



ORIGINAL ARTICLE

White-Tailed Deer (*Odocoileus virginianus*) Exclusion Shifts Soil Carbon Dynamics in Mature Oak-Dominated and Hemlock-Dominated Forest Stands

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ABSTRACT

While the direct effects of white-tailed deer (*Odocoileus virginianus*) on vegetation have been intensively studied, less is known about the indirect and interactive effects of herbivory on lower trophic levels, such as soil microbes and their processing of carbon pools. We explored how carbon dynamics shift with release from over-browsing by white-tailed deer in two mature stands of oak and hemlock trees. We measured soil carbon pools (for example, soil organic matter, carbon stocks, litter biomass, and litter stabilization) and fluxes (for example, soil respiration, methane uptake, microbial substrate use, and litter decomposition) using a spatially balanced survey design inside and outside two 24-year-old deer exclosures, one in each forest

stand. Soil carbon pools were higher inside the exclosures than in deer-browsed plots in both forest stands, but the effect of deer herbivory on fine-scale spatial patterning of soil carbon pools and mean carbon fluxes varied by forest type. Release from deer herbivory in the oak stand increased the patchiness of soil pools and led to higher litter decomposition, soil respiration, and methane uptake rates. Release from deer herbivory in the hemlock stand did not affect the spatial structure of soil pools, had little effect on methane uptake, and had negative effects on litter decomposition and soil respiration. These differences may be due in part to the interactive effects of two herbivores, deer and the hemlock woolly adelgid (*Adelges tsugae*), that appear to be limiting regeneration and promoting the proliferation of monodominant hay-scented fern (*Dennstaedtia punctilobula*) in the hemlock-dominated stand. Our work suggests that future efforts consider multiple zoogeochemical stressors simultaneously, in addition to variation in environmental templates, to explain uncertainties

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in carbon pools and fluxes in temperate forested ecosystems.

Key words: Soil heterogeneity; Carbon storage; Methane uptake; Soil respiration; Hemlock woolly adelgid; Hay-scented fern.

HIGHLIGHTS

- Deer exclusion improves soil carbon storage in temperate forest stands.
- Deer exclusion enhances methane uptake in oak-dominated stands.
- Herbivores interact to drive carbon dynamics in hemlock-dominated stands.

INTRODUCTION

Upland forests are important for climate mitigation through their fixation of carbon dioxide (CO_2) into woody plant biomass, storage of carbon (C) in soils (McKinley and others 2011; Canham and others 2024), and uptake of methane (CH_4) (Feng and others 2023). However, the effects of zoogeoch- emical stressors on forest C dynamics are uncertain (Schmitz and others 2014; Leroux and others 2020). The distribution and abundance of large herbivores can influence short-term and long-term C pools and fluxes across multiple scales (Schmitz and others 2018), but effect sizes and directionality vary across studies and ecosystems (Andriuzzi and Wall 2018; Forbes and others 2019; Ibañez-Alvarez and others 2022). In forests, some uncertainty may be caused by environmental variation and the particular zoogeoch- emical stressors experienced by dominant tree species. Here, we explored how release from over-browsing of white-tailed deer (*Odocoileus virginianus*) affects C dynamics in oak-dominant and hemlock-dominant forest stands.

Deer are keystone herbivores who alter forest structure and function (Husheer and others 2003; Côté and others 2004) across biomes (Ramirez 2021). Systematic studies of deer browsing began with investigations of deer densities (Leopold and others 1947) and have expanded to studies on trophic dynamics (Alverson and others 1988; Rooney and Waller 2003; Tanentzap and Coomes 2012). A main focus has been on deer browsing's negative effects on seedling recruitment and understory development (Gill and Beardall 2001;

Russell and others 2017). Further, by reducing species richness and abundance, deer browsing has been shown to alter long-term regeneration trajectories, resulting in lower overstory diversity, taller stands with lower basal areas, and more open canopies (Reed and others 2022).

While these vegetative shifts have the potential to affect C cycling, direct links between deer browsing intensity and soil C dynamics are less certain. Studies have found positive, negative, and neutral effects of ungulate browsing on both soil C pools (Wardle and others 2001; Mohr and others 2005; Stark and others 2010; Stephan and others 2017; Kolstad and others 2018; Chollet and others 2021b) and fluxes (Kielland and others 1997; Ellis and Leroux 2017; Kolstad and others 2018; Ramirez and others 2021; Ibañez-Alvarez and others 2022). Ungulates affect the quality of litter through shifts in composition (Chollet and others 2021b) by avoiding vegetation with hard-to-decompose lignified structures (Wardle and others 2002). However, they also provide nutrient additions to soils (for example, feces and urine) that accelerate decomposition (Hobbs 1996). Further, tree species can differ in compensatory growth after browsing (Kupferschmid 2017), leading to variation in the biomass available for decomposition. The response of C stocks to browsing can also depend on the environmental template, such as soil or forest type (Popma and Nadelhoffer 2020; Ibañez-Alvarez and others 2022).

In addition to changes in mean soil C pools and fluxes, herbivores, such as deer, can affect the spatial and temporal distribution of soil resources and microbial activity within forest stands (Augustine and Frank 2001; Abbas and others 2012) through excretion additions, compaction (Tanentzap and Coomes 2012), canopy gap formation, shifts in litter chemistry (Stadler and others 2006), and selective browsing. For example, black cherry (*Prunus serotina*), which contains cyanogenic glycosides (Swain and Poulton 1994), is avoided during browsing and may affect nearby seedling recruitment due to negative plant-microbial associated feedback loops (Royo and others 2021). This can lead to spatial restructuring of C both above- and below-ground. Nevertheless, few studies have explored the effects of ungulates on soil resource redistribution at the fine scales that affect herbaceous and seedling regeneration (Murray and others 2013).

In temperate forests of the eastern U.S., increased levels of white-tailed deer browsing are having adverse effects on palatable tree species such as oaks (*Quercus sp.*) and eastern hemlock (*Tsuga*

canadensis L.) (Hosley and Ziebarth 1935; Dahlberg and Guettinger 1956; Rooney and Waller 2003). Losing these foundational species can alter energy flows, nutrient cycling, and C storage (Ellison and others 2005). The eastern hemlock, a long-lived shade-tolerant (Hough 1960) conifer, creates low-light forest conditions (Merrill and Hawley 1924) and has lower transpiration rates per leaf area compared to deciduous trees (Catovsky and others 2002), promoting slower nutrient cycling and enhancing C storage (Mladenoff 1987; Ellison and others 2005). Northern red oak (*Quercus rubra*), a long-lived shade-intolerant broad-leaved tree, has high photosynthetic rates and can have higher canopy C uptake rates compared to eastern hemlock and red maple (*Acer rubrum*) at certain times of the year (Catovsky and Bazzaz 2000).

If browsing pressure is removed from this landscape, a simple reversal is not likely to occur (Carter and others 2015). Oak regeneration is dependent on suitable light and moisture regimes, active management of competitive species, and fire disturbance (Iverson and others 2017; Beasley and others 2022). Further, eastern hemlock regeneration is affected by the hemlock woolly adelgid (HWA), an invasive insect that can cause tree death within 4 to 10 years (Orwig 2002). Oaks and hemlock have higher woody debris (Campbell and Gower 2000; McGarvey and others 2015), litter lignin-to-nitrogen ratios (Finzi and Canham 1998), and soil carbon-to-nitrogen (C:N) ratios compared to potential replacement species such as sugar maple (*Acer saccharum*), white ash (*Fraxinus americana*), and red maple (Finzi and others 1998). Furthermore, red maple has litter tannin chemistry

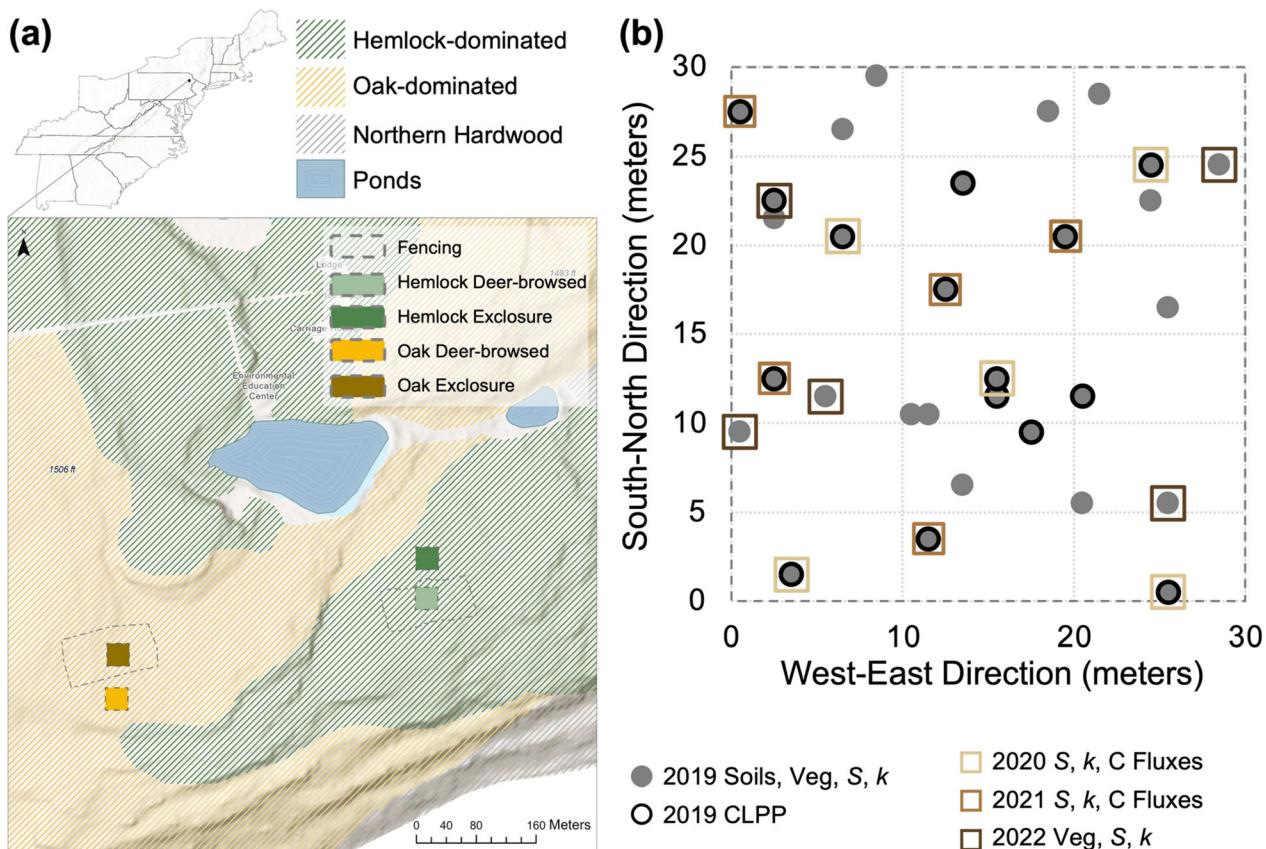


Figure 1. **a** Map of experimental deer exclusion area on a southern slope crest at Lacawac Sanctuary in Wayne County, Pennsylvania, USA. Forest composition data was provided by Green Leaf Consulting, Inc. **b** Schematic of plot sampling design used to measure carbon pools and fluxes across years. Gray circles identify the sampling points ($n = 30$) used for soil (that is, soil organic matter, soil total carbon, soil carbon stock, soil total nitrogen, soil nitrogen stock, and gravimetric water content), vegetation (percent ground cover), standard litter stabilization (S), and standard litter decomposition (k) measurements in 2019. Black circle outlines identify the subset ($n = 15$) of these sampling points used in community-level physiological profiling (CLPP). Boxes identify the random subset of sampling points ($n = 5$) used in 2020 (light brown), 2021 (medium brown), and 2022 (dark brown) for additional S , k , and vegetative assessments, along with soil carbon flux measurements (C Flux).

that stimulates higher microbial respiration compared to both eastern hemlock and oaks (Talbot and Finzi 2008), and black birch (*Betula lenta*), another replacement species, can take up more N, stimulating soil decomposition through lower litter C:N ratios (Finzi and others 2014). However, replacement stands can also have soil respiration rates similar to secondary eastern hemlock stands due to their less well-developed root biomass (Finzi and others 2014). Thus, the long-term effects of replacing eastern hemlock and oaks on C cycling remain uncertain.

We investigated how release from over-browsing by white-tailed deer affects C dynamics by comparing plots inside and outside two long-term deer exclosures situated within two distinct stands, one dominated by northern red oak and the other by eastern hemlock. Both stands are experiencing HWA infestation, with infection rates of over 90% in 2024. We hypothesized that in both stands, release from deer browsing (H1) allows for the development of fine-scale patchiness of soil C pools; (H2) improves long-term soil C storage; and (H3) enhances variation in bacterial communities, driven by an increase in ground cover complexity. We expected the differences inside and outside the deer exclosure to be less pronounced in the hemlock stand, due to low recovery of hemlock saplings. We also hypothesized that (H4) release from deer browsing has an ecosystem-specific effect on C fluxes. We predicted the oak exclosure would have higher litter decomposition rates, soil respiration rates, and more CH₄ uptake than the browsed plot due to denser understory and higher moisture retention. The hemlock stand has canopy gaps created by HWA infestation, which has created compositional differences in the understory layer; non-palatable hay-scented fern (*Dennstaedtia punctilobula*) appears in areas with deer and common successional saplings appear in the exclosure. Because the deer-browsed plots have a denser, more microbially labile ground cover, we expected it would have higher litter decomposition rates, soil respiration rates, and more CH₄ uptake than the exclosure.

MATERIALS AND METHODS

Study Area and Design

We conducted our study at Lacawac Sanctuary, Wayne County, Pennsylvania, USA, which consists of ~ 200 ha of secondary growth forest within the Allegheny Plateau (Figure 1a). This region has a humid continental climate with forests represent-

ing a transition between the Appalachian oak and northern hardwood forest types (Russell and others 1993; Townsend and others 2002). In the past 10 years (2013 – 2023), annual precipitation ranged from 461 to 1413 mm, and the mean annual temperature ranged from 7.8 to 10.2 °C (unpublished data, B. Hargreaves).

In the past 80 to 100 years, there has been minimal forest management on site, resulting in mature eastern hemlock- and northern red oak-dominated forests, with some oak trees estimated to be over 150 years old (Fiad 2017). Seedling density is relatively low (sapling to canopy density ratio of 0.49 recorded in 1991–1992) and attributed to intense browsing by deer (Townsend and others 2002). Deer density at Lacawac was estimated to be between 6.1 and 9.7 deer km⁻² in 2001 (unpublished, Lacawac database), which is similar to current day estimates in Wisconsin (8 to 20 deer km⁻² in Waller and Alverson 1997; Rooney and Waller 2003) and elsewhere in Pennsylvania (11.56 deer km⁻² in San Julian and Smith 2001).

In 1995, two deer exclosures were constructed 306 m apart in mature forest without stand manipulation (Byman 2011). The oak exclosure (Lat. 41.374533, Long. -75.295345, Elv. 444 m.) sits on Wellsboro channery loam (Soil Survey Staff, Natural Resources Conservation Service (NRCS), last accessed 07/2024), and is dominated by northern red oak (basal area (BA): 17 m² ha⁻¹), followed by eastern hemlock (BA: 8 m² ha⁻¹) and chestnut oak (*Quercus montana*, BA: 5 m² ha⁻¹) (Fiad 2017). The hemlock exclosure (Lat. 41.375025, Long. -75.290225, Elv. 439 m) sits on Lordstown channery loam (Soil Survey Staff, NRCS, last accessed 07/2024) and is dominated by eastern hemlock (BA: 20 m² ha⁻¹), followed by northern red oak (BA: 4 m² ha⁻¹) and red maple (BA: 8 m² ha⁻¹).

HWA was documented in Wayne County between 1991 and 2002 (U.S. Forest Service 2018). BA of hemlock did not decline in the stands between 1995 and 2012 and, in some areas, increased due to new sapling growth (unpublished data, D. Townsend). However, by 2012, the health of over 40% of hemlocks was in poor or very poor condition (unpublished data, D. Townsend), and significant defoliation was also reported in 2017 (Fiad 2017).

Exclosures are 60 m × 120 m and have 3 m high fencing. Fence integrity was monitored weekly thru 2012 and periodically through the present study. In recent years, several fallen trees have resulted in fence breaches, which were repaired. As such, exclosures represent areas with a legacy of

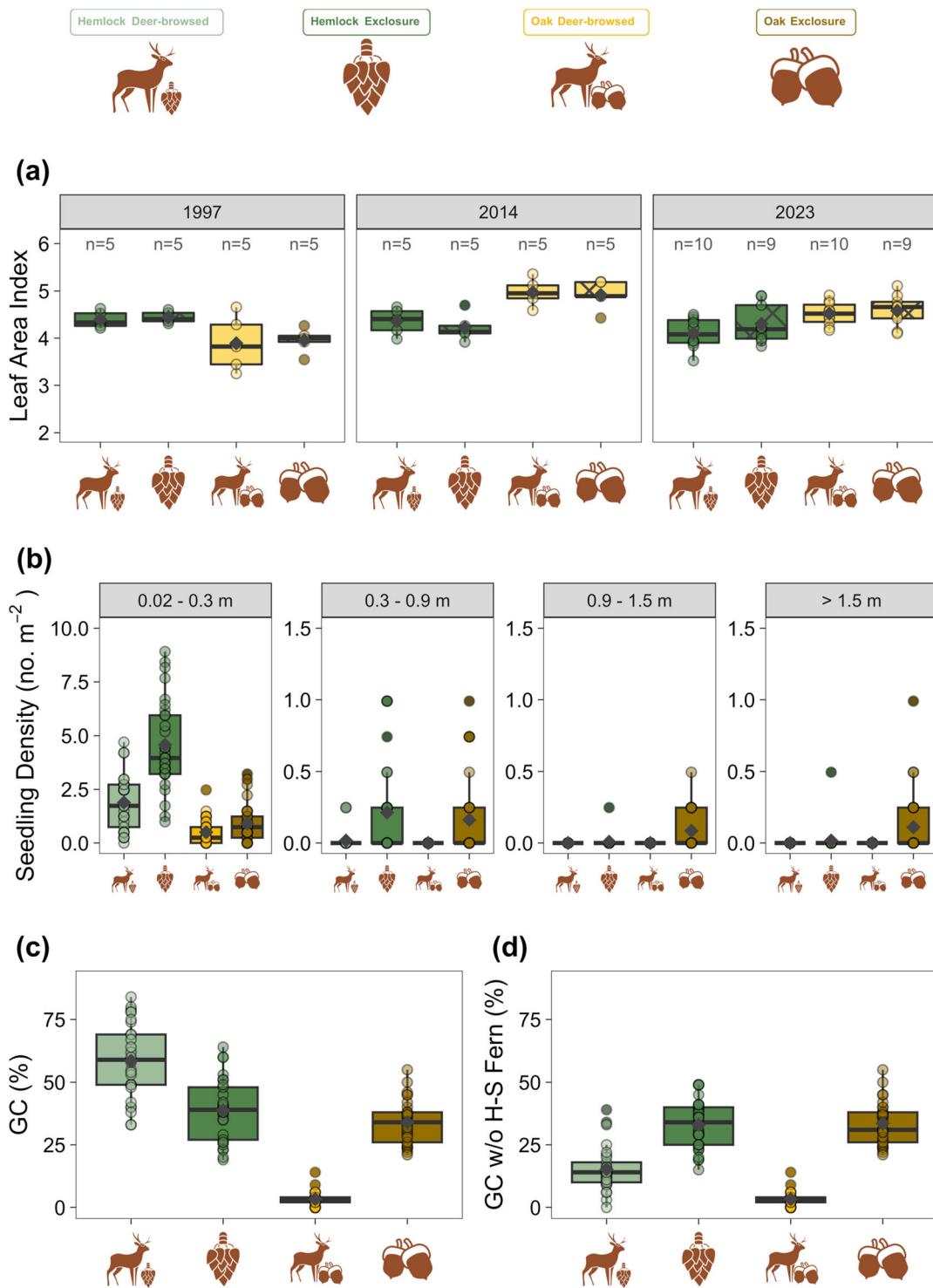


Figure 2. Vegetation surveys within the hemlock-dominated (green/cone) and oak-dominated stands (yellow/acorn), with (deer-browsed, light-color) deer and without (exclosure, dark-color) deer. Surveys included (a) leaf area index (LAI), measured in September of 1997, 2014, and 2023, (b) woody seedlings ($\text{stems } m^{-2}$) classified according to height size classes (that is, 2 to 30 cm, 30 to 90 cm, 90 cm to 1.5 m, and > 1.5 m) in 2022, and (c) ground cover (GC, %) and (d) ground cover excluding hay-scented fern (GC w/o H-S Fern, %) collected in 2022. Twenty-nine sampling points were assessed unless noted otherwise. The boxplots were created in the ggplot2 package (Wickham 2016), representing the mean (diamond), median (horizontal line), interquartile range (IQR, box boundary), variability outside the IQR (whiskers), outliers (filled circles), and raw data points (transparent circles). Icons are the work of Lars Meiertoberen, from the Noun Project, Inc., under a royalty-free license.

deer exclusion (1995–2012) and highly reduced deer influence from 2012 to the present. In 2019, avoiding the fence edge by ~ 10 m, we established a 30 m × 30 m plot inside (exclosure plots) and outside (deer-browsed plots) the two deer exclosures to measure C pools and fluxes (Figure 1b). Thirty sample points were established inside each of the four plots using a generalized tessellation randomized design (Stevens and Olsen 2004). All data was collected at these sampling points except the leaf area index (LAI) measurements described below.

CANOPY MEASUREMENTS

We measured the LAI in September 1997, 2014, and 2023 using a plant canopy analyzer (LiCor, Inc., Lincoln, NB, USA). With little understory in 1997 and 2014, we collected 9 LAI measurements at 20 cm from the ground using the 45-view cap for 5 subplots (each 20 m × 20 m) inside and outside the exclosures. In 2023, we used a 1 m measurement height to avoid the influence of the understory and collected 5 LAI measurements within 10 subplots outside the exclosures and 9 subplots inside the exclosures. Measurements were averaged

per subplot before plots were compared across years.

During the early summer of 2019 (6/29 – 7/2), we took hemispherical photographs using a Nikon D7500 DX-Format Digital SLR equipped with a Sigma AF 4.5 mm f2.8 EX DC HSM Circular Fish-eye Lens with an equisolid angle projection at each sampling point ($n = 120$) to estimate canopy openness, LAI, and light transmittance. We re-took photographs in the mid-summer of 2020 (7/30 – 8/1) at five randomly selected sampling points in each of the four plots. These datasets were used to look for spatial autocorrelation and generalized relationships between aboveground and below-ground measurements collected during 2019 and 2020. Photograph processing details can be found in Appendix 1.

GROUND COVER

Ground cover was also quantified in the early summer of 2019. A 1-m long 10-rod point counter was used in the four cardinal directions from each sampling point to estimate the ground cover at < 50 cm in height (Caratti 2006). These data were used to assess spatial autocorrelation and identify

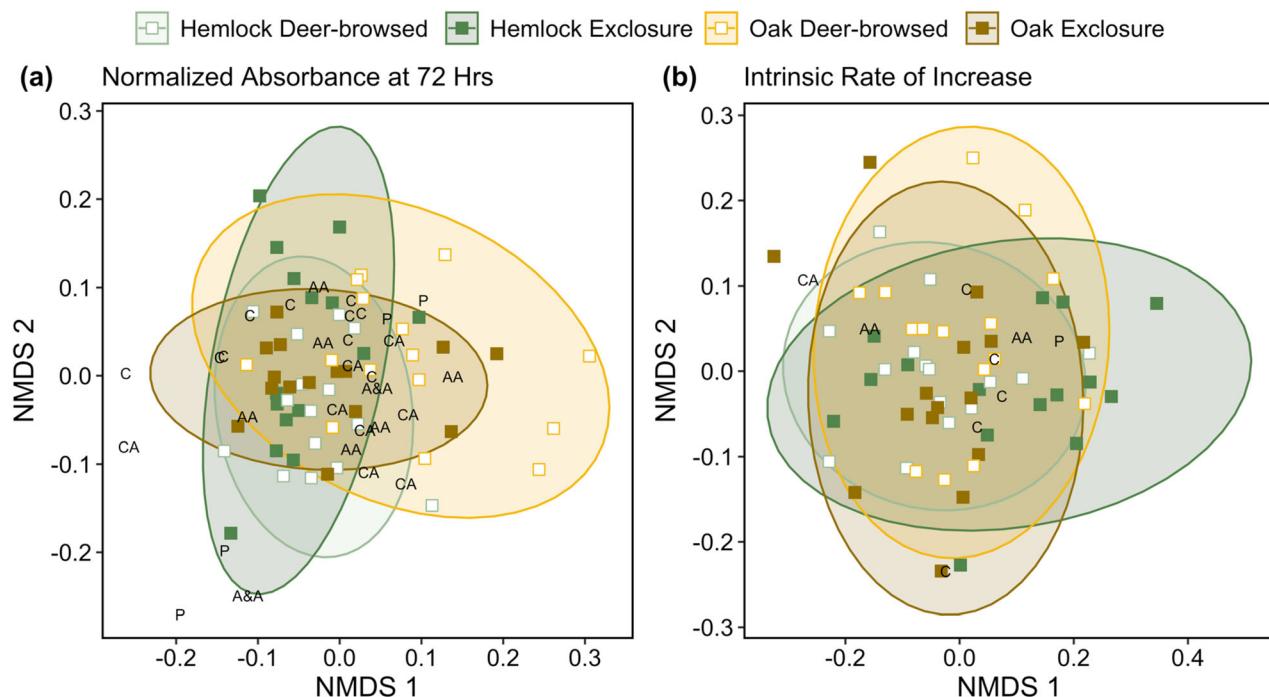


Figure 3. Nonmetric multidimensional scaling (NMDS) for hemlock-dominated (green squares) and oak-dominated (yellow squares) stands, with (deer-browsed, unfilled squares) and without (exclosure, filled squares) deer, using community-level physiological profiling ($n = 15$ per plot). NMDS plots include **a** normalized absorbance data (Garland and Mills 1991) at ~ 72 h of incubation and **b** the intrinsic rate of increase. Carbon substrates are identified as gray letters, with C = carbohydrates, P = polymers, CA = carboxylic and acetic acids, AA = amino acids, and A&A = amines and amides.

relationships between aboveground and below-ground measurements collected that year. Ground cover was reassessed in the summer of 2022, along 11.3 m transects in the four cardinal directions from the center of each sampling point (adapted from Finley and others 2007). Ground cover presence/absence was recorded at each pace (~ 0.5 m) along the transects, as was the occurrence of hemlock seedlings and saplings, and hay-scented fern, which is an indicator of intensive deer browsing in eastern U.S. temperate forests (Reed and others 2022).

In 2022, deer impacts on woody regeneration were also assessed. The number of woody seedlings was quantified within a 4-m² circle centered around each sampling point and classified according to height size classes (2 to 30 cm, 30 to 90 cm, 90 cm to 1.5 m, and > 1.5 m). To estimate current regeneration potential, seedling classes were then weighted according to height and compared to thresholds for stands experiencing either “low” or “high” deer browsing (Finley and others 2007). We summarized this data as the percentage of sampling points within the plot that had enough seedlings of the appropriate height to achieve regeneration in the presence of low deer impacts, as currently experienced in the exclosure plots, or high deer impacts, as currently experienced in the deer-browsed plots. A follow-up assessment of seedling composition was performed in 2024, by identifying all seedlings < 1 m in height within a 4-m² circle at each sampling point.

SOIL AND LITTER POOLS

In June of 2019, we collected soil from the top 5 cm of each sampling point. Fresh soils were passed through a 2-mm sieve and subsampled for a subset of sampling points (n = 15 per plot) for community-level physiological profiling (details below). A subsample from all sampling points was also weighed and dried at 105 °C for gravimetric water content (GWC). The rest of the soil was air-dried, ground, and put through a 0.251-mm sieve for soil organic matter (SOM) using loss on ignition (Nelson and Sommers 1996) and stocks of total carbon (TC) and total nitrogen (TN) using dry combustion (Perkin-Elmer 2400 Series CHNS/O elemental analyzer). Stocks of C and N were calculated using bulk density estimates for the top 5 cm of Wellsboro channery loam (0.82 g cm⁻³) and Lordstown channery loam (0.58 g cm⁻³) (Soil Survey Staff, NRCS, last accessed 07/2024). In June of 2021, we collected litter from a 287-cm² circular area at each

sampling point. The litter was dried at ~ 55 °C to estimate litter biomass (g m⁻²).

COMMUNITY-LEVEL PHYSIOLOGICAL PROFILING

Community-level physiological profiles (CLPPs) identify shifts in microbial communities and their metabolic functional diversity by measuring activity (that is, accumulated respiration) across C substrates (Li and others 2004; Barros and others 2023). For CLPPs we used BIOLOG Eco-plates (Biolog Inc., Hayward, CA), which contain 31 C sources in triplicate, including 6 biochemical groups (carbohydrates, amino acids, carboxylic acids, amines, and phenols). Soil was placed in Butterfield’s phosphate buffer (D590, Dilu-Lok II™, Butterfield’s Buffer), shaken (30 min.), and left to set for one hour before the supernatant was diluted to 0.1% by weight, and 100 µL was placed in each well. We ran samples in triplicate and incubated at 20 °C (Precision Dual Program Illuminated Incubator) under dark, humid conditions. Control Eco-plates were run with buffer-only dilutions and showed no significant color development.

Absorbance was measured every 12 to 24 h at 592 nm on a 96-well plate reader (VICTOR Nivo Multimode Microplate Reader, PerkinElmer) across a 6-day incubation period. We assessed absorbance values at the mid-way point (~ 72 h). To minimize the effects of inoculum density on color development, we normalized absorbance data (Garland and Mills 1991) by calculating the average well-color development at 72 h (AWCD_{72hours}). Kinetic analysis was also performed on each absorbance curve (n = 9 measurements) using the growthcurve package (version 0.3.1, Sprouffske 2020) in R (version 4.3.1, R Core Team 2023). This package fits a curve based on a logistical equation, from which we extracted r, the intrinsic rate of increase. Only curves with a significant fit (p < 0.05) were retained for data analysis.

STABILIZATION AND DECOMPOSITION OF STANDARDIZED LITTER

We use the Tea Bag Index (TBI), developed by Keuskamp and others (2013), to assess decomposition using standard litter sources, green tea (*Camellia sinensis*) and rooibos tea (*Aspalathus linearis*). Tea bags (that is, nonwoven tetrahedron-shaped polypropylene tea bags, Lipton, Unilever) were buried in pairs to 5 cm at all sampling points in the summer of 2019, and at five randomly se-

Table 1. Results From Analysis of Variance (ANOVA) Tests on Soil Organic Matter (SOM, %), Soil Total Carbon (TC, %), Soil Carbon Stock (C Stock, Mg C ha⁻¹), Soil Total Nitrogen (TN, %), Soil TC:TN Ratio, Gravimetric Water Content (GWC, g 100 g⁻¹ soil), Litter Biomass (g m⁻²), and Standard Litter Stabilization (S, unitless)

Soil Pools	Date	Hemlock				Oak				ANOVA P-values				
		Browsed		Exclosure		Diff (%)	Browsed		Exclosure		Forest	Deer	Int.	
		M	(SE)	M	(SE)		M	(SE)	M	(SE)				
SOM	2019	47.9	(3.8)	53.5	(4.3)	11.0	38.7	(3.7)	52.0	(7.0)	29.3	.248	.042*	0.40
Soil TC	2019	24.5	(1.8)	27.9	(2.0)	12.8	22.2	(2.0)	27.8	(3.6)	22.4	.621	.056	0.63
Soil Carbon Stock ^{††}	2019	71.1	(5.3)	80.8	(5.9)	12.8	91.2	(8.3)	114.1	(14.8)	22.4	.057	.345	0.62
Soil TN	2019	1.2	(0.1)	1.4	(0.1)	15.7	0.9	(0.1)	1.2	(0.2)	30.4	.011*	.015*	0.60
Soil Nitrogen Stock	2019	3.4	(0.3)	4.0	(0.3)	15.7	3.5	(0.3)	4.8	(0.7)	30.4	.209	.010**	0.33
Soil TC:TN	2019	21.2	(0.6)	20.5	(0.6)	3.2	25.3	(0.5)	24.2	(0.6)	4.5	< .001***	.156	0.72
GWC	2019	65.0	(2.1)	66.9	(1.9)	2.8	58.7	(2.1)	70.2	(3.9)	17.8	.582	.017*	0.08
Litter Biomass ^{††}	2020	15.9	(0.7)	13.4	(0.8)	17.4	15.0	(1.1)	12.8	(0.6)	15.8	.364	.001***	0.73
S	2019 ^{††}	0.21	(0.01)	0.18	(0.01)	15.5	0.20	(0.01)	0.20	(0.01)	1.2	.622	.049*	0.12
	2020	0.22	(0.02)	0.19	(0.01)	16.8	0.21	(0.02)	0.16	(0.02)	26.0	.366	.025*	0.67
	2021	0.20	(0.04)	0.18	(0.02)	11.2	0.22	(0.01)	0.25	(0.01)	12.4	.046*	.855	0.25
	2022	0.27	(0.01)	0.25	(0.03)	8.8	0.31	(0.02)	0.29	(0.02)	7.4	.068	.290	0.99
Average ^{††}		0.22	(0.01)	0.19	(0.01)	12.5	0.21	(0.01)	0.21	(0.01)	3.7	.506	.035*	0.29

Means (M) and standard errors (SE) are provided for each plot, along with percentage differences (Diff %) between treatments in each stand. P-values are provided for forest-type effects, deer treatment effects, and interactions. The table was constructed using the *rempsc* package (Thériault 2023). [†] Full dataset for plot. When spatial autocorrelation was present, a randomly selected set of sampling points was selected for statistical analysis. Sampling points were geometrically further apart than the estimate of spatial autocorrelation.. ^{††} Used Aligned Rank Transformed data, due to a violation of the unequal variances. Assumption * p < .05, ** p < .01, *** p < .001

lected sampling points within each plot in the summer of 2020, 2021, and 2022. Burial was not as deep as recommended (8 cm) due to the rocky soils. Tea bags were incubated in soils between 82 and 98 days, and after collection, roots and soil particles were removed. Tea was dried between 55 and 60 °C for 48 h to obtain mass loss.

The mass loss of the two teas was used to calculate a stabilization factor (S , unitless) and decomposition rate constant (k , d^{-1}) for each sampling point. Equations for calculating S and k can be found in Keuskamp and others (2013). S is a measure of the chemically labile fraction (that is, hydrolyzable) that remains after incubation and is assumed to be incorporated into the recalcitrant pool. In other words, S is a measure of the soil environment's ability to store C. Bags with visible holes due to large roots or macrofauna (Egginton and others 2020), were not included in the final analyses.

CARBON FLUXES

We measured soil respiration and CH_4 uptake in July of 2020, and in June, July, and August of 2021 at 5 sampling points, paired with tea bags within each plot. Fluxes were measured using a LiCor $\text{CH}_4/\text{CO}_2/\text{H}_2\text{O}$ Trace Gas Analyzer (LI-7810, LiCor Biosciences) with a LiCor Survey Smart Chamber (8200-01S, LiCor Biosciences). Soil collars (287 cm^2) were sampled for three consecutive days in each month in the late morning and late afternoon, for a total of 6 samples per sampling event per sampling point, which were subsequently averaged for each month-year before data analyses. Litter was left inside the collars for sampling and collected in August to measure final litter biomass (g m^{-2}). Soil temperature, moisture, and conductivity were also collected (HydraProbe, Stevens), and flux rates were calculated in SoilFluxPro™ software (ver. 4.2.1) using an exponential function.

STATISTICAL ANALYSIS

All statistical analyses were conducted in R (version 4.3.1, R Core Team 2023). We used the Moran's I autocorrelation index in the ape package (Gittleman 1990; Paradis and Schliep 2019) and permutation tests in the spdep package (Pebesma and Bivand 2023) to test for spatial structure within our plots (H1) for canopy metrics, ground cover, k , S , and soil property data (that is, SOM, GWC, TC, TN, and stocks) collected in 2019, and litter biomass collected in 2021. When spatial autocorrelation was significant, semivariogram analysis was used

with weighted least squares modeling to estimate the *beta*, *range*, *sill*, and *nugget* in the geoR package (version 1.9–2, Ribeiro and others 2022). *Beta* estimates the mean for the variable, the *range* estimates the scale over which spatial autocorrelation occurs, while the *nugget:sill* ratio determines the proportion of unexplained variance at scales finer than the closest sampling points (that is, 1 m) and includes measurement error.

We also used spatial autocorrelation as an assessment of sampling point independence. When the range of spatial autocorrelation was $< 6.5 \text{ m}$, we used the spatialEco package (Evans and Murphy 2023) to randomly select sampling points geometrically further apart than the estimate of spatial autocorrelation. This left us with a maximum of 14 randomly selected sampling points that could be used as replicates within a plot to run the statistical analyses described below. When the range of spatial autocorrelation was $> 6.5 \text{ m}$, we described plot means and variances, but did not test for statistical differences among these means, due to the small sample size (< 14 sampling points) that would be available as replicates within a plot. No spatial autocorrelation analyses were conducted on soil respiration and CH_4 uptake measurements. However, the 5 sampling points selected in each plot per year were $> 7 \text{ m}$ apart, and all but one sampling point pair were $> 10 \text{ m}$ apart.

We used factorial analysis of variance (ANOVA) tests to identify differences in mean plot C pools (H2) and fluxes (H4) between forest types and deer browsing treatments. When a significant interaction occurred, we used orthogonal contrasts in the afex package (version 1.3–0, (Singmann and others 2023) with Bonferroni correction. Repeated measure ANOVAs were used for C pools (H2) and fluxes (H4) to test for interactive effects of forest type and deer browsing when multiple samples were collected from the same plots across time (years or months). Statistical assumptions were evaluated with Levene's tests and Q-Q plots. When assumptions weren't met, we used align-and-rank data with nonparametric factorial ANOVAs (Wobbrock et al. 2011) in the ARTool package (Kay and others 2021).

We used CLPPs to determine whether deer exclusion affects the dispersion of heterotrophic bacterial profiles in a plot (H3). To visualize differences in CLPPs, AWCD_{72hours} and r values were ordinated with non-metric multidimensional scaling (NMDS). Permutational multivariate ANOVA (PERMANOVA) and multivariate homogeneity of group dispersion (H3) were used to test for the effects of forest type and deer browsing. All tests were

Table 2. Results From Analysis of Variance (ANOVA) Tests on Standard Litter Decomposition (d^{-1}) From 2019 – 2022, and Soil Respiration ($\text{CO}_2 \mu\text{g m}^{-2} \text{s}^{-1}$) and Soil Methane Uptake ($\text{CH}_4 \text{ nmol m}^{-2} \text{s}^{-1}$) From 2020 – 2021

Carbon Flux & Date	Hemlock						Oak						ANOVA P-values		
	Browsed		Exclosure		p [‡]	Browsed		Exclosure		p [‡]	Forest	Deer	Int.		
	M	(SE)	M	(SE)		M	(SE)	M	(SE)						
Litter Decomposition (d^{-1})															
2019	0.021	(0.001)	0.017	(0.001)	.106	0.016	(0.001)	0.020	(0.002)	.085	.523	.983	.006**		
2020	0.014	(0.001)	0.012	(0.001)		0.012	(0.001)	0.016	(0.004)		.398	.652	.109		
2021	0.029	(0.010)	0.017	(0.002)		0.017	(0.001)	0.020	(0.005)		.320	.288	.083		
2022	0.014	(0.002)	0.013	(0.002)		0.016	(0.003)	0.015	(0.002)		.315	.752	.928		
Average	0.217	(0.010)	0.191	(0.007)	.011*	0.214	(0.006)	0.206	(0.008)	.277	.525	.320	.003**		
Soil Respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$)															
Jul-2020	8.16	(0.69)	4.55	(0.57)	.258	6.89	(1.80)	10.72	(2.48)	.217	.144	.945	.033*		
Jun-2021	13.15	(1.27)	6.79	(1.05)	.019*	6.56	(1.23)	10.49	(2.25)	.174	.356	.437	.004**		
Jul-2021	12.82	(1.90)	7.48	(1.14)	.167	7.55	(1.50)	13.95	(3.09)	.084	.773	.799	.011*		
Aug-2021	11.73	(1.89)	6.10	(0.95)	.111	7.17	(1.48)	12.65	(2.87)	.124	.614	.969	.011*		
Jul-2020 & 2021	10.49	(1.23)	6.01	(0.78)	.046*	7.22	(1.11)	12.33	(1.94)	.020*	.260	.812	.001***		
Soil Methane Uptake ($\text{nmol m}^{-2} \text{s}^{-1}$)															
Jul-2020	– 1.46	(0.34)	– 1.79	(0.48)		– 1.95	(0.32)	– 3.46	(0.56)		.024*	.051	.198		
Jun-2021	– 2.43	(0.39)	– 2.14	(0.35)		– 1.94	(0.19)	– 2.66	(0.21)		.974	.485	.112		
Jul-2021	– 4.34	(1.06)	– 3.48	(0.70)		– 3.00	(0.41)	– 4.01	(0.25)		.559	.914	.188		
Aug-2021	– 3.95	(1.11)	– 3.17	(0.66)		– 2.96	(0.32)	– 4.43	(0.25)		.843	.622	.115		
Jul-2020 & 2021	– 2.90	(0.71)	– 2.63	(0.49)		– 2.48	(0.30)	– 3.73	(0.30)		.485	.309	.123		

Means and standard errors (S.E.) are provided for each plot. P-values are provided for forest-type effects, deer treatment effects, and interactions. The table was constructed using the *rempscyc* package (Thériault 2023). [†] Pairwise comparisons were used when a significant interaction was present. A Bonferroni correction was used to estimate the p-value

* p < .05, ** p < .01, *** p < .001

performed using Bray–Curtis dissimilarity matrices in the vegan package (version 2.6–4, (Oksanen and others 2022) and the pairwise Adonis package (version 0.4.1., Martinez Arbizu 2017) with a Bonferroni adjustment for pairwise comparisons.

RESULTS

Leaf Area Index

In 1997, there was a 12% difference in LAI between the hemlock and oak stands, with the hemlock stand having a higher LAI compared to the oak stand ($t_{66} = 3.39, p = 0.004$). However, in 2014 ($t_{66} = -4.50, p < 0.001$) and 2023 ($t_{66} = -3.29, p = 0.005$), the oak stand had a higher LAI than the hemlock stand (Figure 2a). This was not due to a decrease in the LAI in the hemlock stand but rather a 26% increase in the LAI in the oak stand by 2014, resulting in a 14% difference between stands. There was no significant difference inside and outside the exclosures within any year.

GROUND COVER

Hemlock (mean \pm s.d.: 19.45 ± 8.33 no. seedlings m^{-2}) and oak exclosures (5.14 ± 4.10 no. seedlings m^{-2}) had higher densities of saplings compared to the hemlock (7.69 ± 5.27 no. seedlings m^{-2}) and oak (2.03 ± 2.47 no. seedlings m^{-2}) deer-browsed plots. While the hemlock exclosure had higher densities of saplings between 0.02 and 0.30 m in height, 59% of the oak exclosure sampling points had saplings above 0.9 m in height, compared to 7% of hemlock exclosure sampling points (Figure 2b). In 2024, more than 70% of saplings, less than 1 m in height, were maple species in both exclosures. Only 9% of saplings in the oak exclosure were oak species, and 1% of saplings in the hemlock exclosure were hemlocks, with 12% being pine.

The hemlock deer-browsed plot had more ground cover than the exclosure plot due to the cover of hay-scented fern (Figure 2c). Hay-scented fern was 7 \times higher outside ($43.34 \pm 18.22\%$) the exclosure compared to inside ($5.86 \pm 8.53\%$). Excluding hay-scented fern, ground cover was higher inside both exclosures than in respective deer-browsed plots (Figure 2d).

Considered areas of low-deer-impact (Finley and others 2007), we found 45% and 76% of sampling points were sufficiently vegetated for regeneration to proceed in the oak and hemlock exclosures, respectively. However, if deer were reintroduced at current watershed densities, only 28% (oak

exclosure) and 1% (hemlock exclosure) of sampling points would have enough vegetation to proceed with regeneration (that is, escape browsing), suggesting low regeneration potential, even after 24 years of exclusion.

(H1) Release from Deer Browsing Allows For The Development of Fine-Scale Patchiness of Soil C Pools.

The presence of spatial autocorrelation in properties varied among the four sampling plots (Appendix 2). Both hemlock plots had positive spatial autocorrelation (clumping) in percent ground cover but lacked spatial autocorrelation in canopy LAI, soil, and litter properties. Spherical variogram models estimated a ground cover *range* at 16.5 and 17.1 m, for the hemlock exclosure and deer-browsed plots, respectively. The oak deer-browsed plot lacked spatial autocorrelation for all properties except LAI. LAI had an estimated *range* of 23.7 m. The oak exclosure had positive spatial autocorrelation in soil TN, N stocks, and GWC. Given all three of these soil properties were correlated with soil TC, C stocks, and SOM, we also evaluated spherical variograms for these properties. Spherical variogram models estimated *ranges* between 5 and 6 m for soil TN, TC, stocks, and SOM. Soil GWC produced a plot-wide trend. There was also no significant autocorrelation for LAI, percent ground cover, *S*, or litter biomass in the oak exclosure.

(H2) Release from Deer Browsing Improves Long-Term Soil C Storage.

Soil Pools

There were positive correlations between SOM, TC, TN, and GWC across sampling points within each plot (Appendix 3). A subset of randomly selected data from the oak exclosure ($n = 14$) was used to test for differences in soil properties due to the spatial autocorrelation in this plot (described above). As predicted, mean SOM ($f_{1,100} = 4.26, p = 0.042$) was higher inside of exclosures compared to paired deer-browsed plots, and mean soil TC ($f_{1,100} = 3.75, p = 0.056$) was marginally higher inside exclosures compared to paired deer-browsed plots (Table 1). It should be noted that mean C stocks were also significantly higher inside the exclosures ($f_{1,100} = 4.04, p = 0.047$), but not after transforming the data (aligned rank transformed:

$F_{1,100} = 0.90, p = 0.345$) for non-equal variances (Levene's test: $f_{3,100} = 3.62, p = 0.016$) caused by the disproportionately large variance in the oak enclosure. In the oak enclosure, C pools were lower at sampling points with a more open canopy (Appendix 3). Like C pools, soil TN ($f_{1,100} = 6.09, p = 0.015$) and N stocks ($f_{1,100} = 6.98, p = 0.010$) were higher inside exclosures compared to paired deer-browsed plots. In all cases, there was a greater difference between the plot means in the oak stand compared to the hemlock stand. While slightly higher inside the exclosures, soil TC:TN ratios were not influenced by deer browsing but were higher in the hemlock stand than the oak stand ($f_{1,100} = 38.29, p < 0.001$).

Litter Biomass and Stabilization

In 2021, litter biomass followed patterns opposite those found in SOM and TC stocks in 2019. Litter biomass was significantly higher in deer-browsed plots than exclosures (aligned rank transformed: $f_{1,116} = 11.66, p = 0.001$). Similarly, mean S was higher in deer-browsed plots compared to the paired exclosures in 2019 (aligned rank transformed: $f_{1,101} = 3.97, p = 0.049$) and 2020 ($f_{1,16} = 6.11, p = 0.025$). However, in 2021, the mean S was only higher in the oak stand compared to the hemlock stand ($f_{1,15} = 4.74, p = 0.046$), and no differences were found in 2022.

(H3) Release from Deer Browsing Enhances Stand-Level Variation in Heterotrophic Bacteria.

CLPPs were used to examine the variation in heterotrophic bacterial community activity across sampling points within each plot. There weren't significant differences in dispersion for $AWCD_{72\text{hours}}$ values among the four plots (permutations = 999, $F_{3,56} = 1.54, p = 0.239$). However, there were significant differences between the oak control plot, and both the hemlock control plot (Pairwise Adonis $adj. p = 0.006$) and the hemlock enclosure plot (Pairwise Adonis $adj. p = 0.006$). All soil pools (that is, SOM, soil TC and TN, GWC, with $p < 0.01$; and C and N stocks with $p < 0.05$) were related to the 2D NMDS plot (Figure 3a, stress 0.175, Sheppard's plot non-metric $R^2 = 0.969$, Linear Fit $R^2 = 0.873$).

A subset of nine C sources (that is, carbohydrates = 5, amino acids = 2, Polymer = 1, and carboxylic acid = 1) was used to compare r across plots. There was no significant difference in dispersion among the four plots (permutations = 999,

$F_{3,56} = 1.33, p = 0.31$) and no difference between plots. Soil properties (that is, SOM, TC, TN, and GWC, all $p < 0.01$) and S ($p = 0.02$) were significantly related to the 2D NMDS plot (Figure 3b, stress 0.150, Sheppard's plot non-metric $R^2 = 0.978$, Linear Fit $R^2 = 0.9$).

(H4) Release from deer browsing leads to ecosystem-specific effects on soil C fluxes.

Decomposition of Standardized Litter

The decay constant, k , varied from 0.008 to 0.050 d^{-1} across all measurements and was highest on average in 2021 (Table 2). Across most years, mean k was higher in the deer-browsed plot compared to the exclosure of the hemlock stand, but higher in the exclosure plot compared to the deer-browsed plot of the oak stand. These differences weren't statistically significant for each year, but when averaged across years, there was a statistically significant effect of deer in the hemlock stand ($t_{100} = -2.82, p = 0.011$).

Soil Respiration

Like k , mean soil respiration rates ($\text{CO}_2 \mu\text{mol m}^{-2} \text{s}^{-1}$) were higher in the deer-browsed plot compared to the exclosure of the hemlock stand, but lower in the deer-browsed plot compared to the exclosure of the oak stand. There was an interaction between forest type and deer browsing across all year-months sampled. However, the only statistically significant differences between plots were in June of 2021 ($t_{16} = -2.95, p = 0.019$). However, when independent sampling points were combined across years (July 2020 & July 2021), there was a statistically significant difference in deer treatments for both the hemlock ($t_{36} = -2.37, p = 0.046$) and oak ($t_{36} = 2.71, p = 0.021$) stands. Soil respiration was not related to soil moisture, soil temperature, or soil conductivity, but was related to litter biomass across all sampling plots (Figure 4a, using July datasets from 2020 and 2021: $t_{38} = 4.21, p < 0.001, adj. R^2 = 0.30$) and by forest stand (oak: $t_{18} = 4.00, p < 0.001, adj. R^2 = 0.44$; hemlock: $t_{18} = 3.35, p = 0.004, adj. R^2 = 0.35$).

Soil Methane Uptake

All sampling points exhibited CH_4 uptake during the study and there was no interaction between forest type and deer browsing for CH_4 uptake across

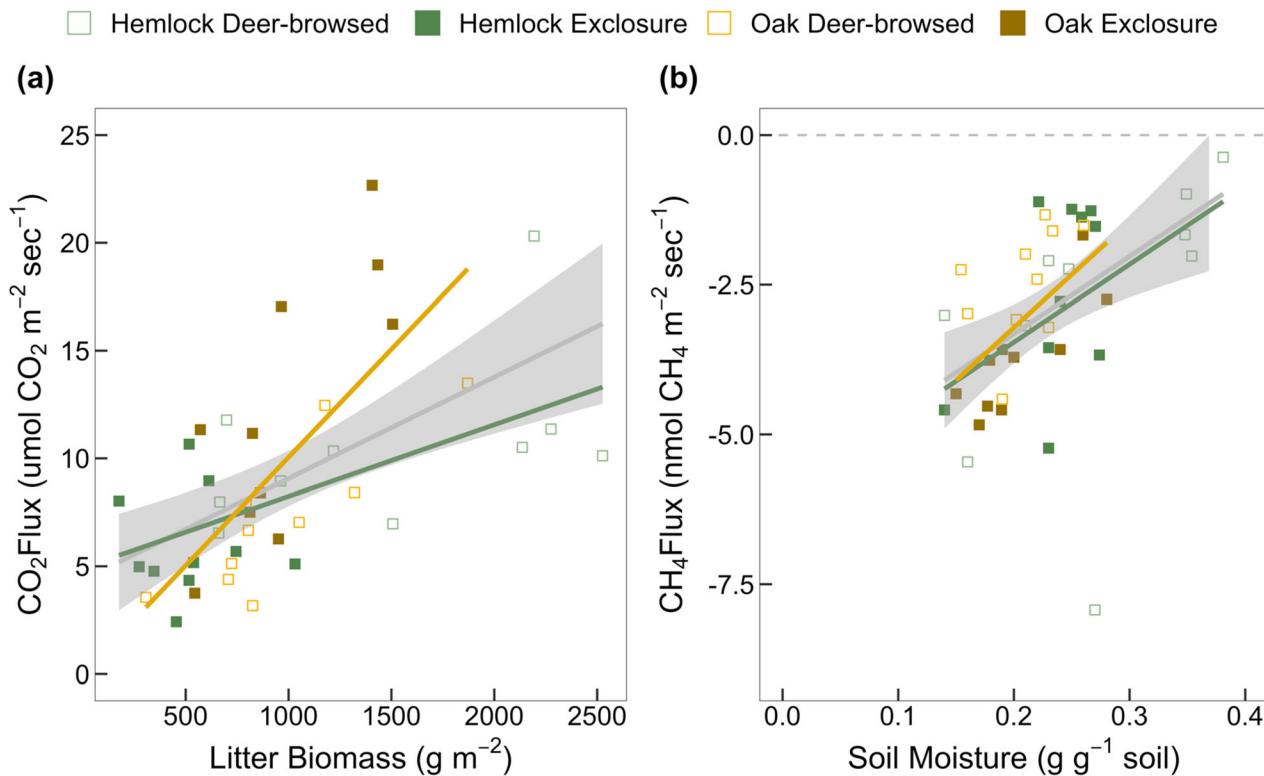


Figure 4. **a** Soil respiration (CO_2 , $\mu\text{mol m}^{-2} \text{s}^{-1}$) as a function of litter biomass (g m^{-2}) and **(b)** soil methane uptake (CH_4 , $\text{nmol m}^{-2} \text{s}^{-1}$) as a function of soil moisture (% v:v) across 2020 and 2021 in hemlock-dominated stands (green) and oak-dominated stands (yellow), with (deer-browsed, open symbols) and without (exclosure, closed symbols) deer. Plots were created in the ggplot2 package (Wickham 2016).

all year-months sampled. There was significantly more uptake in exclosures compared to deer-browsed plots in July of 2020 ($f_{1,16} = 6.18$, $p = 0.024$), and marginally more uptake in the oak stand compared to the hemlock stand ($f_{1,16} = 4.45$, $p = 0.051$). There were no statistically significant differences in 2021; however, mean differences in the oak stand were consistently stronger than in the hemlock stand. CH_4 uptake was not related to soil temperature or litter biomass but was positively related to soil moisture across all sampling plots (Figure 4b, July datasets from 2020 and 2021: $t_{38} = 3.47$, $p = 0.001$, adj. $R^2 = 0.22$), and by forest stand (oak: $t_{18} = 3.01$, $p = 0.008$, adj. $R^2 = 0.30$; hemlock: $t_{18} = 2.21$, $p = 0.040$, adj. $R^2 = 0.17$).

DISCUSSION

Synopsis

We found unique patterns in C dynamics with deer exclusion across the two forest stands studied. Excluding deer from the oak stand led to higher stand-level soil respiration, decomposition rates,

CH_4 uptake, (significance varied by sampling period), and SOM. Deer exclusion in the oak stand also promoted fine-scale spatial structure in soil C pools, likely stimulating habitat complexity as forest recovery continues. Excluding deer from the hemlock stand led to higher SOM, and higher CH_4 uptake in the first year of sampling. However, the effect was much lower than in the oak stand, and fine-scale spatial structure in soil C pools was not evident. Further, soil respiration and decomposition rates were higher with deer presence. We hypothesize that the trajectories found in the hemlock stand are the result of interactions between deer browsing and the effects of HWA on the regeneration potential of hemlocks.

(H1) Release From Deer Browsing Allows For The Development of Fine-Scale Patchiness of Soil C Pools.

While some studies have shown that deer grazing can increase the heterogeneity of the vegetative community (Chollet and others 2021a) and below-ground biogeochemistry (Ibañez-Alvarez and oth-

ers 2022) across plots, little attention has been paid to the patchiness of soil properties within plots (Murray and others 2013). Fine-scale patterning is essential for understanding forest recovery (Fraterrigo and others 2005), which can affect niche availability and competition among plants and soil biota (Hutchings and others 2003). We predicted that excluding deer would increase woody plants through sapling regeneration and, thus, the patchiness of soil C pools; previous studies have shown that increases in woody plants can increase the patchiness of microclimates and soil resources by creating fertility islands (Schlesinger and others 1990).

As predicted, we found spatial structure in soil C pools within the oak enclosure, creating resource patchiness. Variation in soil C pools across sampling points was not correlated with variation in ground cover (< 0.5 m height) but negatively correlated with canopy openness, an integrative measure of the mature canopy and understory development. The lack of relationship between C pools and recent seedling growth highlights the slow nature of autochthonous C accumulation. Others have attributed the development of patchiness in forest soil C pools to the accumulation of litter from the canopy (Suchewaboripont and others 2015) and the acceleration of soil respiration and mineralization in canopy gaps (Tong and others 2024).

We did not find spatial structure in soil C pools in the hemlock enclosure. This was likely due to the low regeneration of the sapling layer, caused by high hemlock sapling mortality due to the HWA infestation (Orwig and Foster 1998) and/or intolerance of replacement species to acidic soils common in hemlock stands (Sharpe and Halofsky 2004). Regeneration may have also been delayed by low light availability (Tremblay and others 2007), as there was little change in LAI between 1997 and 2023. This was unexpected, as hemlock mortality generally occurs within 2 to 12 years of HWA infestation (Mayer and others 2002). While mortality can be patchy, surviving trees are often significantly defoliated (Orwig and Foster 1998), and mixed hardwoods establish (Orwig and Foster 1998; Small and others 2005).

However, HWA infestation may not always lead to canopy openings within initial decades. For example, the average LAI in our hemlock stand with known HWA infestation ($4.3 \text{ m}^2 \text{ m}^{-2}$) was similar to stands with suspected (4.46 to $4.52 \text{ m}^2 \text{ m}^{-2}$, Spaulding and Rieske 2010), low (Table 2 in Zhao and others 2011; Kim and others 2017) and no HWA infestation (3.0 to $4.2 \text{ m}^2 \text{ m}^{-2}$, Hadley and Schedlbauer 2002). This is possible if mature

replacement species (for example, red maple, northern red oak, chestnut oak, and American beech, *Fagus grandifolia*) offset needle mortality in LAI measurements.

(H2) Release from Deer Browsing Improves Long-Term Soil C Storage.

Reforestation and natural forest management are estimated to have great potential for climate mitigation through C sequestration (Fargione and others 2018). However, we are still uncertain about how ungulate exclusion will affect long-term forest soil C pools. Ungulate exclusion can lead to soil C gains (Stephan and others 2017; Chollet and others 2021b) through reduced erosion by browsing and trampling (Mohr and Topp 2001), or deposition of urine and dung (Mohr and others 2005). Exclusion can also have no effect (Kolstad and others 2018), or effects can vary by forest and across time (Wardle and others 2001; Stark and others 2010). Forest-specific selective browsing can shift vegetative community composition, producing litter that is less advantageous (that is, lignified) or more advantageous (that is, increasing foliar nutrient concentrations) for detritivores (Tanentzap and Coomes 2012).

We predicted that deer exclusion would increase mean C pools in our forests through inputs from recalcitrant woody sapling regeneration. As predicted, deer exclusion led to higher C storage (significance level varied by C pool and analysis used), but the magnitude of this response was smaller in the hemlock stand compared to the oak stand. This may reflect the low regeneration potential of the sapling layer in the hemlock stand. However, C pools were comparable between the two enclosures, and others have found that these species can reach similar soil C stocks (Finzi and others 1998). Instead, the difference appeared to be due to larger soil C pools in the hemlock deer-browsed plot compared to the oak deer-browsed plot.

The mechanisms driving the difference in the effect size between stands deserve further attention. Losses in soil C pools where browsing has occurred may be delayed near hemlocks compared to oaks due to litter quality, as woody evergreens have slower return rates than woody deciduous species (Cornwell and others 2008). Hemlock stands also develop cooler, moister microclimates (Ellison and others 2005) and deep acidic soil layers (Lovett and others 2006) that can slow decomposition. Litter biomass and litter stabilization were higher in the browsed plot compared to the

exclosure and could, with time, lead to soil C gains without deer exclusion. However, our results contradict others who found no effect of ungulate exclusion on stabilizing labile C (Kolstad and others 2018; Ibañez-Alvarez and others 2022).

Similar to others, we found minimal changes in soil TC:TN ratios with herbivore exclusion (Kolstad and others 2018; Ibañez-Alvarez and others 2022). Soil TC:TN ratios were lower in the hemlock stand compared to the oak stand. This contrasts with single species plots in the Catskill Mountains, NY, USA, where hemlock stands had higher soil TC:TN ratios than oak stands (Lovett and others 2004). However, others have found that forests infested with HWA can have lower soil TC:TN ratios compared to uninfected hemlock areas, and their ratios (mean range across sites: 22.2 to 25.5) were in line with ratios found in our study (Orwig and others 2008).

(H3) Release from deer browsing enhances stand-level variation in heterotrophic bacteria.

Deer browsing can negatively affect tree species diversity, evenness, and richness (Horsley and others 2003; Habeck and Schultz 2015; Reed and others 2022), particularly in height classes accessible to deer (Simončič and others 2019). We predicted that improved woody sapling richness, caused by release from browsing, would lead to higher belowground dissimilarity in heterotrophic bacterial communities. However, deer exclusion didn't affect the dispersion of our CLPP measurements. Limitations of the CLPPs method may obscure biological effects, such as the bias of the technique towards culturable and fast-growing groups. It is also possible that substrates did not represent unique microbial habitats in our plots (Garland 1999). For example, fungal communities, which were visible (J. Moon personal observations) but not measured in CLPPs, can be strongly affected by browsing (Burke and others 2019; Zhu and others 2022; Magarzo and others 2024). Microbes with direct symbiotic associations and those that play a functional role in the initial stages of tree litter decomposition may be most sensitive to our stage of stand reorganization. We suggest that future work use techniques that are more inclusive, such as microbial eDNA metabarcoding (for example, Kadowaki and others 2023).

(H4) Release from Deer Browsing Leads To Ecosystem-Specific Effects on Soil C Fluxes.

Unlike soil C pools, we expected soil C fluxes to be context-specific (Forbes and others 2019), responding to local conditions, which can exhibit high spatial variation in forested ecosystems. Ungulate exclusion can have positive (some litter types in Ramirez and others 2021), negative (Kielland and others 1997), or neutral (Ellis and Leroux 2017; Kolstad and others 2018; Ibañez-Alvarez and others 2022) effects on litter decomposition rates. Similarly, some have found herbivores stimulate soil respiration (Chen and others 2013), while others have reported that decomposition and soil respiration vary by vegetation type and herbivore compilation (Harrison and Bardgett 2003; Risch and others 2013). Our results support the context-specific nature of these dynamic C fluxes.

Soil Respiration and Decomposition

Effects of browsing were stand-specific for litter decomposition and soil respiration. Rates were generally higher with browsing in the hemlock stand but higher with deer exclusion in the oak stand. These patterns coincided with the percent ground cover found across plots. Specifically, the higher rates found in the deer-browsed plot of the hemlock stand were associated with the dense cover of hay-scented fern. Hay-scented ferns can trap litter (George and Bazzaz 1999) and can have higher N content when grown under low light conditions or with oak seedlings (Dustin and Cooper-Driver 1992; Lyon and Sharpe 2003), both of which may stimulate decomposition and soil respiration.

The specific impacts hay-scented fern will have on long-term C cycling deserve further attention in this region. It has been described as a "native invasive" and "invasive weed" in the northeastern United States (Lyon and Sharpe 1996, 2003; Fei and others 2010) and is a strong competitor in low Ca:Al ratio soils ideal for hemlock growth (Sharpe and Halofsky 2004). Hay-scented fern can limit the absorption of nutrients by herbivores through its foliar chemistry, and limit hardwood regeneration through shading and effects on light quality (Horsley 1993; Smallidge 2015). Further, it affects root growth and mycorrhizal infection rates (Lyon and Sharpe 1996) and causes nutrient imbalances in competing species such as red oak (Lyon and Sharpe 2003).

Soil Methane Uptake

While ungulate exclusion has been investigated in CH₄-producing systems (Falk and others 2015; Fischer and others 2022), our study is one of few investigating the response of CH₄ uptake to exclusion in forests. Such investigations are timely, as model estimates show temperate forests have the highest rates of CH₄ uptake compared to all other climate zones (Feng and others 2023). While the response in the hemlock stand was generally weak, CH₄ uptake significantly improved with deer exclusion in the oak stand.

Our results support the well-accepted paradigm that soil moisture is a primary driver of both CH₄ production and oxidation as it controls the diffusion of CH₄ and oxygen in soil (Liu and others 2019). However, our results also suggest that additional factors that vary with browsing might play a role in CH₄ uptake. Sampling points with similar soil moisture had higher CH₄ uptake inside the oak exclosure compared to the deer-browsed plot. Understory vegetation, which was present in the exclosure but absent from the deer-browsed plot, has been associated with a two-fold increase in CH₄ uptake in sessile oak systems (Plain and others 2019). Vegetation enhance methanotrophy directly through gas transport (Subke and others 2018; Plain and others 2019) and indirectly through transpiration, which decreases soil water content and increases soil air-filled pore space (Plain and others 2019). Further, increased root growth can reduce soil compaction, allowing for greater gas diffusion (Epron and others 2016) and uptake of ammonium, which lowers the competitiveness of microbial oxidation of ammonium versus CH₄ in soils (Dunfield and Knowles 1995). Oak roots and associated ectomycorrhiza have also been shown to cause a 40% increase in CH₄ uptake compared to bulk soils and may be related to enhanced methanotrophic communities, delivery of labile C sources, and soil pore connectivity (Subke and others 2018).

CONCLUSIONS

Our findings indicate release from deer browsing can positively affect C pools across forest stands. However, soil CH₄ uptake and development of habitat complexity may be delayed when other zoogeochemical stressors are present, as appeared to occur in the hemlock stand. This study didn't allow us to disentangle patterns driven by differences in the environmental template between stands from the zoogeochemical stress of the HWA,

due to the lack of an exclosure in a non-infested hemlock stand. However, with over 3,540 non-native insect species established in the U.S. and Canada (Yamanaka and others 2015) alone, future studies should consider the interactive effects of multiple zoogeochemical stressors when attempting to explain variation in C dynamics across ecosystems. Understanding their role in driving uncertainty in C dynamics may be useful in establishing the confidence levels on future model predictions. Further, while we found that deer exclusion enhanced soil C storage, soil CH₄ uptake, and soil habitat complexity in the oak-dominated stand, oak species are only a minor portion of established seedlings to date. Future work is needed to assess longer-term patterns with and without interventions such as thinning and fire disturbance to release oak species from competition (Iverson and others 2017).

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AUTHOR'S CONTRIBUTION

JBM, BN, and NF designed the study; JBM, ES, BN, and NF conducted fieldwork; JBM and SF conducted soil and microbial analyses; JBM, ES, BN, and NF processed datasets; JBM and NF ran statistical analyses; JBM lead writing with significant contributions from BN, ES, and NF, and edits from all authors; all authors approved the paper.

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DATA AVAILABILITY

Data has been uploaded to Dryad. <https://doi.org/10.5061/dryad.4qrfj6qmk>

Declarations

Conflict of interest The authors have no conflicts of interest to report.

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REFERENCES

- Abbas F, Merlet J, Morellet N, Verheyden H, Hewison AJM, Cargnelutti B, Angibault JM, Picot D, Rames JL, Lourtet B, Aulagnier S, Daufresne T. 2012. Roe deer may markedly alter forest nitrogen and phosphorus budgets across Europe. *Oikos* 121:1271–1278.
- Alverson WS, Waller DM, Solheim SL. 1988. Forests too deer: Edge effects in northern Wisconsin. *Conservation Biology* 2:348–358.
- Andriuzzi WS, Wall DH. 2018. Soil biological responses to, and feedbacks on, trophic rewinding. *Philosophical Transactions of the Royal Society B: Biological Sciences* 373.
- Augustine DJ, Frank DA. 2001. Effects of migratory grazers on spatial heterogeneity of soil nitrogen properties in a grassland ecosystem. *Ecology* 82:3149–3162.
- Barros DJ, Carvalho GA, de Chaves MG, Vanzela LS, Kozusny-Andreani DI, Guarda EA, Neu V, de Moraes PB, Tsai SM, Navarrete AA. 2023. Microbial metabolic activity in Amazon floodplain forest and agricultural soils. *Frontiers in Microbiology* 14:1–12.
- Beasley C, Carter DR, Coates TA, Keyser TL, Greenberg CH. 2022. Impacts of oak-focused silvicultural treatments on the regeneration layer nine years posttreatment in a productive mixed-oak southern Appalachian forest. *Journal of the Torrey Botanical Society* 149:166–180.
- Burke DJ, Carrino-Kyker SR, Hoke A, Cassidy S, Bialic-Murphy L, Kalisz S. 2019. Deer and invasive plant removal alters mycorrhizal fungal communities and soil chemistry: Evidence from a long-term field experiment. *Soil Biology and Biochemistry* 128:13–21.
- Byman D. 2011. The effects of deer exclosures on voles and shrews in two forest habitats. *Northeastern Naturalist* 18:509–520.
- Campbell JL, Gower ST. 2000. Detritus production and soil N transformations in old-growth eastern hemlock and sugar maple stands. *Ecosystems* 3:185–192.
- Canham CD, Murphy L, Hansen WD. 2024. Successional dynamics of carbon sequestration in forests of the eastern United States. *Ecosphere* 15:e4838.
- Caratti JF. 2006. Point Intercept (PO) Sampling Method. www.fs.usda.gov/treesearch/pubs/24062
- Carter DR, Fahey RT, Dreisilker K, Bialecki MB, Bowles ML. 2015. Assessing patterns of oak regeneration and C storage in relation to restoration-focused management, historical land use, and potential trade-offs. *Forest Ecology and Management* 343:53–62.
- Catovsky S, Bazzaz FA. 2000. Contributions of coniferous and broad-leaved species to temperate forest carbon uptake: A bottom-up approach. *Canadian Journal of Forest Research* 30:100–111.
- Catovsky S, Holbrook NM, Bazzaz FA. 2002. Coupling whole-tree transpiration and canopy photosynthesis in coniferous and broad-leaved tree species. *Canadian Journal of Forest Research* 32:295–309.
- Chen J, Wang Q, Li M, Liu F, Li W, Yin L. 2013. Effects of deer disturbance on soil respiration in a subtropical floodplain wetland of the Yangtze River. *European Journal of Soil Biology* 56:65–71.
- Chollet S, Baltzinger C, Maillard M, Martin JL. 2021a. Deer exclusion unveils abiotic filtering in forest understorey plant assemblages. *Annals Botany* 128:371–381.
- Chollet S, Maillard M, Schörghuber J, Grayston SJ, Martin JL. 2021b. Deer slow down litter decomposition by reducing litter quality in a temperate forest. *Ecology* 102:1–14.
- Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Pérez-Harguindeguy N, Quested HM, Santiago LS, Wardle DA, Wright IJ, Aerts R, Allison SD, Van Bodegom P, Brokvink V, Chatain A, Callaghan TV, Díaz S, Garnier E, Gurvich DE, Kazakou E, Klein JA, Read J, Reich PB, Soudzilovskaia NA, Vaieretti MV, Westoby M. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* 11:1065–1071.
- Côté SD, Rooney TP, Tremblay J-P, Dussault C, Waller DM. 2004. Ecological impacts of deer overabundance. *Annual Reviews of Ecology, Evolution, and Systematics* 35:113–147.
- Dahlberg BL, Guettinger RC. 1956. The white-tailed deer in Wisconsin. Madison, Wisconsin: Game Management Division, Wisconsin Conservation Department Technical Bulletin. 282 pp.
- Dunfield P, Knowles R. 1995. Kinetics of inhibition of methane oxidation by nitrate, nitrite, and ammonium in a humisol. *Applied Environmental Microbiology* 61:3129–3135.
- Dustin CD, Cooper-Driver GA. 1992. Changes in phenolic production in the hay-scented fern (*Dennstaedtia punctilobula*) in relation to resource availability. *Biochemical Systematics and Ecology* 20:99–106.

- Eggleton P, Griffiths H, Ashton L, Law S, Evans TA, Parr K. 2020. Not our cup of tea: the Tea Bag Index (Kueskamp et al. 2013) for assessing decomposition is problematic in most environments, due to macrofauna. *Authorea*:1–6.
- Ellis NM, Leroux SJ. 2017. Moose directly slow plant regeneration but have limited indirect effects on soil stoichiometry and litter decomposition rates in disturbed maritime boreal forests. *Functional Ecology* 31:790–801.
- Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR, Foster DR, Kloeppele BD, Knoepp JD, Lovett GM, Mohan J, Orwig DA, Rodenhouse NL, Sobczak WV, Stinson KA, Stone JK, Swan CM, Thompson J, Von Holle B, Webster JR. 2005. Loss of foundation species: Consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3:479–486.
- Epron D, Plain C, Ndiaye FK, Bonnaud P, Pasquier C, Ranger J. 2016. Effects of compaction by heavy machine traffic on soil fluxes of methane and carbon dioxide in a temperate broad-leaved forest. *Forest Ecology and Management* 382:1–9.
- Evans J, Murphy M. 2023. spatialEco. R package version 2.0–1, <[<https://github.com/jeffreyevans/spatialEco>](https://github.com/jeffreyevans/spatialEco)
- Falk JM, Schmidt NM, Christensen TR, Ström L. 2015. Large herbivore grazing affects the vegetation structure and greenhouse gas balance in a high arctic mire. *Environmental Research Letters* 10:45001. <https://doi.org/10.1088/1748-9326/10/4/045001>.
- Fargione JE, Bassett S, Boucher T, Bridgman SD, Conant RT, Cook-Patton SC, Ellis PW, Falcucci A, Fourqurean JW, Gopalakrishna T, Gu H, Henderson B, Hurteau MD, Kroeger KD, Kroeger T, Lark TJ, Leavitt SM, Lomax G, McDonald RI, Megonigal PJ, Miteva DA, Richardson CJ, Sanderman J, Shoch D, Spawn SA, Veldman JW, Williams CA, Woodbury PB, Zganjar C, Baranski M, Elias P, Houghton RA, Landis E, McGlynn E, Schlesinger WH, Siikamaki JV, Sutton-Grier AE, Griscom BW. 2018. Natural climate solutions for the United States. *Science Advances* 4:eaat1869.
- Fei S, Gould P, Kaeser M, Steiner K. 2010. Distribution and dynamics of the invasive native hay-scented fern. *Weed Science* 58:408–412.
- Feng H, Guo J, Peng C, Ma X, Kneeshaw D, Chen H, Liu Q, Liu M, Hu C, Wang W. 2023. Global estimates of forest soil methane flux identify a temperate and tropical forest methane sink. *Geoderma* 429:116239.
- Fiad J. 2017. Forest Management Plan Lacawac Sanctuary. Green Leaf Consulting Services, LLC.
- Finley JC, Stout SL, Pierson TG, McGuinness BJ. 2007. Managing timber to promote sustainable forests: A second-level course for the sustainable forestry initiative of Pennsylvania. USDA Forest Service: General Technical Report - Northern Research Station. p 47.
- Finzi AC, Canham CD. 1998. Non-additive effects of litter mixtures on net N mineralization in a southern New England forest. *Forest Ecology and Management* 105:129–136.
- Finzi AC, Van Breemen N, Canham CD. 1998. Canopy tree-soil interactions within temperate forests: Species effects on soil carbon and nitrogen. *Ecological Applications* 8:440–446.
- Finzi AC, Raymer PCL, Giasson MA, Orwig DA. 2014. Net primary production and soil respiration in New England hemlock forests affected by the hemlock woolly adelgid. *Ecosphere* 5.
- Fischer W, Thomas CK, Zimov N, Göckede M. 2022. Grazing enhances carbon cycling but reduces methane emission during peak growing season in the Siberian Pleistocene Park tundra site. *Biogeosciences* 19:1611–1633.
- Forbes ES, Cushman JH, Burkepile DE, Young TP, Klope M, Young HS. 2019. Synthesizing the effects of large, wild herbivore exclusion on ecosystem function. *Functional Ecology* 33:1597–1610.
- Fraterrigo JM, Turner MG, Pearson SM, Dixon P. 2005. Effects of past land use on spatial heterogeneity of soil nutrients in southern Appalachian forests. *Ecological Monographs* 75:215–230.
- Garland JL, Mills AL. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied Environmental Microbiology* 57:2351–2359.
- Garland JL. 1999. Potential and limitations of BIOLOG for microbial community analysis. In: *Microbial Biosystems: New Frontiers*, Proceedings of the 8th International Symposium on Microbial Ecology. pp 1–7.
- George LO, Bazzaz FA. 1999. The fern understory as an ecological filter: Emergence and establishment of canopy-tree seedlings. *Ecology* 80:833–845.
- Gill RMA, Beardall V. 2001. The impact of deer on woodlands: The effects of browsing and seed dispersal on vegetation structure and composition. *Forestry* 74:209–218.
- Gittleman J. 1990. Adaptation: Statistics and a null model for estimating phylogenetic effects. *Systematic Zoology* 39:227–241.
- Habeck CW, Schultz AK. 2015. Community-level impacts of white-tailed deer on understorey plants in North American forests: A meta-analysis. *AoB Plants* 7:plv119.
- Hadley JL, Schedlbauer JL. 2002. Carbon exchange of an old-growth eastern hemlock (*Tsuga canadensis*) forest in central New England. *Tree Physiology* 22:1079–1092.
- Harrison KA, Bardgett RD. 2003. How browsing by red deer impacts on litter decomposition in a native regenerating woodland in the Highlands of Scotland. *Biology and Fertility of Soils* 38:393–399.
- Hobbs NT. 1996. Modification of ecosystems by ungulates. *Journal of Wildlife Management* 60:695–713.
- Horsley SB. 1993. Mechanisms of interference between hay-scented fern and black cherry. *Canadian Journal of Forest Research* 23:2059–2069.
- Horsley SB, Stout SL, DeCalesta DS. 2003. White-tailed deer impact on the vegetation dynamics of a northern hardwood forest. *Ecological Applications* 13:98–118.
- Hosley NW, Ziebarth RK. 1935. Some winter relations of the white-tailed deer to the forests in north central Massachusetts. *Ecology* 16:535–553.
- Hough AF. 1960. Silvical characteristics of eastern hemlock (*Tsuga canadensis*). Station Paper NE-132. Upper Darby, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 23 pp.
- Husheer SW, Coomes DA, Robertson AW. 2003. Long-term influences of introduced deer on the composition and structure of New Zealand *Nothofagus* forests. *Forest Ecology and Management* 181:99–117.
- Hutchings MJ, John EA, Wijesinghe DK. 2003. Toward understanding the consequences of soil heterogeneity for plant populations and communities. *Ecology* 84:2322–2334.
- Ibañez-Alvarez M, Baraza E, Serrano E, Romero-Munar A, Cardona C, Bartolome J, Krumins JA. 2022. Ungulates alter plant cover without consistent effect on soil ecosystem functioning. *Agriculture, Ecosystems and Environment* 326:107796.

- Iverson LR, Hutchinson TF, Peters MP, Yaussy DA. 2017. Long-term response of oak-hickory regeneration to partial harvest and repeated fires: Influence of light and moisture. *Ecosphere* 8:e01642.
- Kadowaki K, Honjo MN, Nakamura N, Kitagawa Y, Ishihara MI, Matsuoka S, Tachiki Y, Fukushima K, Sakaguchi S, Mizuki I, Fujiki D, Sakai M, Takayanagi A, Yamasaki M, Tokuchi N, Takahashi D, Nagasawa K, Masuda K. 2023. eDNA metabarcoding analysis reveals the consequence of creating ecosystem-scale refugia from deer grazing for the soil microbial communities. *Environmental DNA* 5:1732–1742.
- Kay M, Elkin L, Higgins J, Wobbrock J. 2021. ARTTool: Aligned Rank Transform for Nonparametric Factorial ANOVAs. R package version 0.11.1, <<https://github.com/mjskay/ARTool>>.
- Keuskamp JA, Dingemans BJJ, Lehtinen T, Sarneel JM, Hefting MM. 2013. Tea Bag Index: A novel approach to collect uniform decomposition data across ecosystems. *Methods in Ecology and Evolution* 4:1070–1075.
- Kielland K, Bryant JP, Ruess RW. 1997. Moose herbivory and carbon turnover of early successional stands in interior Alaska. *Oikos* 80:25–30.
- Kim J, Hwang T, Schaaf CL, Orwig DA, Boose E, Munger JW. 2017. Increased water yield due to the hemlock woolly adelgid infestation in New England. *Geophysical Research Letters* 44:2327–2335.
- Kolstad AL, Austrheim G, Solberg EJ, Venete AMA, Woodin SJ, Speed JDM. 2018. Cervid exclusion alters boreal forest properties with little cascading impacts on soils. *Ecosystems* 21:1027–1041.
- Kupferschmid AD. 2017. Central European tree species in response to leader shoot browsing. In: Menendez A, Sands N, Eds. *Ungulates: Evolution, Diversity and Ecology*. Inc: Nova Science Publishers. pp 1–64.
- Leopold A, Sowls KK, Spencer DL. 1947. A survey of over-populated deer ranges in the United States. *Journal of Wildlife Management* 11:162–177.
- Leroux SJ, Wiersma YF, Vander Wal E. 2020. Herbivore impacts on carbon cycling in boreal forests. *Trends in Ecology and Evolution* 35:1001–1010.
- Li Q, Allen HL, Wollum AG II. 2004. Microbial biomass and bacterial functional diversity in forest soils: Effects of organic matter removal, compaction, and vegetation control. *Soil Biology and Biochemistry* 36:571–579.
- Liu L, Estiarte M, Peñuelas J. 2019. Soil moisture as the key factor of atmospheric CH₄ uptake in forest soils under environmental change. *Geoderma* 355:113920.
- Lovett GM, Weathers KC, Arthur MA, Schultz JC. 2004. Nitrogen cycling in a northern hardwood forest: Do species matter? *Biogeochemistry* 67:289–308.
- Lovett GM, Canham CD, Arthur MA, Weathers KC, Fitzhugh RD. 2006. Forest ecosystem responses to exotic pests and pathogens in eastern North America. *Bioscience* 56:395–405.
- Lyon J, Sharpe WE. 1996. Hay-scented fern (*Dennstaedtia punctilobula* (Michx.) Moore) interference with growth of northern red oak (*Quercus rubra* L.) seedlings. *Tree Physiology* 16:923–932.
- Lyon J, Sharpe WE. 2003. Impacts of hay-scented fern on nutrition of northern red oak seedlings. *Journal of Plant Nutrition* 26:487–502.
- Magarzo A, Olsson S, Sanz-Benito I, Mediavilla O, Oria-de-Rueda JA, Villafuerte-Jordán R, Martínez-Jauregui M, Mar-tín-Pinto P. 2024. Wild ungulate effects on soil fungal diversity in Mediterranean mixed forests. *Forest Ecology and Management* 562:121928.
- Martinez Arbizu P. 2017. pairwiseAdonis: Pairwise Multilevel Comparison using Adonis.
- Mayer M, Chianese R, Scudder T, White J, Vongpaseuth K, Ward R. 2002. Thirteen years of monitoring the hemlock woolly adelgid in New Jersey forests. *Hemlock Woolly Adelgid in the Eastern United States Symposium*: 50–60.
- McGarvey JC, Thompson JR, Epstein HE, Shugart HHJ. 2015. Carbon storage in old-growth forests of the Mid-Atlantic: Toward better understanding the eastern forest carbon sink. *Ecology* 96:311–317.
- McKinley DC, Ryan MG, Birdsey RA, Giardina CP, Harmon ME, Heath LS, Houghton RA, Jackson RB, Morrison JF, Murray BC, Pataki DE, Skog KE. 2011. A synthesis of current knowledge on forests and carbon storage in the United States. *Ecological Applications* 21:1902–1924.
- Merrill PH, Hawley RC. 1924. Hemlock: Its place in the silviculture of the southern New England forest. *Yale School of Forestry Bulletin* 68 pp.
- Mladenoff DJ. 1987. Dynamics of nitrogen mineralization and nitrification in hemlock and hardwood treefall gaps. *Ecology* 68:1171–1180.
- Mohr D, Topp W. 2001. Forest soil degradation in slopes of the low mountain range of Central Europe — Do deer matter? *European Journal of Forest Research* 120:220–230.
- Mohr D, Cohnstaedt LW, Topp W. 2005. Wild boar and red deer affect soil nutrients and soil biota in steep oak stands of the Eifel. *Soil Biology and Biochemistry* 37:693–700.
- Murray BD, Webster CR, Bump JK. 2013. Broadening the ecological context of ungulate-ecosystem interactions: The importance of space, seasonality, and nitrogen. *Ecology* 94:1317–1326.
- Nelson DW, Sommers LE. 1996. Total carbon, organic carbon, and organic matter. In: *Methods of soil analysis: Part 3 Chemical methods 5*. SSSA Book Series. pp 961–1010.
- Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J. 2022. *vegan: Community Ecology Package*.
- Orwig DA, Foster DR. 1998. Forest response to the introduced hemlock wooly adelgid in southern New England, USA. *Torrey Botanical Society* 125:60–73.
- Orwig DA, Cobb RC, D'Amato AW, Kizlinski ML, Foster DR. 2008. Multi-year ecosystem response to hemlock woolly adelgid infestation in southern New England forests. *Canadian Journal of Forest Research* 38:834–843.
- Orwig DA. 2002. Stand dynamics associated with chronic hemlock woolly adelgid infestations in southern New England. In: *Symposium on the Hemlock Woolly Adelgid in Eastern North America Proceedings*. pp 36–46.
- Paradis E, Schliep K. 2019. *ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R*. *Bioinformatics* 35:526–528.
- Pebesma E, Bivand R. 2023. *Spatial data science with applications in R*. Chapman and Hall/CRC.

- Plain C, Ndiaye F-K, Bonnaud P, Ranger J, Epron D. 2019. Impact of vegetation on the methane budget of a temperate forest. *New Phytologist* 221:1447–1456.
- Popma J, Nadelhoffer K. 2020. Deer browsing effects on temperate forest soil nitrogen cycling shift from positive to negative across fertility gradients. *Canadian Journal of Forest Research* 50:1281–1288.
- R Core Team. 2023. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <<https://www.R-project.org/>>.
- Ramirez JI. 2021. Uncovering the different scales in deer-forest interactions. *Ecology and Evolution* 11:5017–5024.
- Ramirez JI, Jansen PA, den Ouden J, Moktan L, Herdoiza N, Poorter L. 2021. Above- and below-ground cascading effects of wild ungulates in temperate forests. *Ecosystems* 24:153–167.
- Reed SP, Royo AA, Fotis AT, Knight KS, Flower CE, Curtis PS. 2022. The long-term impacts of deer herbivory in determining temperate forest stand and canopy structural complexity. *Journal of Applied Ecology* 59:812–821.
- Ribeiro PJ, Diggle P, Christensen O, Schlather M, Bivand R, Ripley B. 2022. geoR: Analysis of Geostatistical Data. R package version 1.9–2, <<https://CRAN.R-project.org/package=geoR>>.
- Risch AC, Haynes AG, Busse MD, Filli F, Schütz M. 2013. The response of soil CO₂ fluxes to progressively excluding vertebrate and invertebrate herbivores depends on ecosystem type. *Ecosystems* 16:1192–1202.
- Rooney TP, Waller DM. 2003. Direct and indirect effects of white-tailed deer in forest ecosystems. *Forest Ecology and Management* 181:165–176.
- Royo AA, Vickers LA, Long RP, Ristau TE, Stoleson SH, Stout SL. 2021. The forest of unintended consequences: Anthropogenic actions trigger the rise and fall of black cherry. *Bioscience* 71:683–696.
- Russell EWB, Davis RB, Anderson RS, Rhodes TE, Anderson DS. 1993. Recent centuries of vegetational change in the glaciated north-eastern United States. *Journal of Ecology* 81:647.
- Russell MB, Woodall CW, Potter KM, Walters BF, Domke GM, Oswalt CM. 2017. Interactions between white-tailed deer density and the composition of forest understories in the northern United States. *Forest Ecology and Management* 384:26–33.
- San Julian GJ, Smith SS. 2001. White-tailed deer. PennState Extension. 4 pp.
- Schlesinger WH, Reynolds JF, Cunningham GL, Huenneke LF, Jarrell WM, Virginia RA, Whitford WG. 1990. Biological feedbacks in global desertification. *Science* 247:1043–1048.
- Schmitz OJ, Raymond PA, Estes JA, Kurz WA, Holtgrieve GW, Ritchie ME, Schindler DE, Spivak AC, Wilson RW, Bradford MA, Christensen V, Deegan L, Smetacek V, Vanni MJ, Wilmers CC. 2014. Animating the carbon cycle. *Ecosystems* 17:344–359.
- Schmitz OJ, Wilmers CC, Leroux SJ, Doughty CE, Atwood TB, Galetti M, Davies AB, Goetz SJ. 2018. Animals and the zoolgeochemistry of the carbon cycle. *Science* 362:eaar3213.
- Sharpe WE, Halofsky JE. 2004. Hay-Scented fern (*Dennstaedtia punctilobula*) and sugar maple (*Acer Saccharum*) seedling occurrence with varying soil acidity in Pennsylvania. In: Proceedings of the 14th Central Hardwoods Forest Conference. pp 265–70.
- Simončič T, Bončina A, Jarník K, Klopčič M. 2019. Assessment of the long-term impact of deer on understory vegetation in mixed temperate forests. *Journal of Vegetation Science* 30:108–120.
- Singmann H, Bolker B, Westfall J, Aust F, Ben-Shachar M. 2023. afex: Analysis of Factorial Experiments. R package version 1.3–0,
- Small MJ, Small CJ, Dreyer GD. 2005. Changes in a hemlock-dominated forest following woolly adelgid infestation in southern New England. *Journal of the Torrey Botanical Society* 132:458–470.
- Smallidge P. 2015. Native Invasive: Hay-Scented Fern. the Overstory 26:1–7.
- Soil Survey Staff, Natural Resources Conservation Service USDA. Soil Survey Geographic (SSURGO) Database.
- Spaulding HL, Rieske LK. 2010. The aftermath of an invasion: Structure and composition of Central Appalachian hemlock forests following establishment of the hemlock woolly adelgid, *Adelges tsugae*. *Biological Invasions* 12:3135–3143.
- Sprouffske K. 2020. growthcurver: Simple metrics to summarize growth curves. R package version 0.3.1.
- Stadler B, Müller T, Orwig D. 2006. The ecology of energy and nutrient fluxes in hemlock forests invaded by hemlock woolly adelgid. *Ecology* 87:1792–1804.
- Stark S, Männistö MK, Smolander A. 2010. Multiple effects of reindeer grazing on the soil processes in nutrient-poor northern boreal forests. *Soil Biology and Biochemistry* 42:2068–2077.
- Stephan JG, Pourazari F, Tattersall K, Kobayashi T, Nishizawa K, De Long JR. 2017. Long-term deer exclosure alters soil properties, plant traits, understory plant community and insect herbivory, but not the functional relationships among them. *Oecologia* 184:685–699.
- Stevens DL, Olsen AR. 2004. Spatially balanced sampling of natural resources. *Journal of the American Statistical Association* 99:262–278.
- Subke J-A, Moody CS, Hill TC, Voke N, Toet S, Ineson P, Teh YA. 2018. Rhizosphere activity and atmospheric methane concentrations drive variations of methane fluxes in a temperate forest soil. *Soil Biology and Biochemistry* 116:323–332.
- Suchewaboripont V, Ando M, Iimura Y, Yoshitake S, Ohtsuka T. 2015. The effect of canopy structure on soil respiration in an old-growth beech-oak forest in central Japan. *Ecological Research* 30:867–877.
- Swain E, Poulton JE. 1994. Utilization of amygdalin during seedling development of *Prunus serotina*. *Plant Physiology* 106:437–445.
- Talbot JM, Finzi AC. 2008. Differential effects of sugar maple, red oak, and hemlock tannins on carbon and nitrogen cycling in temperate forest soils. *Oecologia* 155:583–592.
- Tanentzap AJ, Coomes DA. 2012. Carbon storage in terrestrial ecosystems: Do browsing and grazing herbivores matter? *Biological Reviews* 87:72–94.
- Thériault R. 2023. rempsyc: Convenience functions for psychology. *Journal of Open Source Software*, 8:5466. <<http://doi.org/10.21105/joss.05466>>
- Tong R, Ji B, Wang GG, Lou C, Ma C, Zhu N, Yuan W, Wu T. 2024. Canopy gap impacts on soil organic carbon and nutrient dynamic: A meta-analysis. *Annals of Forest Science* 81:12.
- Townsend DS, Seva JS, Hee-seagle C, Mayers G. 2002. Structure and composition of a northern hardwood forest exhibiting regeneration failure. *Philadelphia Botanical Club* 61:1–13.

- Tremblay JP, Huot J, Potvin F. 2007. Density-related effects of deer browsing on the regeneration dynamics of boreal forests. *Journal of Applied Ecology* 44:552–562.
- U.S. Forest Service. 2018. Hemlock woolly adelgid invasion and hemlock volume, growth, removals, mortality estimates. <<https://hub.arcgis.com/maps/6cf9e726020b48cc85d5210291ea0d93/explore?location=39.499524%2C-76.911200%2C5.00>>
- Waller DM, Alverson WS. 1997. White-tailed deer: A keystone herbivore. *Wildlife Society Bulletin* 25:217–226.
- Wardle DA, Barker GM, Yeates GW, Bonner KI, Ghani A. 2001. Introduced browsing mammals in New Zealand natural forests: Aboveground and belowground consequences. *Ecological Monographs* 71:587–614.
- Wardle DA, Bonner KI, Barker GM. 2002. Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. *Functional Ecology* 16:585–595.
- Wickham H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York, <<https://ggplot2.tidyverse.org>>.
- Wobbrock JO, Findlater L, Gergle D, Higgins JJ. 2011. The aligned rank transform for nonparametric factorial analyses using only ANOVA procedures. *Proceedings of the ACM Conference on Human Factors in Computing Systems (CHI '11)*. Vancouver, British Columbia (May 7–12, 2011). New York: ACM Press, pp. 143–146.
- Yamanaka T, Morimoto N, Nishida GM, Kiritani K, Moriya S, Liebhold AM. 2015. Comparison of insect invasions in North America, Japan and their Islands. *Biological Invasions* 17:3049–3061.
- Zhao F, Yang X, Schull MA, Román-Colón MO, Yao T, Wang Z, Zhang Q, Jupp DLB, Lovell JL, Culvenor DS, Newnham GJ, Richardson AD, Ni-Meister W, Schaaf CL, Woodcock CE, Strahler AH. 2011. Measuring effective leaf area index, foliage profile, and stand height in New England forest stands using a full-waveform ground-based lidar. *Remote Sensing of Environment* 115:2954–2964.
- Zhu Y, Wu Y, An Y. 2022. Effects of David deer grazing on soil bacterial and fungal communities in an eastern coastal wetland of China. *Diversity* 14:976.