New Phytologist **Supporting Information**

Article title: **Altered plant carbon partitioning enhanced forest ecosystem carbon storage after 25 years of nitrogen additions**

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Fig. S1 Mean percent basal area of eight dominant species in the reference watershed (light green) and the fertilized watershed (dark green) at the beginning of the experiment (1990-1991) and end of the experiment (2018). Data are from 25 permanent growth plots that were censused in 1990-1991 and 2018 (see Fig 1 and methods). Error bars represent +/- 1 se. Species codes: ACRU *Acer rubra*, ACSA *Acer saccharum*, BELE *Betula lenta*, LITU *Liriodendron tulipifera*, MAFR *Magnolia fraseri,* PRSE *Prunus serotina*, QURU *Quercus rubrum,* and ROPS *Robinia psuedoacacia* (symbiotic N fixer).

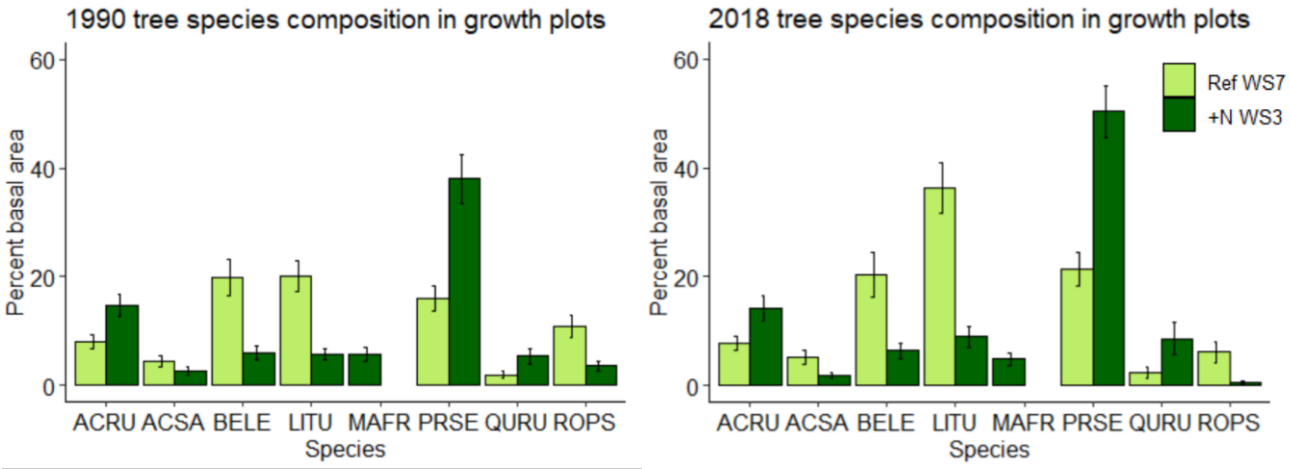

Fig. S2 Mean measurements and estimates of fine earth soil bulk density. Measured (solid symbols) and estimated (open symbols) of fine earth bulk density in +N WS3 (dark green), Ref WS7 (light green), and a nearby site at the Fernow (blue). Estimates of fine earth bulk density were constructed using a linear relationship between mean measured bulk density and soil depth at 0-5 cm and 30-45 cm depths. The orange diamond is an additional mean measurement made in Ref WS7 using soil cores in 30 locations (Kelly, 2010) and shows that the linear regression matches this measurement and is likely a reasonable approach to estimate bulk density in the absence of robust measurements in both watersheds.

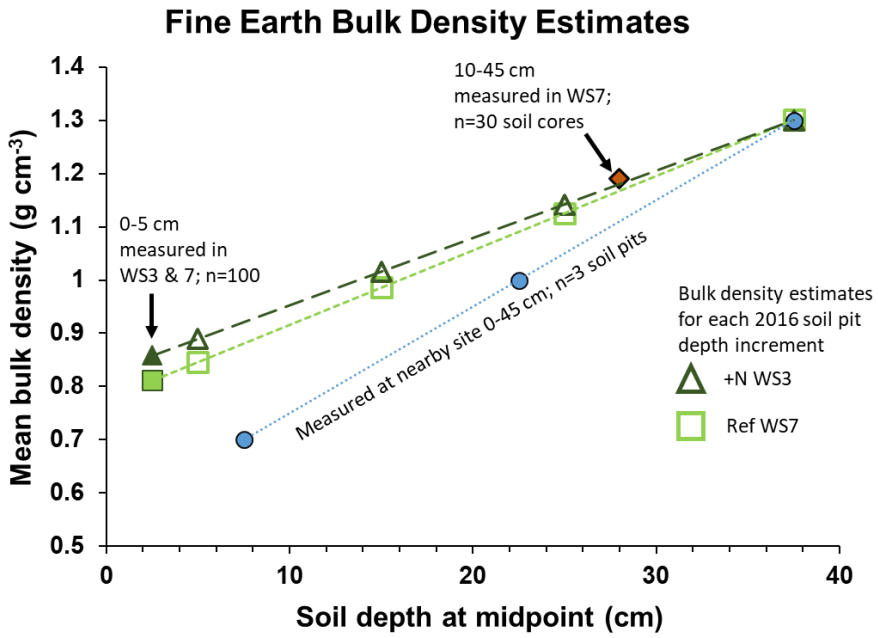

Fig. S3 Mean fine root C stocks in the organic horizon (a) and surface mineral soil (b) were variable across 5 different years of measurement in the reference WS7 (light green) and +N WS3 (dark green). Error bars represent +/- one standard error. Labels above bars are p-values comparing watershed root stocks for each year and soil depth (t-one-way ANOVA). 1991 values are from Adams et al. (2006); 2015 values are adapted from Carrara et al. (2018).

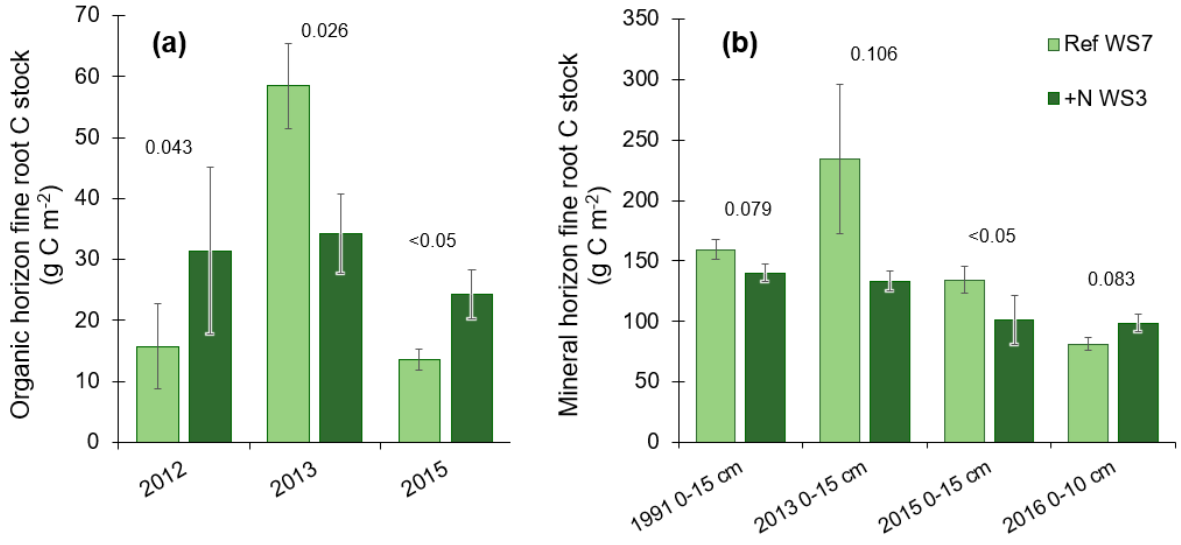

Fig. S4 Time series of soil respiration, temperature, and moisture data from 2016-2017 at the Fernow Watershed Fertilization study. Mean (a) soil respiration rates, (b) soil temperature at 10 cm soil depth, and (c) soil percent moisture at 0-10 cm soil depth for Ref WS7 (light green, dotted lines) and +N WS3 (dark green, dashed lines) (n=40) over two years (x-axis date format: month/day/year).

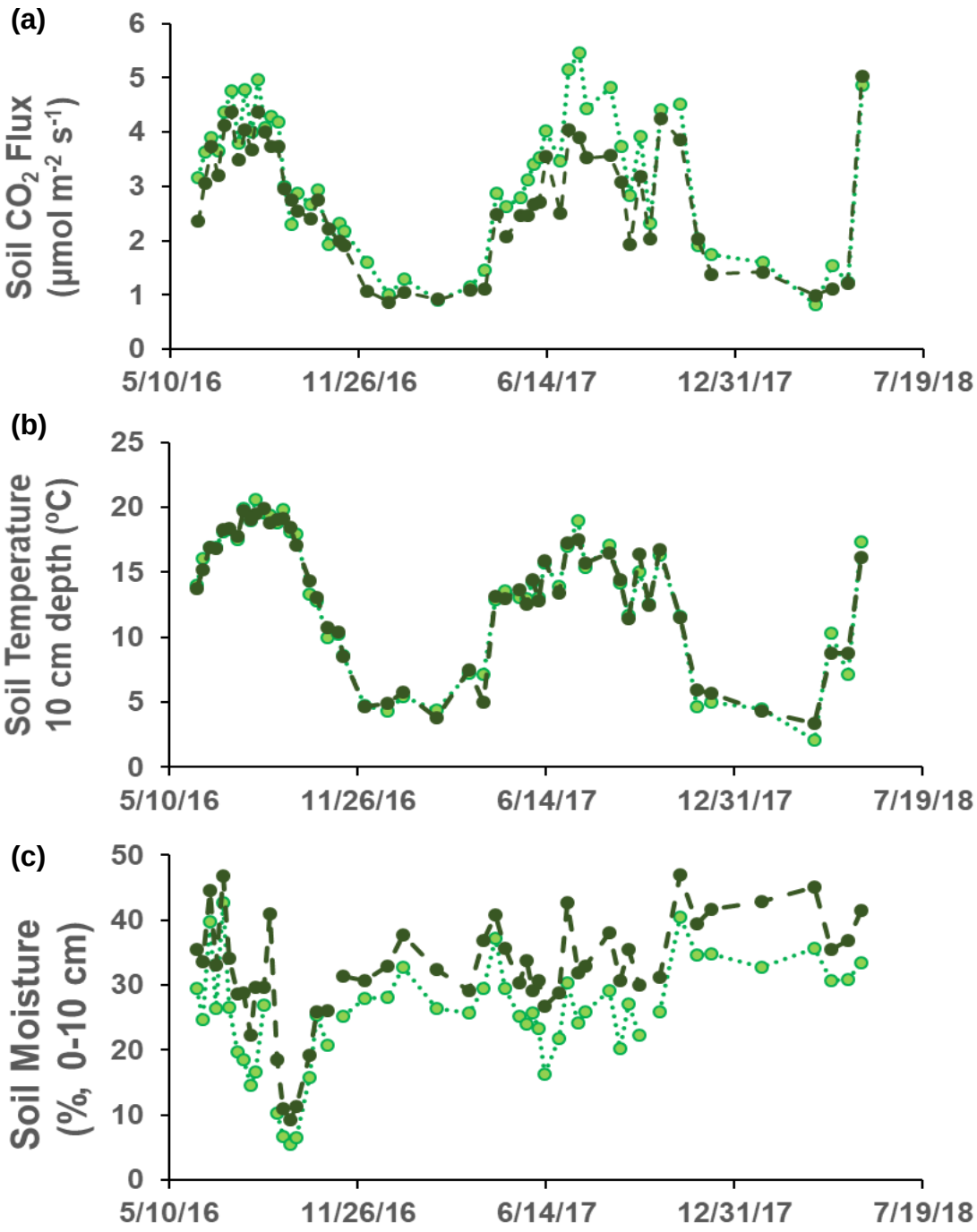

Fig. S5 Hydrologic inorganic N budgets for reference watershed 7 (left) and fertilized watershed 3 (right) over 34 calendar years. N inputs (dark gray) include total ambient N deposition (CASTNET, NADP) and experimental N additions (for +N WS3). N outputs (light gray) include total NO3- -N and NH4+-N discharged in streamwater. The apparent N retention (black lines) is the difference between inputs and outputs. The red, dashed line indicates the start of experimental N additions to +N WS3. Watershed-scale N budgets reveal enhanced losses of inorganic N in streamwater, as well as significant levels of N retention (~3 g N m-2 y-1) in the fertilized watershed that have persisted for more than 25 years.

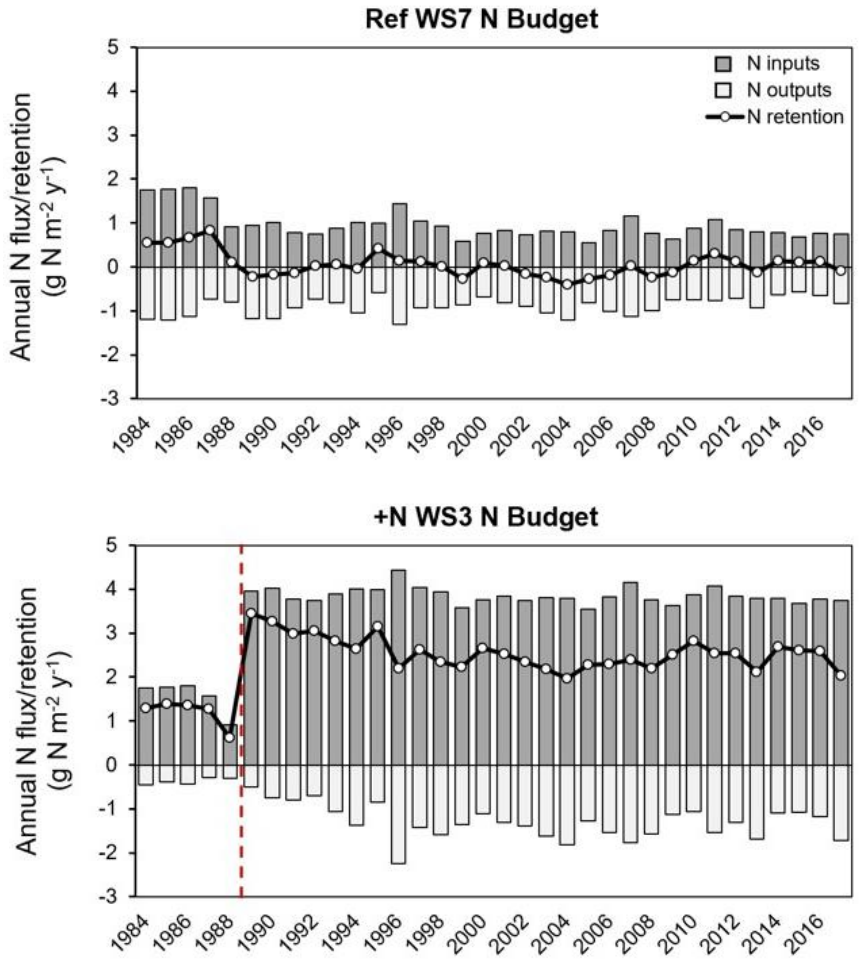

Fig. S6 Top: Basal area increment (BAI; mm2) of four dominant species in +N WS3 vs. Ref WS7 during pretreatment years (1973-1988) suggest faster growth (regression slopes >1) in all species in +N WS3 except *Liriodendron tulipifera* prior to the start of fertilizer application. Bottom: Mean observed minus mean predicted BAI for four dominant species in +N WS3 show an enhancement in growth early in N addition experiment, followed by relative decrease in tree growth later in the experiment. Predicted BAI was estimated from pretreatment relationships of BAI between watersheds, as determined from increment cores (Top). Observed BAI was estimated from increment cores and permanent growth plot data. Method and pretreatment data through 2000 for *Acer rubra*, *Prunus serotina* and *L. tulipifera* were from DeWalle *et al.* (2006). Data past 2000 from permanent growth plot data. All increment core data for *Betula lenta* from M.B. Burnham & W.T. Peterjohn, unpublished.

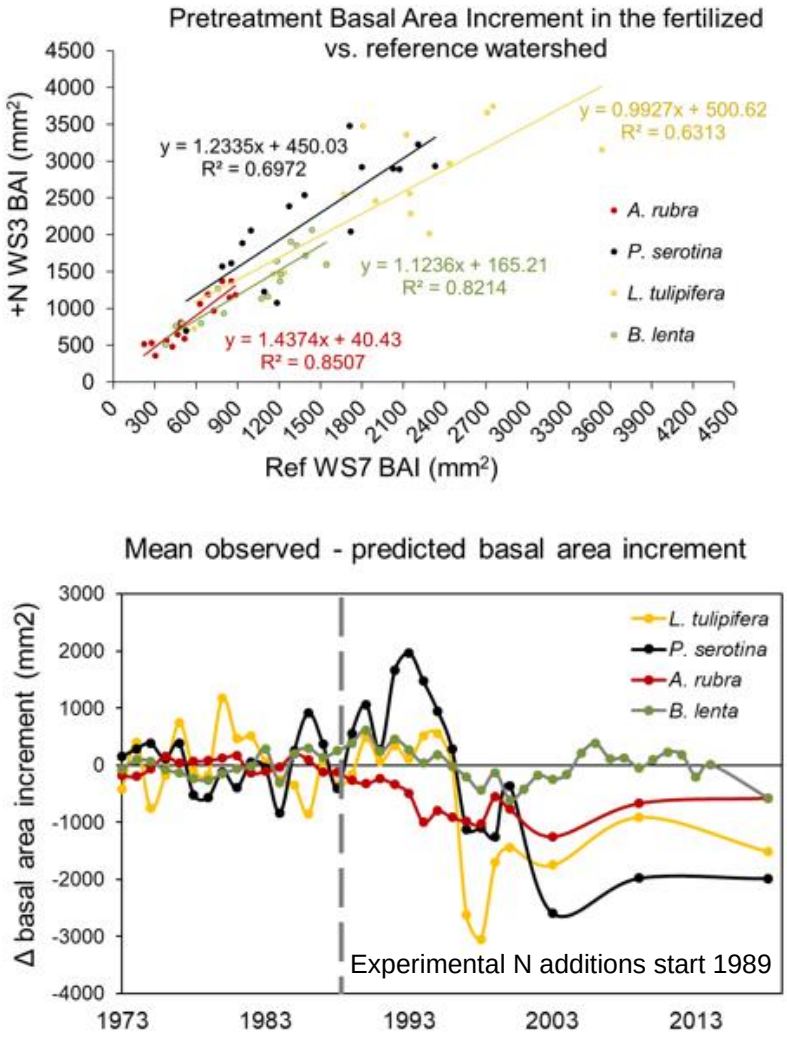

Fig. S7 Mean black locust (*Robinia pseudoacia*) stem density and annual estimated N fixation flux from black locust in Ref WS7 (light green) and +N WS3 (dark green). Mean stem densities are reported as measured at each growth plot census (1991-2018, n=25). Annual N fixation rates were estimated from N fixation rates and stem densities reported by Boring and Swank (1984). N fixation rates were assumed to be proportional to black locust stem density.

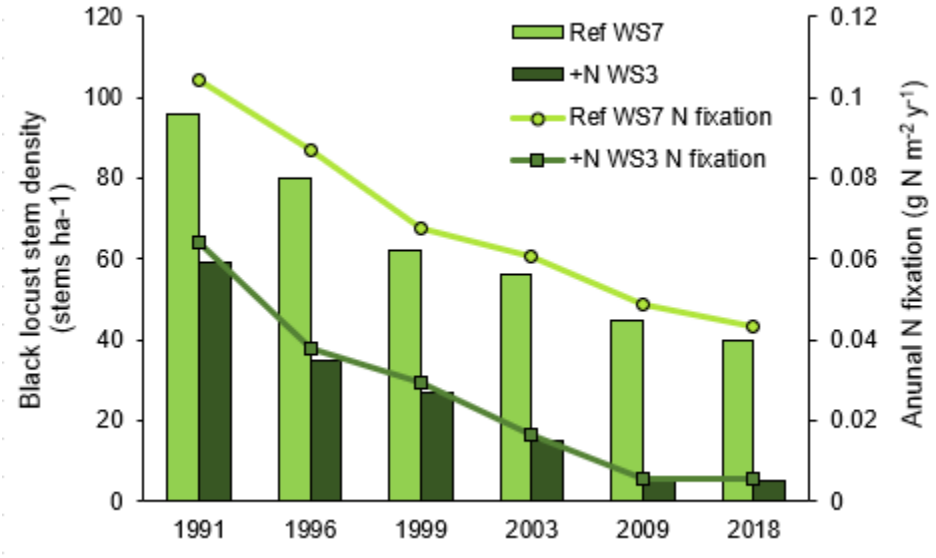

Table S1. Mean (+/- se) tree wood carbon and nitrogen concentrations in the outer 1 cm of bolewood, and sample sizes (*n*) for the reference WS7 and fertilized WS3. Results from 2-way ANOVA with Watershed and Species as main effects: bold values differed between watersheds ($p<0.05$) and superscripts of different letters were different among species ($p<0.05$; Tukey HSD test).

Watershed	Species	%C	%N	*n*
Reference WS7				
	Acer rubrum	**46.51 (0.08)**	0.145 (0.008)^{ab}	10
	Betula lenta	**46.21 (0.07)**	0.125 (0.013)^{a}	10
	Liriodendron tulipifera	**46.17 (0.08)**	0.189 (0.018)^{b}	8†
	Prunus serotina	**45.97 (0.08)**	0.110 (0.005)^{a}	10
Fertilized WS3				
	Acer rubrum	**47.53 (0.19)**	0.129 (0.007)^{ab}	10
	Betula lenta	**47.39 (0.26)**	0.107 (0.011)^{a}	10
	Liriodendron tulipifera	**47.05 (0.15)**	0.160 (0.014)^{b}	10
	Prunus serotina	**47.45 (0.10)**	0.098 (0.004)^{a}	10

†Two outliers removed for unusually high values.

Table S2. Mean (+/- se) leaf litter carbon and nitrogen concentrations, C:N ratios, mass (g m-2) and sample size (*n*) for the reference WS7 and fertilized WS3.

Watershed	Species	Year	%C	%N	C:N ratio	litter mass (g m-2)	*n*†
Reference WS7	*Acer rubrum*	2015	47.2 (0.2)	1.09 (0.09)	46.7 (4.3)	44 (18)	10
		2016	48.4 (0.3)	0.89 (0.05)	56.2 (3.2)	41 (16)	10
		2017	47.8 (0.1)	0.99 (0.07)	50.6 (3.6)	48 (17)	10
	Betula lenta	2015	49.5 (0.6)	1.41 (0.03)	35.2 (0.9)	87 (12)	10
		2016	51.2 (1.5)	1.3 (0.04)	40.7 (1.3)	69 (14)	10
		2017	50.3 (0.9)	1.34 (0.03)	37.8 (1.0)	114 (51)	10
	Liriodendron tulipifera	2015	47.5 (0.3)	1.38 (0.04)	34.8 (1.2)	60 (12)	10
		2016	49.5 (0.8)	1.02 (0.02)	48.9 (1.2)	87 (19)	10
		2017	48.5 (0.5)	1.20 (0.02)	40.7 (1.1)	93 (23)	10
	Prunus serotina	2015	50.7 (1.0)	1.13 (0.04)	45.2 (1.5)	42 (9)	10
		2016	49.0 (0.7)	1.31 (0.05)	37.9 (1.3)	37 (9)	10
		2017	49.9 (0.7)	1.22 (0.03)	41.1 (1.0)	37 (8)	10
	Quercus rubra	2015	48.8 (0.6)	0.82 (0.03)	60.6 (2.7)	48 (20)	10
		2016	51.4 (2.4)	0.87 (0.05)	59.6 (3.1)	22 (9)	7
		2017	49.8 (1.0)	0.85 (0.02)	58.9 (1.3)	28 (12)	9
Fertilized WS3	*Acer rubrum*	2015	45.9 (1.8)	1.37 (0.07)	34.4 (2.3)	72 (13)	10
		2016	48.1 (0.2)	0.88 (0.04)	55.9 (2.6)	64 (16)	10
		2017	47.0 (0.8)	1.12 (0.05)	42.6 (2.1)	65 (14)	10
	Betula lenta	2015	48.5 (0.4)	1.82 (0.12)	27.6 (1.6)	31 (6)	10
		2016	49.9 (0.7)	1.38 (0.04)	36.3 (1.2)	31 (7)	10
		2017	49.2 (0.4)	1.60 (0.07)	31.2 (1.4)	45 (12)	10
	Liriodendron tulipifera	2015	47.2 (0.4)	1.77 (0.26)	29.3 (3.2)	6 (4)	7
		2016	49.2 (0.4)	1.38 (0.09)	36.7 (2.1)	9 (4)	9
		2017	48.2 (0.1)	1.53 (0.10)	32.6 (1.8)	21 (14)	10
	Prunus serotina	2015	49.8 (0.3)	1.42 (0.08)	36.1 (2.1)	105 (13)	10
		2016	51.7 (1.0)	1.68 (0.05)	31.0 (0.9)	68 (9)	10
		2017	50.7 (0.6)	1.55 (0.04)	33.0 (0.9)	135 (35)	10
	Quercus rubra	2015	48.6 (0.6)	1.18 (0.12)	44.4 (3.7)	41 (11)	10
		2016	49.4 (0.4)	1.11 (0.06)	45.2 (2.0)	39 (8)	10
		2017	49.0 (0.3)	1.15 (0.06)	43.5 (1.9)	35 (9)	10

†Sample size for litter chemistry based on plot-level litter collection baskets. If n<10, there were no leaves of that species collected from one or more litter baskets (plots) that year.

Table S3. Methods for fine root measurements at the Fernow Experimental Forest Watershed Fertilization Experiment.

Organic horizon

Date	Sampling scheme	Sample dimensions	Sample processing
June 2012	2 subsamples from two locations in 7 plots	25 x 25 cm square divided in half by steel frame	Fine roots (<2mm diameter) were picked by hand and dried at 65'C for >48 hours.
June 2013	2 subsamples from two locations in 7 plots	25 x 25 cm square divided in half by steel frame	Fine roots (<2mm diameter) were picked by hand, dried at 65'C for >48 hours, and ground in mill to #20 mesh for %C and %N analysis
June, July & August 2015[a]	1 sample in 10 plots	10 x 10 cm	Fine roots (<2 mm diameter) were picked by hand, washed in deionized water, and dried

Mineral horizon

Date	Sampling scheme	Core diameter (cm)	Depth (cm)	Sample processing
May & September 1991[b]	1 soil core in 17 plots	5.08	45.72	Fine roots were picked by hand and washed with water. Live roots were separated into fine (<2mm diameter) and coarse (>2mm diameter). Roots were oven dried at 70'C for 24 hours
June 2013	2 subsamples in 7 plots where O-horizon was sampled	4	15	Fine roots (<2mm diameter) were picked by hand, dried at 65'C for >48 hours, and ground in mill to #20 mesh for %C and %N analysis
June, July & August 2015[a]	3 subsamples in 10 plots where O horizons sampled	5	15	Fine roots (<2mm diameter) were picked by hand, washed in deionized water, and dried
June 2016	6 subsamples in 10 plots	4.5	10	Fine roots (<2mm diameter) were picked by hand, washed with deionized water, dried at 65'C for >48 hours, and ground to #20 mesh for %C and %N analysis

[a]From Carrara et al., 2018

[b]From Adams, 2016

Table S4. Mean (+/- se) pre-senescence foliar carbon and nitrogen concentrations and C:N ratios and sample size (*n*) for the reference WS7 and fertilized WS3.

Watershed	Species	%C	%N	C:N ratio	*n*
Reference WS7					
	Acer rubrum	49.7 (0.47)	2.19 (0.03)	22.8 (0.35)	30
	Betula lenta	49.7 (0.41)	2.93 (0.05)	17.1 (0.27)	30
	Liriodendron tulipifera	49.0 (0.47)	3.25 (0.08)	15.3 (0.36)	30
	Prunus serotina	49.4 (0.32)	2.93 (0.06)	17.1 (0.38)	30
	Quercus rubra[†]	49.8 (0.40)	2.87 (0.12)	17.6 (0.66)	8
Fertilized WS3					
	Acer rubrum	48.6 (0.16)	2.24 (0.04)	21.8 (0.36)	30
	Betula lenta	49.0 (0.15)	2.93 (0.05)	16.8 (0.28)	30
	Liriodendron tulipifera	47.9 (0.21)	3.46 (0.09)	14.1 (0.43)	30
	Prunus serotina	49.4 (0.13)	3.04 (0.08)	16.6 (0.51)	30
	Quercus rubra[†]	48.1 (0.72)	2.40 (0.10)	20.3 (0.97)	11

[†]All leaves sampled in July 2012 except *Quercus rubra* leaves were sampled in July 2016

Methods S1 Methods for propagating error when combining datasets across various years or plots.

Standard errors were propagated analytically following the methods of Lehrter & Cebrian (2010). such that when means are added or subtracted, $z = x + y$, errors (δx, δy) are summed in quadrature:

$$\delta z = \sqrt{\delta x^2 + \delta y^2} \quad \text{Eq.1}$$

and when means are multiplied or divided, $z = x * y$, fractional errors ($\delta x/x$, $\delta y/y$) are summed in quadrature:

$$\frac{\delta z}{z} = \sqrt{\left(\frac{\delta x}{x}\right)^2 + \left(\frac{\delta y}{y}\right)^2} \quad \text{Eq. 2}$$

Methods S2 Leaf litterfall collection and chemical analysis for 2015-2017.

Leaf litter collections baskets were placed in the center of 10 plots in each of the reference (Ref WS7) and fertilized (+N WS3) watersheds (Fig. 1). Litter collection baskets were 0.56 m x 0.40 m (0.224 m2). Litter from baskets were collected in the autumns of 2015-2017 every one or two weeks from September through the first week in November. Leaves were air dried in 2015 for over 5 days, and oven-dried at 65°C in 2016 and 2017 for over 48 hours. In all three years, dried leaves were separated by species, weighed, and leaves of five species (*Acer rubrum, Betula lenta, Liriodendron tulipifera, Prunus serotina, and Quercus rubra*) were ground and analyzed for C and N concentrations with Dumas combustion using an elemental analyzer (NA 1500 Series 2, Carlo Erba Instruments). We estimated plot-level estimates of total C and N litterfall for each year using the species-level mass and C and N concentrations, and for those species from which C and N concentrations were not measured, we applied the mean plot C and N concentrations to this remaining (other species) mass. Summarized data are presented in **Table S2**. These C and N fluxes were applied to the long-term leaf litterfall mass data. Litter trap sizes and litter pickup schedule were similar between the long-term traps (Adams, 2008) and the 10 additional traps. The locations of the 10 additional traps were selected to correspond with the location of soil respiration and other soil measurements (see Fig. 1).

Methods S3 Green foliage collection and chemical analysis.

Green canopy leaves were collected with a shotgun in July of 12 from ten plots in Ref WS7 and +N WS3. At each plot, three leaves were collected from one canopy tree from each of four species (*Acer rubrum, Betula lenta, Liriodendron tulipifera, and Prunus serotina*) at the high, mid, and low canopy. Leaf samples were kept on ice and stored in a cold room for transportation back to the lab and before analysis (~24 hours after collection). Leaves were dried at 65°C for 48 hours and ground through a # 20 mesh screen (0.841 m) prior to C and N analysis using an elemental analyzer (NA 1500 Series 2, Carlo Erba Instruments). Values from the three canopy leaves per tree were averaged and considered one observation. In the July of 2016, green foliage from an additional species (Quercus rubra) was collected with a shotgun from 8 canopy trees in Ref WS7 and 11 canopy trees in +N WS3. Leaves were dried at 60°C for 48 hours, ground through a #40 mesh screen (0.425 mm) before analysis for C and N using Dumas combustion elemental analyzer (NC 2500, Carlo Erba Instruments) at the University of Maryland Central Appalachian Stable Isotope Facility. Summarized data for all foliage are presented in **Table S3**.

Methods S4 Soil respiration measurements and annual CO2 efflux estimates

Estimates of annual soil CO_2 efflux used soil respiration measurements that were made year-round from June 2016-May 2017 at four respiration collars in each of 10 plots per watershed (Fig. 1), for a total of 40 measurements per treatment on each measurement date. Respiration collars (10 cm diameter, 5 cm height PVC) were inserted 2.5 cm into the soil approximately one week before the first respiration measurement. Soil respiration ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) was measured with an infrared gas analyzer (LI-8100A, LI-COR®, Inc., Lincoln, NE) weekly during the growing season, and biweekly to monthly during the non-growing season and snow-free period. In tandem with soil respiration measurements, soil temperature at 5- and 10-cm depths and soil moisture to a depth of 10 cm was measured. Additionally, buried soil temperature loggers at a depth of 5 cm in the center of each plot recorded soil temperature every hour over the course of the 2-year measurement period (HOBO Pendant® Temperature Data Logger, Onset Computer Corporation, Bourne, MA). These continuous soil temperature measurements were

used to model annual respiration, using a first-order exponential relationship (ae^{bT}, T= soil temperature, and *a* and *b* are parameters optimized to each watershed using Guass-Newton optimization; van't Hoff 1898). All analyses were done in R (version 3.0.2) and SAS JMP (JMP® Pro ver. 12.2.0).

References

Adams MB. **2008**. Long-term leaf fall mass from three watersheds on the Fernow Experimental Forest, West Virginia. *16th Central Hardwoods Forest Conference, USDA Forest Service* **304**: 179–186.

Adams MB. 2016. *Fernow Experimental Forest Watershed Acidification Root Data, 1991.* Parsons, WV, USA: Department of Agriculture, Forest Service, North Central Research Station.

Adams MB, DeWalle DR, Hom JL (Eds.). 2006. *The Fernow Watershed Acidification Study.* Springer Netherlands, Dordrecht.

Boring LR, Swank WT. **1984**. Symbiotic nitrogen fixation in regenerating black locust (Robinia pseudoacacia L.) stands. *Forest Science* **30**: 528–537.

Carrara JE, Walter CA, Hawkins JS, Peterjohn WT, Averill C, Brzostek ER. 2018. Interactions among plants, bacteria, and fungi reduce extracellular enzyme activities under long-term N fertilization. *Global Change Biology* **24**:2721–2734.

DeWalle DR, Kochenderfer JN, Adams MB, Miller GW, Gilliam FS, Wood F, Odenwald-Clemens SS, Sharpe WE. **2006**. Vegetation and Acidification. In: Adams MB, Dewalle DR, Hom JL, eds. The Fernow Watershed Acidification Study. Netherlands: Springer, 137–188.

Kelly CN. **2010**. *Carbon and nitrogen cycling in watersheds of contrasting vegetation types in the Fernow Experimental Forest, West Virginia*. Doctoral dissertation, Virginia Tech.

Lehrter JC, Cebrian J. **2010**. Uncertainty propagation in an ecosystem nutrient budget. *Ecological Applications* **20**: 508–524.

van't Hoff JH. 1898. Lectures on theoretical and physical chemistry. *Chemical Dynamics Part I.* 224-229. Edward Arnold, London.