Botanica Neerlandica 45:461–490.

Baskin, C. C., and J. M. Baskin. 2001. Seeds: ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego, California, USA.

Beckage, B., J. S. Clark, B. D. Clinton, and B. L. Haines. 2000. A long-term study of tree seedling recruitment in southern Appalachian forests: the effects of canopy gaps and shrub understories. Canadian Journal of Forest Research 30:1617–1631.

Bekker, R., I. Knevel, J. Tallowin, E. Troost, and J. Bakker. 1998. Soil nutrient input effects on seed longevity: a burial experiment with fen-meadow species. Functional Ecology 12:673–682.

Blazich, F. A., S. L. Warren, J. R. Acedo, and W. M. Reece. 1991. Seed germination of *Rhododendron catawbiense* and *Rhododendron maximum*: influence of light and temperature. Journal of Environmental Horticulture 9:5–8.

Boring, L. R., and W. T. Swank. 1986. Hardwood biomass and net primary production following clearcutting in the Coweeta Basin. Pages 43–50 *in* R. T. Brooks Jr., editor. Proceedings of the 1986 Southern Forest Biomass Workshop, 16–19 June 1986, Knoxville, Tennessee. Tennessee Valley Authority, Norris, Tennessee, USA.

Brown, C., C. Harper, N. Muldoon, and S. Cladis. 2015. Procedures for chemical analysis. Coweeta Hydrologic Laboratory, Otto, North Carolina, USA.

Champness, S. S., and K. Morris. 1948. The population of buried viable seeds in relation to contrasting pasture and soil types. Journal of Ecology 36:149–173.

Chatterjee, S., and A. S. Hadi. 2015. Regression analysis by example. John Wiley & Sons, New York, New York, USA.

Clark, J. S., M. Silman, R. Kern, E. Macklan, and J. Hille Ris Lambers. 1999. Seed dispersal near and far: patterns across temperate and tropical forests. Ecology 80:1475–1494.

Clinton, B. D. 1995. Temporal variation in photosynthetically active radiation (PAR) in mesic southern Appalachian hardwood forest with and without rhododendron understories. Pages 534–540 *in* K. W. Gottschalk and S. L. Fosbroke, editors. Proceedings of the 10th Central Hardwood Forest Conference, 5–8 March 1995, Morgantown, West Virginia. NE GTR-197. USDA Forest Service, Northeastern Forest Experiment Station, Radnor, Pennsylvania, USA.

Clinton, B. D., and L. R. Boring. 1994. Regeneration patterns in canopy gaps of mixed-oak forests of the southern Appalachians—influences of topographic position and evergreen understory. American Midland Naturalist 132:308–319.

Day, F. P., D. L. Phillips, and C. D. Monk. 1988. Introduction and site description. Pages 141–149 *in* W. T. Swank and D. A. Crossley, editors. Forest hydrology and ecology at Coweeta. Springer-Verlag, New York, New York, USA.

Dukes, J. S., et al. 2009. Responses of insect pests, pathogens, and invasive plant species to climate change in the forests of northeastern North America. Canadian Journal of Forest Research 39: 231–248.

Eide, I., F. Westad, I. Nilssen, F. S. de Freitas, N. G. dos Santos, F. dos Santos, M. M. Cabral, M. C. Bicego, R. Figueira, and S. Johnsen. 2017. Integrated environmental monitoring and multivariate data analysis—a case study. Integrated Environmental Assessment and Management 13:387–395.

Elliott, K. J., L. R. Boring, and W. T. Swank. 2002. Aboveground biomass and nutrient accumulation 20 years after clear-cutting a southern Appalachian watershed. Canadian Journal of Forest Research 32:667–683.

Elliott, K. J., and J. D. Knoepp. 2005. The effects of three regeneration harvest methods on plant diversity and soil characteristics in the southern Appalachians. Forest Ecology and Management 211:296–317.

Elliott, K. J., and J. M. Vose. 2011. The contribution of the Coweeta Hydrologic Laboratory to developing an understanding of long-term (1934–2008) changes in managed and unmanaged forests. Forest Ecology and Management 261:900–910.

Elliott, K. J., and J. M. Vose. 2012. Age and distribution of an evergreen clonal shrub in the Coweeta Basin: *Rhododendron maximum* L. Journal of the Torrey Botanical Society 139:149–166.

Elliott, K. J., J. M. Vose, J. D. Knoepp, B. D. Clinton, and B. D. Kloeppel. 2015. Functional role of the herbaceous layer in eastern deciduous forest ecosystems. Ecosystems 18:221–236.

Elliott, K. J., J. M. Vose, and D. Rankin. 2014. Herbaceous species composition and richness of mesophytic cove forests in the southern Appalachians: synthesis and knowledge gaps. Journal of the Torrey Botanical Society 141:39–71.

Ellison, A. M., et al. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment 3:479–486.

Ford, C. R., K. J. Elliott, B. D. Clinton, B. D. Kloeppel, and J. M. Vose. 2012. Forest dynamics following eastern hemlock mortality in the southern Appalachians. Oikos 121:523–536.

Gauch, H. G. 1982. Multivariate analysis in community ecology. Cambridge University Press, Cambridge, UK.

Gilliam, F. S. 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems. BioScience 57:845–858.

Gioria, M., and P. Pyšek. 2016. The legacy of plant invasions: changes in the soil seed bank of invaded plant communities. BioScience 66:40–53.

Gleason, H. A., and A. Cronquist. 1991. Manual of vascular plants of northeastern United States and adjacent Canada. Second edition. The New York Botanical Garden, New York, New York, USA.

Graham, M. H. 2003. Confronting multicollinearity in ecological multiple regression. Ecology 84:2809–2815.

Hille Ris Lambers, J., J. S. Clark, and M. Lavine. 2005. Implications of seed banking for recruitment of southern Appalachian woody species. Ecology 86:85–95.

Horton, J. L., B. D. Clinton, J. F. Walker, C. M. Beier, and E. T. Nilsen. 2009. Variation in soil and forest floor characteristics along gradients of ericaceous, evergreen shrub cover in the southern Appalachians. Castanea 74:340–352.

Kaiser, H. F. 1960. The application of electronic computers to factor analysis. Educational and Psychological Measurement 20:141–151.

Keyser, T. L., T. Roof, J. L. Adams, D. Simon, and G. Warburton. 2012. Effects of prescribed fire on the buried seed bank in mixed-hardwood forests of the southern Appalachian Mountains. Southeastern Naturalist 11:669–688.

Kurz, W. A., C. C. Dymond, G. Stinson, G. J. Rampley, E. T. Neilson, A. L. Carroll, T. Ebata, and L. Safranyik. 2008. Mountain pine beetle and forest carbon feedback to climate change. Nature 452: 987–990.

Laseter, S. H., C. R. Ford, J. M. Vose, and L. W. Swift. 2012. Long-term temperature and precipitation trends at the Coweeta Hydrologic Laboratory, Otto, North Carolina, USA. Hydrology Research 43:890–901.

Lausch, A., M. Heurich, and L. Fahse. 2013. Spatio-temporal infestation patterns of *Ips typographus* (L.) in the Bavarian Forest National Park, Germany. Ecological Indicators 31:73–81.

Leck, M. A., V. T. Parker, and R. L. Simpson. 1989. Ecology of soil seed banks. Academic Press, San Diego, California, USA.

Leckie, S., M. Vellend, G. Bell, M. J. Waterway, and M. J. Lechowicz. 2000. The seed bank in an old-growth, temperate deciduous forest. Canadian Journal of Botany 78:181–192.

Lei, T. T., E. T. Nilsen, and S. W. Semones. 2006. Light environment under *Rhododendron maximum* thickets and estimated carbon gain of regenerating forest tree seedlings. Plant Ecology 184:143–156.

Lei, T. T., S. W. Semones, J. F. Walker, B. D. Clinton, and E. T. Nilsen. 2002. Effects of *Rhododendron maximum* thickets on tree seed dispersal, seedling morphology, and survivorship. International Journal of Plant Sciences 163:991–1000.

Lévesque, D., C. Hudson, P. M. A. James, and P. Legendre. 2017. Environmental factors structuring benthic primary producers at different spatial scales in the St. Lawrence River (Canada). Aquatic Sciences 79:345–356.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 2004. SAS system for mixed models. Sixth edition. SAS Publishing, Cary, North Carolina, USA.

Lovett, G. M., et al. 2016. Nonnative forest insects and pathogens in the United States: impacts and policy options. Ecological Applications 26:1437–1455.

Maclean, J. E., R. J. Mitchell, D. F. R. P. Burslem, D. Genney, J. Hall, and R. J. Pakeman. 2018*a*. Understorey plant community composition reflects invasion history decades after invasive rhododendron has been removed. Journal of Applied Ecology 55:874–884.

Maclean, J. E., R. J. Mitchell, D. F. R. P. Burslem, D. Genney, J. Hall, and R. J. Pakeman. 2018*b*. Invasion by *Rhododendron ponticum* depletes the native seed bank with long-term impacts after its removal. Biological Invasions 20:375–384.

Mallik, A. U. 2003. Conifer regeneration problems in boreal and temperate forests with ericaceous understory: role of disturbance, seedbed limitation, and keystone species change. Critical Reviews in Plant Sciences 22:341–366.

Martin, J. G., B. D. Kloeppel, T. L. Schaefer, D. L. Kimbler, and S. G. McNulty. 1998. Aboveground biomass and nitrogen allocation of ten deciduous southern Appalachian tree species. Canadian Journal of Forest Research 28:1648–1659.

Meilleur, A., H. Véronneau, and A. Bouchard. 1994. Shrub communities as inhibitors of plant succession in southern Quebec. Environmental Management 18:907–921.

Milberg, P., and L. Andersson. 1998. Does cold stratification level out differences in seed germinability between populations? Plant Ecology 134:225–234.

Monk, C. D., D. T. McGinty, and F. P. Day Jr. 1985. The ecological importance of *Kalmia latifolia* and *Rhododendron maximum* in the deciduous forest of the southern Appalachians. Bulletin of the Torrey Botanical Club 112:187–193.

Muller, R. N. 1978. The phenology, growth and ecosystem dynamics of *Erythronium americanum* in the northern hardwood forest. Ecological Monographs 48:1–20.

Nilsen, E. T., B. D. Clinton, T. T. Lei, O. K. Miller, S. W. Semones, and J. F. Walker. 2001. Does *Rhododendron maximum* L. (Ericaceae) reduce the availability of resources above and belowground for canopy tree seedlings? American Midland Naturalist 145:325–343.

Orwig, D. A., D. R. Foster, and D. L. Mausel. 2002. Landscape patterns of hemlock decline in New England due to the introduced hemlock woolly adelgid. Journal of Biogeography 29:1475–1487.

Poland, T. M., and D. G. McCullough. 2006. Emerald ash borer: invasion of the urban forest and the threat to North America's ash resource. Journal of Forestry 104:118–124.

Roberts, S. W., R. Tankersley, and K. H. Orvis. 2009. Assessing the potential impacts to riparian ecosystems resulting from hemlock mortality in Great Smoky Mountains National Park. Environmental Management 44:335–345.

Royo, A. A., and W. P. Carson. 2006. On the formation of dense understory layers in forests worldwide: consequences and implications for forest dynamics, biodiversity, and succession. Canadian Journal of Forest Research 36:1345–1362.

Saatkamp, A., P. Poshold, and D. L. Venable. 2014. The functional role of soil seed banks in natural communities. Pages 263–295 *in* R. S. Gallagher, editor. Seeds: the ecology of regeneration in plant communities, Third edition. CAB International, Oxfordshire, UK.

Schuler, T. M., M. T. Van-Gundy, M. B. Adams, and W. M. Ford. 2010. Seed bank response to prescribed fire in the central Appalachians. Research Paper NRS-9. USDA Forest Service, Northern Research Station, Newtown Square, Pennsylvania, USA.

Small, C. J. and B. C. McCarthy. 2010. Seed bank variation under contrasting site quality conditions in mixed oak forests of southeastern Ohio, USA. International Journal of Forestry Research. https://doi.org/10.1155/2010/419482

Staaf, H., M. Jonsson, and L. G. Olsén. 1987. Buried germinative seeds in mature beech forests with different herbaceous vegetation and soil types. Holarctic Ecology 10:268–277.

Tabachnick, B. G., and L. S. Fidell. 2001. Using multivariate statistics, Fourth edition. Allyn & Bacon, Boston, Massachusetts, USA.

Thomas, D. 1996. Soil survey of Macon County, North Carolina. USDA Natural Resources Conservation Service, Washington, D.C., USA.

Thompson, K. 1987. Seeds and seed banks. New Phytologist 106:23–34.

Thompson, K., and J. P. Grime. 1979. Seasonal variation in seed banks of herbaceous species in ten contrasting habitats. Journal of Ecology 67: 893–921.

Wagner, H. H. 2013. Rethinking the linear regression model for spatial ecological data. Ecology 94:2381–2391.

Welch, N. T., J. M. Belmont, and J. Randolph. 2007. Summer ground layer biomass and nutrient contribution to above-ground litter in an Indiana temperate deciduous forest. American Midland Naturalist 157:11–26.

Wurzburger, N., and R. L. Hendrick. 2007. Rhododendron thickets alter N cycling and soil extracellular enzyme activities in southern Appalachian hardwood forests. Pedobiologia 50:563–576.

Wurzburger, N., and R. L. Hendrick. 2009. Plant litter chemistry and mycorrhizal roots promote a nitrogen feedback in a temperate forest. Journal of Ecology 97:528–536.

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