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# Forest N Dynamics after 25 years of Whole Watershed N Enrichment: The Bear Brook Watershed in Maine

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We examine the temporal trend of input–output N fluxes and net ecosystem N retention, and estimate a mass balance for ecosystem soil and vegetation pools, after 25 yr of chemical manipulation at the Bear Brook Watershed in Maine (BBWM). The BBWM is a paired whole watershed manipulation experiment designed to study the effects of elevated N and S deposition on forest ecosystem function. Starting in 1989, 25.2 kg N ha<sup>-1</sup> was added annually to the West Bear (treated) watershed. The N additions in West Bear stimulated N loss through stream exports, and West Bear retained 81% of the annual N inputs, compared to 94% retention in the reference, East Bear. After 25 yr of N additions, the West Bear watershed had accumulated ~700 kg ha<sup>-1</sup> more N than East Bear in soils and vegetation, with ~10% of the accumulated N stored in forest biomass. The treatment increased recent biomass N accumulation rates in the hardwood stands, but not in the softwoods. Soils did not show detectable differences in total N content between watersheds, although the organic soils had greater N in West Bear. This paper presents a unique set of findings from one of the few long-term whole forest ecosystem N enrichment studies in the world. While N dynamics were clearly altered in West Bear, with evidence of accelerated N cycling, the treated watershed did not attain an advanced stage of N saturation during the study period, based on the evidence from forest growth and stream N exports.

**Abbreviations:** BBWM, Bear Brook Watershed in Maine; DIN, dissolved inorganic nitrogen; DON, dissolved organic nitrogen.

**T**emperate forest productivity is typically nitrogen (N)-limited (Fenn et al., 1998; Thomas et al., 2010). Many forests in North America and Europe have shown increased growth rates in response to elevated N deposition (Thomas et al., 2010). Some ecosystems, however, have exceeded the critical loads beyond which negative effects occur (McNulty et al., 2007; Pardo et al., 2011; Tietema et al., 1998). Environmental monitoring and experimental manipulation studies show that N-enriched ecosystems have increased N losses via leaching (Lovett and Goodale, 2011; Lu et al., 2011; Templer et al., 2012a; Niu et al., 2016) and denitrification (Morse et al., 2015; Templer et al., 2012b), increased tree stress and fine root mortality (Ferretti et al., 2015; Gundersen et al., 1998; Minocha et al., 2015; Smithwick et al., 2013), and altered microbial community structure and

## Core Ideas

- Chronic N enrichment increased N exports, although ecosystem N retention was still high.
- Soil was the largest ecosystem N pool, but it was not significantly altered by chronic N enrichment.
- Chronic N enrichment increased biomass N accumulation in hardwood but not in softwood stands.

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function (Carrara et al., 2018; Kopáček et al., 2013; Morrison et al., 2018; Treseder, 2008). The responses varied with the magnitude of N inputs, vegetation type, disturbance history, geographic location, climate, and the duration of the experiments.

Small watersheds are useful as ecological observatories, and long-term watershed studies allow us to understand complex and interactive ecological responses. Multi-decadal monitoring of N processes in the United States and Europe has demonstrated that short-term effects may not scale up to longer time scales. Studies at the Hubbard Brook Experimental Forest in New Hampshire, USA, revealed changes in N source–sink relationships between the 1970s and the early 2000s (Yanai et al., 2013). Multi-decadal N addition experiments at the Harvard Forest (Massachusetts, USA) and Mount Ascutney (Vermont, USA) documented accelerated growth of red spruce (*Picea rubens* Sarg.) trees in the initial years of treatment, followed by declines in productivity and increased mortality in the later years (Magill et al., 2004; McNulty et al., 2017). In the NITREX experiment in Gårdsjön, Sweden, N additions induced stream nitrate ( $\text{NO}_3^-$ ) leaching, although isotopic analysis indicated that the source of the leached N changed with time. Initially, leached N originated from the added N, but in the later years, the leached N originated from nitrification of the ‘old’ N present in the ecosystem prior to the manipulation (Moldan and Wright, 2011). Nave et al. (2009) reported significant temporal lags in soil response to N additions; mineral soils showed significant increases in C and N pools only after 15 to 20 yr of treatment. Climate variability and stochastic events in-

crease nitrification rates and stream  $\text{NO}_3^-$  exports, adding to the complexity of interpreting ecosystem N processes (Casson et al., 2010; Mitchell et al., 1996). The current climate trajectory in the northeastern United States of increasing temperature and precipitation, as well as increasing climate variability (Hayhoe et al., 2007; Salinger, 2005; Wuebbles et al., 2017), can also influence N transformations and availability. There is emergent evidence of N oligotrophication in temperate forests due to increased primary production, and plant and microbial N uptake, and declining N deposition (McLauchlan et al., 2017; Craine et al., 2018; Groffman et al., 2018).

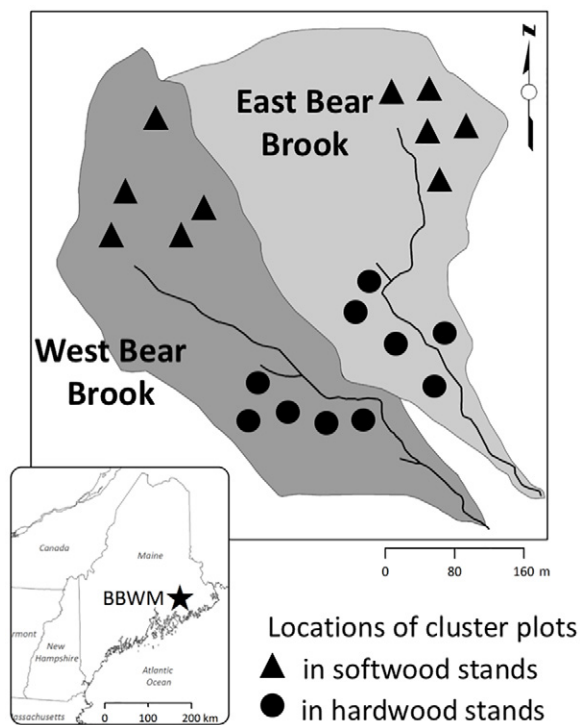
The Bear Brook Watershed in Maine (BBWM) is one of the few multi-decadal whole-ecosystem manipulation experiments in the world examining forest ecosystem response to chronic elevated N and sulfur (S) deposition. There, experimental bi-monthly additions of  $(\text{NH}_4)_2\text{SO}_4$  to one watershed have increased foliar N concentrations and tree growth (Elvir et al., 2005, 2006); increased soil available N (Jefts et al., 2004; Wang and Fernandez, 1999; Patel and Fernandez, 2018); altered microbial community composition and enzyme activity (Fatemi et al., 2016; Mineau et al., 2014; Stone et al., 2012; Tatariw et al., 2018; Wallenstein et al., 2006); altered soil organic matter composition and decomposition rates (Hunt et al., 2008; Ohno et al., 2007); and increased base cation leaching (Fernandez et al., 2010) and stream  $\text{NO}_3^-$  exports (Fernandez et al., 2010; Simon et al., 2010). Given the complex and often multi-decadal nature of ecosystem N processes, it is important to understand the interactions among ecosystem components, how these may change through time, and how these changes alter N dynamics within the ecosystem.

In this paper, we (i) examine the multi-decadal trend of input–output N fluxes and net ecosystem N retention, and (ii) estimate a contemporary mass balance for ecosystem soil and vegetation pools, after 25 yr of experimental whole-ecosystem N additions at BBWM.

## MATERIALS AND METHODS

### Site Description

The BBWM is a long-term whole watershed acidification experiment in eastern Maine, USA ( $44^\circ 52' \text{ N}$ ,  $68^\circ 06' \text{ W}$ ), established to study the effects of elevated N and S deposition on ecosystem processes (Fig. 1). The BBWM has two paired watersheds, the reference East Bear Brook (11.0 ha) and the manipulated West Bear Brook (10.3 ha), which was treated with bi-monthly applications of ammonium sulfate  $[(\text{NH}_4)_2\text{SO}_4]$  fertilizer from above the canopy, at the rate of  $28.8 \text{ kg S ha}^{-1} \text{ yr}^{-1}$  and  $25.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , beginning in November 1989 and continuing throughout the period of study reported here (1989–2014). Both watersheds are drained by first-order streams. Bedrock consists dominantly of quartzite, phyllite, and calc-silicate low-grade metasediments, and minor granite dikes (Norton et al., 1999; SanClements et al., 2010). Soils, developed from till, are coarse-loamy, mixed, frigid Typic and Aquic Haplorthods. Average annual air temperature (2005–2014) at the site was  $5.6^\circ \text{C}$  (Patel



**Fig. 1. Design and location (inset) of Bear Brook Watershed in Maine (BBWM), with the paired watersheds East Bear Brook (reference) and West Bear Brook (treated). Map data sources not generated by BBWM team are as follows: United States state outlines are from the National Atlas of the United States; Canadian Provinces are from the National Weather Service.**

et al., 2018a, 2018b), and average annual precipitation (2005–2014) was 140 cm. Vegetation was similar in both watersheds, with lower elevations dominated by hardwood species, primarily *Fagus grandifolia* Ehrh. (American beech), *Acer saccharum* Marsh. (sugar maple), *Acer rubrum* L. (red maple), and *Betula alleghaniensis* Britt. (yellow birch); higher elevations were dominated by softwood species, *Picea rubens* Sarg. (red spruce) and *Abies balsamea* L. (balsam fir) (Wang and Fernandez, 1999). The research site was divided into four compartments by N treatment and forest type: East Bear hardwoods, East Bear softwoods, West Bear hardwoods, and West Bear softwoods. Five cluster plots (10 m by 15 m) were established in each compartment.

## Input–Output Nitrogen Fluxes

Precipitation and stream samples were collected during 1989 to 2014 to calculate input and output N fluxes, respectively. Wet-only precipitation was collected using an AeroChem-Metrics precipitation collector (Norton et al., 1999). Stream samples were collected as grab samples and event samples (e.g., during snowmelt and rain events) using ISCO automated samplers from both East Bear and West Bear streams to calculate dissolved inorganic N (DIN) exports. During the first half of the study period, precipitation and stream samples were collected weekly and during high stream-flow events (up to 300 samples per year). During the second half of the study period, sampling frequency was reduced, and precipitation and stream samples (>50 samples per year) were collected biweekly or monthly, and during selected hydrologic events. Measured stream chemistry from base sampling plus ISCO event samplers was interpolated between collections and coupled with hourly stream discharge to develop monthly and annual streamwater fluxes (Kahl et al., 1999; Norton et al., 2010). Precipitation and stream samples were analyzed for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  using ion chromatography at the University of Maine Sawyer Environmental Research Center. Dry deposition was not measured at the site, so for this analysis we used reported estimates of annual dry deposition from the CASTNET Howland station (HOW132), located ~60 km northwest of BBWM (CASTNET, 2018). The annual wet deposition overlapping values for the Howland station and BBWM were strongly correlated (Pearson's  $r = 0.7735$ ,  $p < 0.0001$ ). Annual ecosystem inorganic N retention was:

$$\text{retention, kg ha}^{-1} = \text{input} - \text{output} \quad [1]$$

$$\text{retention, \%} = (\text{input} - \text{output})/\text{input} \times 100 \quad [2]$$

where input refers to (wet + dry) inorganic N deposition and output refers to stream inorganic N exports. Ecosystem N retention values are reported in Table 1.

Organic N does not contribute significantly to the N retention mass balance at our site (see the Discussion section), and therefore inorganic N retention closely approximates total N retention at BBWM. Annual inputs, outputs, and retention were calculated for the water year from October 1 to September 30. The calculations for ecosystem inorganic N retention in Eq. [1]

**Table 1. Estimated input and output fluxes of inorganic N for East Bear and West Bear watersheds. Input–output fluxes are calculated for the 5-yr period from 2010 to 2014.**

| Input–output fluxes      | East Bear Brook                        | West Bear Brook |
|--------------------------|--|-----------------|
|                          | kg N ha <sup>-1</sup> yr <sup>-1</sup> |                 |
| Input (deposition)       | 2.0                                    | 27.0            |
| Output (stream export)   | 0.2                                    | 7.0             |
| Retention (input–output) | 1.8                                    | 20.0            |

and [2] use (wet + dry) inorganic N ( $\text{NH}_4^+$ -N plus  $\text{NO}_3^-$ -N) deposition as annual inputs and stream inorganic N exports as annual outputs. We assumed that both watersheds received similar amounts of ambient N deposition, with West Bear receiving an additional 25.2 kg N ha<sup>-1</sup> yr<sup>-1</sup> as treatment. These calculations do not account for forest canopy influence on inputs or fluxes such as N fixation or denitrification, both of which are likely small. These unaccounted fluxes are discussed in detail in the Discussion section.

## Ecosystem Nitrogen Pools

Samples for chemical characterization of soils, trees, loose litter, and ground vegetation were collected from 2010 to 2014. We calculated ecosystem N pools for each component by multiplying the mass per area (kg ha<sup>-1</sup>) by total N concentrations (%).

### Soils

Soils were collected from 40 quantitative pits (ten in each of four compartments) in the summer of 2010 using methods comparable to those of SanClements et al. (2010). The pits were excavated to the bottom of the solum (1 m) or to contact with basal till or bedrock. Organic soils (O horizon) were quantitatively removed to the top of the mineral soil within 71 cm by 71 cm frames. Mineral soil (B horizon) was quantitatively removed in depth increments 0 to 5 cm, 5 to 25 cm, and 25 cm to the top of the C horizon. Grab samples were collected from the C horizon. Where present, the E horizon depth was measured but not sampled for chemistry. Soils were transported to the laboratory, air-dried, sieved (<6 mm screen for organic soil, and <2 mm screen for mineral soil), and analyzed for total N concentrations using a LECO CN-2000 Analyzer at the Maine Agriculture and Forest Experiment Station (MAFES) Analytical Laboratory. The soil total N concentrations are presented in Supplemental Table S4. The soil N pool was calculated as the sum of organic (coarse and fine) and mineral (fine) fraction.

### Tree Biomass

Tree biomass for leaf, branch, stem, and stump-root components was calculated using allometric equations (Young et al., 1980):

$$\ln(\text{biomass, lbs}) = B_0 + B_1 * \ln(\text{DBH, in}) \quad [3]$$

where DBH is the diameter at breast height, and  $B_0$  and  $B_1$  are species-specific coefficients for each tree component (Supplemental Table S1). Tree DBH was measured in July 2014 for 861 trees in 20 plots of 400 m<sup>2</sup>, centered around and inclu-



sive of the five cluster plots in each compartment. Biomass was converted to kilograms for further calculation and analysis. The stem biomass values were divided into wood and bark using data from Freedman et al. (1982), who reported that wood comprised 88% of total stem biomass in maple–beech–birch stands, and 87% of total stem biomass in red spruce–balsam fir stands. Individual tree biomass values within each 400 m<sup>2</sup> plot were added to determine biomass per area (kg 400 m<sup>−2</sup>) and then scaled up to hectare (kg ha<sup>−1</sup>). These values (kg ha<sup>−1</sup>) were averaged to calculate compartment-level estimates. Watershed-level (East Bear and West Bear) biomass was calculated as weighted averages of the compartment-level values, using the relative distribution of the two forest types in each watershed (Wang and Fernandez, 1999). The forest cover in West Bear was 51.6% hardwood and 48.4% softwood, and in East Bear was 63.7% hardwood and 36.3% softwood.

### Tree Chemistry

Foliage was sampled from the mid-crown in the upper canopy (i.e., sun-lit leaves) using pole pruners in August of 2010, 2012, and 2013. Five trees of each dominant species (American beech, sugar maple, red maple, yellow birch, and red spruce) were sampled in each watershed (i.e., 25 trees per watershed) each year. Leaves showing signs of disease were excluded. Foliar samples were rinsed with deionized water and blotted dry. Wood and bark samples were obtained from tree cores collected in 2011. One hundred trees were sampled across the five dominant species in each watershed. For a subset of these cores, rings from 2009 to 2011 were processed for wood N. Stump–root and branch samples were not collected for chemical analysis. We used wood N values for stump–roots and woody litterfall (i.e., twigs) values for branches. Woody litter was collected during 2012 and 2013, using four traps (52.5 cm by 37 cm) in each cluster plot. The samples were dried and sorted into hardwood and softwood tissues prior to analysis.

### Loose Litter

Loose litter was surface litter tissues that were not yet mechanically attached to the forest floor and could be readily brushed aside without resistance. Loose litter was collected during the growing season of 2012. Samples were collected in triplicate from each cluster plot using a 10 cm by 10 cm template, and pooled for each cluster plot for chemical analysis.

### Understory Vegetation

Understory vegetation was sampled during the growing season of 2012. The five most dominant species (<60 cm high) were clipped at the ground surface from a 5 m by 5 m area within each cluster plot. The samples were rinsed with deionized water and blotted dry separately by species.

All vegetation samples were dried in a drying room at ~60°C, weighed, ground to <1 mm using a Wiley mill, ground in a ball mill, and analyzed for total N using an elemental analyzer at the University of California Davis Stable Isotope Facility. The

N concentrations for each vegetation component by species are reported in Supplemental Table S3.

### Tree Nitrogen Accumulation

The tree increment cores collected (from 100 trees) in 2011 were used to determine increment growth. Increment cores were digitized and ring widths were measured using WinDENDRO (Regent Instruments, Inc.). The average ring width values for 2007 to 2011 were used as estimates of recent tree ring increment for the five dominant tree species. Tree ring increment data were used to “hindcast” historical annual biomass accumulation based on the 2014 values (Supplemental Table S2). To calculate N accumulation rates in trees, we multiplied the biomass growth rate by the average N concentrations described above. These N concentration values represent the 2014 sampling effort, and N accumulation rates in trees are therefore a result of biomass accumulation.

### Statistical Analysis

Two-tailed *t* tests were used to compare total N concentrations and accumulation rates between watersheds for each given ecosystem component (Tables 2 and 3). Statistical significance was determined at  $\alpha = 0.05$ . Linear regressions were performed to compare annual stream N exports with temperature and precipitation, and we report the Pearson correlation coefficients (*r*) for these relationships, with *r* values >0.50 indicating strong correlation and *p* < 0.05 indicating statistical significance for strong correlations. All statistical analyses were conducted using JMP software, Version 13.

## RESULTS

### Nitrogen Deposition

Total ambient N deposition (wet + dry) in East Bear and West Bear, as NH<sub>4</sub><sup>+</sup> plus NO<sub>3</sub><sup>−</sup> deposition, ranged from 2.3 to 5.4 kg N ha<sup>−1</sup> yr<sup>−1</sup> (mean 3.7 kg N ha<sup>−1</sup> yr<sup>−1</sup>) for 1990 to 2014 (Fig. 2 and 3). Calculated dry deposition accounted for ~2 to 5% of the annual ambient N inputs during this period. Total ambient NH<sub>4</sub><sup>+</sup> deposition (wet + dry) in East Bear ranged from 1 to 2 kg N ha<sup>−1</sup> yr<sup>−1</sup> and was 28 to 49% of the total annual inorganic N deposition. Total inorganic N inputs (ambient + treatment) in West Bear ranged from 27.5 to 30.9 kg N ha<sup>−1</sup> yr<sup>−1</sup> (mean 29.0 kg N ha<sup>−1</sup> yr<sup>−1</sup>) and NH<sub>4</sub><sup>+</sup> accounted for 88 to 95% of total inorganic N inputs. Total ambient inorganic N deposition declined significantly over the study period from 1990 to 2014 (*r* = −0.6291, *p* = 0.0006) attributable to a 60% decline in NO<sub>3</sub><sup>−</sup> deposition (*r* = −0.7587, *p* < 0.0001), while NH<sub>4</sub><sup>+</sup> deposition remained unchanged (*r* = −0.2253, *p* = 0.2684).

### Stream DIN Export

Stream DIN exports ranged from below-detection to 2.1 kg N ha<sup>−1</sup> yr<sup>−1</sup> (mean 0.4 kg N ha<sup>−1</sup> yr<sup>−1</sup>) in East Bear and from 2 to 10 kg N ha<sup>−1</sup> yr<sup>−1</sup> (mean 5.6 kg N ha<sup>−1</sup> yr<sup>−1</sup>) in West Bear (Fig. 2 and 3). Exports from both watersheds were dominated by NO<sub>3</sub><sup>−</sup> (~86 and ~98% of annual inorganic N export in East Bear and West Bear, respectively). In East Bear, consistent

**Table 2. Nitrogen pools in ecosystem components in each compartment of N treatment and forest type (East Bear hardwoods, East Bear softwoods, West Bear hardwoods, and West Bear softwoods) after 25 yr of N additions.**

| Pool  | East Bear Brook (reference) |      | West Bear Brook (treated) |      |
|---|-----------------------------|------|---------------------------|------|
|   | Mean                        | SE   | Mean                      | SE   |
|   | kg N ha <sup>-1</sup>       |      |                           |      |
|   | <b>Hardwoods</b>            |      |                           |      |
| Total vegetation N  | 640                         | 53   | 969*†                     | 69   |
| Foliage   | 100                         | 7    | 141*                      | 15   |
| Woody biomass   | 459                         | 38   | 710                       | 66   |
| Branches  | 103                         | 8    | 207*                      | 25   |
| Wood  | 207                         | 19   | 261                       | 25   |
| Bark  | 78                          | 6    | 151*                      | 18   |
| Stump-root  | 71                          | 6    | 90                        | 9    |
| Ground vegetation   | 0.19                        | 0    | 0.33                      | 0    |
| Loose litter  | 81                          | 11   | 118                       | 15   |
| Total pedon N   | 8170                        | 1325 | 8157                      | 753  |
| Organic soil  | 1781                        | 228  | 1729                      | 326  |
| Mineral soil  | 6389                        | 1215 | 6428                      | 816  |
|   | <b>Softwoods</b>            |      |                           |      |
| Total vegetation N  | 1028                        | 59   | 1311*                     | 78   |
| Foliage   | 269                         | 18   | 347*                      | 22   |
| Woody biomass   | 607                         | 51   | 837                       | 52   |
| Branches  | 189                         | 15   | 215                       | 14   |
| Wood  | 248                         | 21   | 381*                      | 24   |
| Bark  | 71                          | 7    | 88                        | 5    |
| Stump-root  | 99                          | 8    | 152*                      | 10   |
| Ground vegetation   | 0.24                        | 0    | 2.20                      | 2    |
| Loose litter  | 153                         | 33   | 126                       | 27   |
| Total pedon N   | 10018                       | 1707 | 10421                     | 910  |
| Organic soil  | 2422                        | 335  | 3523*                     | 364  |
| Mineral soil  | 7597                        | 1625 | 6898                      | 1020 |
| <b>Total N in watershed (hardwood and softwood, vegetation and soil)‡</b> |                             |      |                           |      |
|   | 9623                        |      | 10387                     |      |

† Two-tailed *t* tests were used to examine differences between watersheds; asterisks denote significant differences between watersheds at  $\alpha = 0.05$ .

‡ Total N in the watershed refers to the sum of soil and biomass N pools, as aerally weighted averages of the hardwoods and softwoods.

Table 3. Estimated biomass increment N accumulation for East Bear and West Bear watersheds. Tree biomass N accumulation is calculated using tree increment cores for the 5-yr period from 2007 to 2011.

| Tree biomass N pool | East Bear Brook (reference)                        |      | West Bear Brook (treated) |     |
|---------------------|--|------|---------------------------|-----|
|                     | Mean   | SE   | Mean                      | SE  |
|                     | kg N ha <sup>-1</sup> yr <sup>-1</sup>             |      |                           |     |
|                     | <b>Hardwoods</b>                                   |      |                           |     |
| Total tree biomass  | 15.5   | 1.2  | 22.3*†                    | 1.2 |
| Leaves              | 2.6  | 0.2  | 3.3*                      | 0.2 |
| Branches            | 3.0  | 0.4  | 5.7*                      | 0.4 |
| Wood                | 5.7  | 0.30 | 6.8*                      | 0.3 |
| Bark                | 2.3  | 0.3  | 4.2*                      | 0.3 |
| Stump-root          | 1.9  | 0.1  | 2.3*                      | 0.1 |
|                     | <b>Softwoods</b>                                   |      |                           |     |
| Total tree biomass  | 24.6   | 1.9  | 24.1                      | 1.9 |
| Leaves              | 7.3  | 0.6  | 6.7                       | 0.6 |
| Branches            | 5.4  | 0.4  | 4.5                       | 0.4 |
| Wood                | 7.1  | 0.6  | 7.9                       | 0.6 |
| Bark                | 2.0  | 0.1  | 1.9                       | 0.1 |
| Stump-root          | 2.8  | 0.2  | 3.1                       | 0.2 |
|                     | <b>Entire watershed (hardwoods and softwoods)‡</b> |      |                           |     |
|                     | 18.8   |      | 23.2                      |     |

† Two-tailed *t* tests were used to examine differences between watersheds; asterisks denote significant differences between watersheds at  $\alpha = 0.05$ .

‡ Biomass accumulation rates for the entire watershed were calculated as aerially weighted averages of the hardwoods and softwoods.

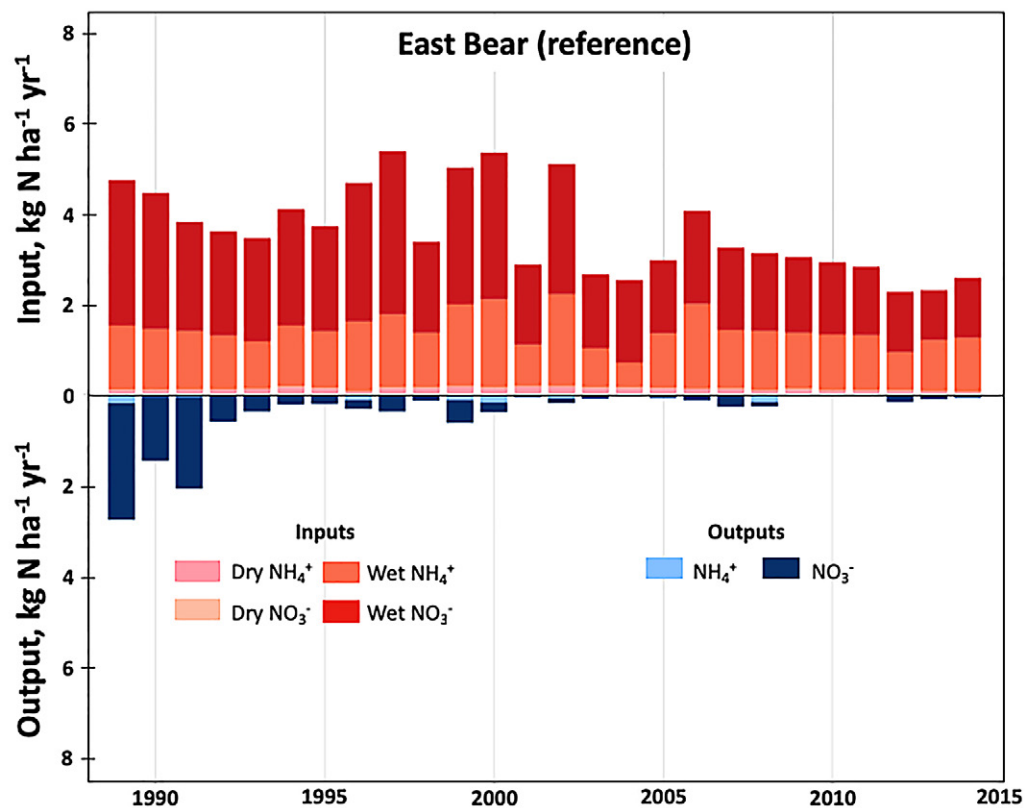


Fig. 2. Input and output inorganic N fluxes for East Bear Brook (reference watershed). The x axis represents the water year, October 1 to September 30.

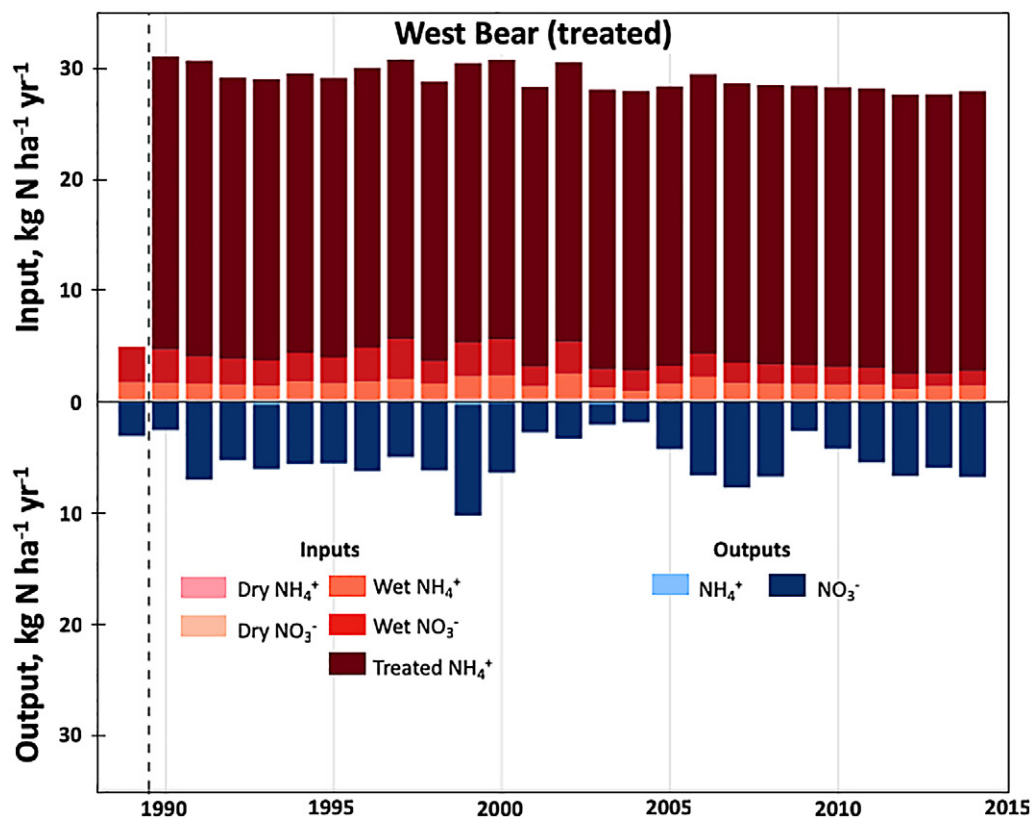
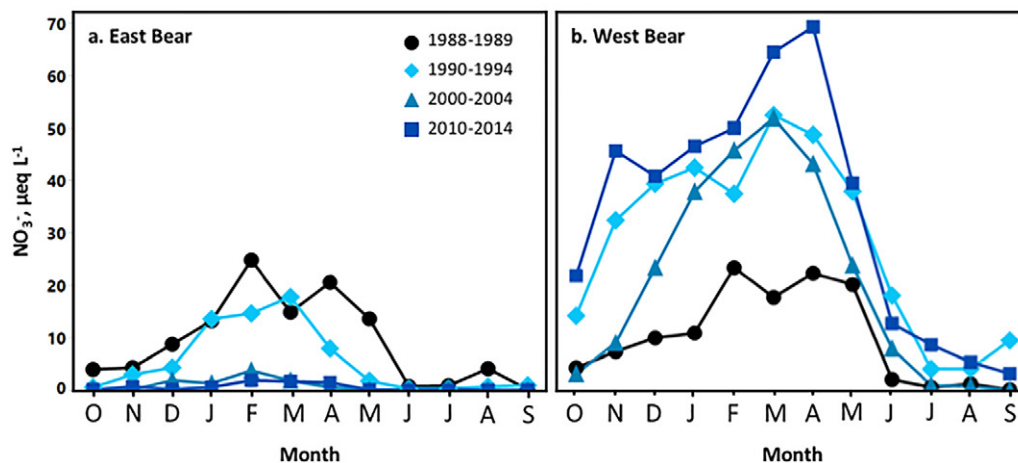


Fig. 3. Input and output inorganic N fluxes for West Bear Brook (treated watershed). The x axis represents the water year, October 1 to September 30. The dashed vertical line represents onset of treatment in West Bear Brook.



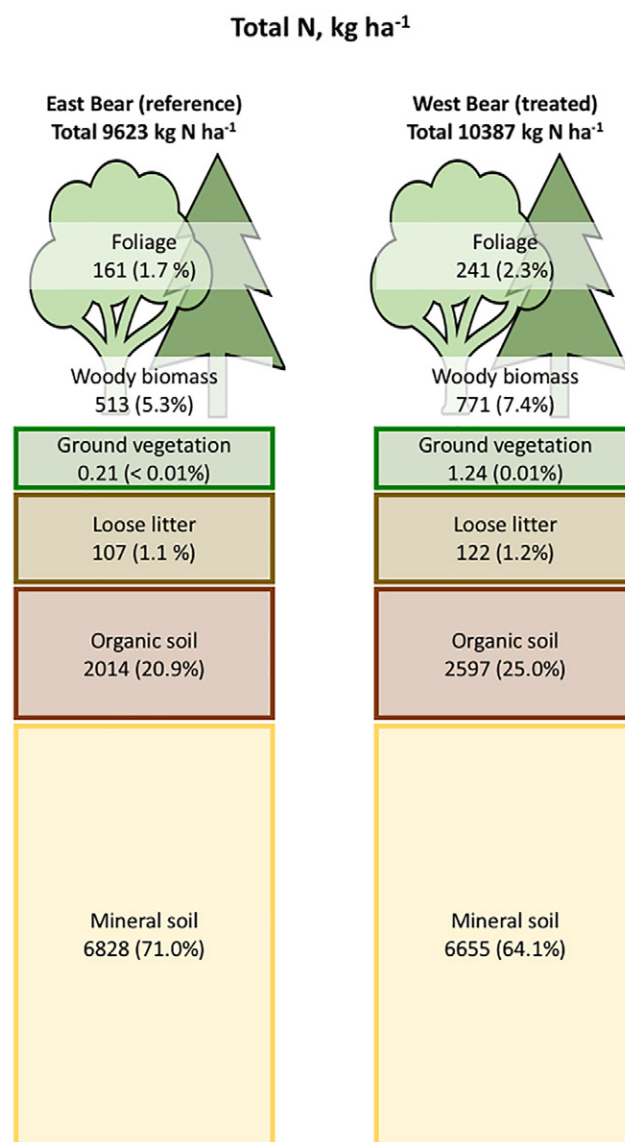
**Fig. 4.** Monthly stream  $\text{NO}_3^-$  concentrations during the study period for (a) East Bear Brook (reference watershed) and (b) West Bear Brook (treated watershed). 1988 to 1989 represents pre-treatment data for West Bear. Data for 1990 to 2014 represent 5-yr intervals during the first, second, and third decades of manipulation.

with temporal trends in ambient N deposition, inorganic N exports declined significantly (87% decline) from 1990 to 2014 ( $r = -0.6557$ ,  $p = 0.0005$ ), but there was no statistically significant long-term trend in West Bear ( $r = 0.0239$ ,  $p = 0.9119$ ). Over the course of the study, East Bear retained 83 to nearly 100% (mean  $94\% \pm \text{SE } 2.5$ ) or  $\sim 3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , and West Bear retained 66 to 93% (mean  $81\% \pm \text{SE } 1.4$ ), or  $\sim 23 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , of total inorganic N inputs. Table 1 shows recent (2010–2014) input-output fluxes and mass balance of inorganic N for both watersheds. Stream  $\text{NO}_3^-$  concentrations showed a distinct seasonal pattern (Fig. 4), peaking during the spring months ( $2.21 \mu\text{eq L}^{-1} \pm \text{SE } 0.24$  in East Bear and  $62.7 \mu\text{eq L}^{-1} \pm \text{SE } 19.8$  in West Bear, for 2010–2014) and decreasing to ‘base flow’ concentrations in the summer months (below-detection in East Bear and  $8.83 \mu\text{eq L}^{-1} \pm \text{SE } 1.94$  in West Bear, for 2010–2014).

### Ecosystem Nitrogen Pools

Figure 5 shows pools of N for each watershed, and Table 2 shows N pools by compartment 20 to 25 yr after the fertilizer treatment was initiated. The vegetation N pool accounted for  $\sim 7$  to 11% of total ecosystem N, across both forest types and both watersheds (Table 2). Overall, vegetation in West Bear had  $\sim 1.3$ -fold more N per hectare than in East Bear. Tree foliage accounted for  $\sim 15\%$  of the total vegetation N pool in the deciduous forests, and  $\sim 26\%$  in the coniferous forests. This was consistent between the reference and the treated watersheds (Table 2). Both foliage and woody biomass had  $\sim 1.4$ -fold more N in West Bear than in East Bear, but the ground vegetation and leaf litter N pools did not differ between the two watersheds. Trees in hardwood stands had greater N accumulation rates in West Bear than in East Bear (Table 3), whereas this was not the case in softwood stands. Overall, biomass accumulation of N was  $\sim 19 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in East Bear and  $\sim 23 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in West Bear for the period 2007 to 2011.

The largest pool of N among the ecosystem components was the mineral soil ( $>6600 \text{ kg N ha}^{-1}$  in both watersheds, 63–70% of total ecosystem N), although N content did not dif-



**Fig. 5.** Ecosystem N pools ( $\text{kg N ha}^{-1}$ ) at Bear Brook Watershed in Maine after 25 yr of N additions. Values in parentheses reflect percentages of the total watershed N pool.



fer significantly between the two watersheds (Table 2; Fig. 5). In the softwood stands, organic horizons in West Bear had ~1.5-fold more N compared to East Bear, but in the hardwood stands, organic horizon N content did not differ significantly between watersheds (Table 2).

## DISCUSSION

### Watershed Dissolved Inorganic N Retention

The high DIN retention in the reference East Bear was likely driven by low N deposition and a high C to N ratio in these soils. Nitrogen is generally retained in forest systems via microbial immobilization (Vitousek and Matson, 1984; Johnson et al., 2000; Perakis et al., 2005; Templer et al., 2005; Piatek and Adams, 2011), incorporation into plant biomass (Fenn et al., 1998; Templer et al., 2005; Emmett, 2007; Schleppi et al., 2017), or abiotic immobilization, mainly incorporation into soil organic matter (Johnson et al., 2000; Fitzhugh et al., 2003; Perakis et al., 2005). The higher N exports in West Bear likely reflected that these N retention mechanisms were exceeded by the higher N inputs. Additions of  $\text{NH}_4^+$  stimulate nitrification by providing labile  $\text{NH}_4^+$  substrate, and also inhibit plant  $\text{NO}_3^-$  uptake by suppressing the production and activity of  $\text{NO}_3^-$  assimilatory enzymes (Emmett, 2007) and by suppressing the N-uptake activity of mycorrhizae (Högberg et al., 2007). These mechanisms accelerate  $\text{NO}_3^-$  leaching losses. At the NITREX study sites across Europe, Dise and Wright (1995) found that  $\text{NO}_3^-$  leaching increased with increasing N deposition, showing that the N retention mechanisms were stronger at sites receiving  $<10 \text{ kg N ha}^{-1}$  annually. At the Fernow Experimental Forest in West Virginia, USA, Gilliam et al. (2018) found that although stream  $\text{NO}_3^-$  increased, soil nitrification rates did not consistently increase in response to N additions. Instead, they attributed increased stream  $\text{NO}_3^-$  exports to reduced biological uptake. At our site, we observed greater nitrification rates in West Bear than in East Bear (Patel and Fernandez, 2018), which likely contributed partially to the elevated stream exports.

### Long-term Watershed Input–Output N Fluxes

Significant declines in ambient atmospheric N deposition at BBWM from 1990 to 2014 were attributable to a combination of (i) emissions reductions from stationary sources resulting from air pollution policies enacted during this period, such as Titles IV ( $\text{SO}_2$ ,  $\text{NO}_x$ ) and VI (ozone) of the US Clean Air Act Amendments (CAAA) of 1990 and Phase I controls of the CAAA in 1995 (Du et al., 2014; Kahl et al., 2004); and (ii) reduced vehicular emissions in the northeastern United States (Butler et al., 2003).

Stream DIN exports in East Bear declined from 1989 to 1993, corresponding to a regional decline of elevated surface water  $\text{NO}_3^-$  concentrations, following severe winter frost prior to 1989 (Mitchell et al., 1996). This short-term phenomenon ended in ~1993 as East Bear exports returned to chronically low values. Subsequent declines in East Bear stream  $\text{NO}_3^-$  largely reflected the long-term trends in declining N inputs, and immobilization

in the soil. The experimental N additions ( $25.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) were originally thought to represent a fourfold increase in N loading in West Bear compared to East Bear, using measured values of wet deposition and estimates of dry deposition at the beginning of the study (Norton et al., 1999). The data reported here suggest ambient plus experimental inputs to West Bear may have been up to ~7-fold the East Bear inputs at the start of the experiment, because of improvements in the estimated dry deposition of N. With declining ambient N deposition, West Bear was receiving up to ~12-fold the East Bear N inputs by 2014 as a result of declining ambient N deposition. Experimental N additions increased  $\text{NO}_3^-$  exports in West Bear within four months after the start of the experiment. Tracer  $^{15}\text{N}$  experiments in 1991 to 1993 revealed that most of the initial excess stream  $\text{NO}_3^-$  exports did not originate from the added  $\text{NH}_4^+$ . Rather, the treatments stimulated nitrification of the resident, 'old' N in the ecosystem (Nadelhoffer et al., 1999). Changes in the isotopic signature of stream  $\text{NO}_3^-$  indicated a greater contribution of the added fertilizer to stream  $\text{NO}_3^-$  after 1993. This is consistent with N mineralization results from BBWM indicating that N mineralization and nitrification rates also increased in West Bear after 1993 (Fernandez et al., 2000; Jeffs et al., 2004; Patel and Fernandez, 2018; Wang and Fernandez, 1999).

Long-term patterns of DIN export in the West Bear stream revealed significant inter-annual variability. Stream N export was not strongly correlated with mean annual precipitation (Pearson's  $r = 0.36$ ,  $p = 0.007$ ) or air temperatures (Pearson's  $r = 0.33$ ,  $p = 0.010$ ). Therefore, other factors likely contributed to the inter-annual variability evident in the long-term record, some of which were stochastic disturbance events. For example, higher DIN exports occurred in 1999, evident in both watersheds, which we attribute to the ice storm of 1998 that deposited ~13 cm of ice in central Maine, a major disturbance event for Maine forests (Irland, 1998). Some damage to the forest canopy at BBWM as a result of the 1998 ice storm occurred in components of the watershed. Anecdotally, greater damage occurred in the hardwood components, although no quantitative studies were done. The ice storm resulted in increased litter inputs to the forest floor and increased forest floor insolation because of damages to the canopy. In combination, these altered processes resulted in a short pulse of DIN losses in BBWM streams (Houlton et al., 2003; SanClements et al., 2010). Additional disturbances from wind storms and defoliations by caterpillar infestations occurred over the period of record, but these were not quantitatively assessed for their effects on the long-term DIN export record.

### Ecosystem Nitrogen Mass Balance

The relative sizes of ecosystem N pools at BBWM reflect the general distribution of N common in temperate upland forests of northeastern North America, with soils comprising the biggest N pools (e.g., Mitchell et al., 1992; Kelly et al., 2011; Yanai et al., 2013). During the 25 yr of treatment, the cumulative retention of DIN in East Bear was  $81 \text{ kg N ha}^{-1}$  and the cumulative retention of DIN in West Bear was  $582 \text{ kg N ha}^{-1}$ .

In the reference East Bear watershed, the vegetation N accumulation rate was an order of magnitude greater than the annual ecosystem N retention (Tables 1 and 3), revealing an additional source of N,  $\sim 17 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , beyond atmospheric N contributions. Trees in northeastern North America have been reported to mine N from the mineral soil during periods of stand development, i.e., the soil acts as a net source of N (Hooker and Compton, 2003; Yanai et al., 2013). Mineral soil N is present as plant-available inorganic forms ( $\text{NH}_4$  and  $\text{NO}_3$ ), or as organic N forms in microbial biomass and soil organic matter, the latter being by far the larger pool (Jefts et al., 2004). Organic N can be mineralized into plant-available forms in support of plant uptake. Mining of inorganic N from soil was documented at BBWM in the early 1990s (Nadelhoffer et al., 1999), and our calculations suggest a net soil N uptake by vegetation persists to at least 2014.

Tracer  $^{15}\text{N}$  isotopic studies during 1991 to 1993 showed that the soil was the major sink of the added labeled N (Nadelhoffer et al., 1999). Based on mass balance calculations for the data presented in this paper, soils continued to be a major sink of the added N even after 25 yr of N enrichment. However, the relative size of the total soil N pools (Fig. 5; Table 3) and the high spatial variability in our soils (see the SE values in Table 1) limit our statistical power to observe significant differences between East Bear and West Bear soil N pools. An analysis of temporal trends in soil total N concentrations also showed that West Bear soil N concentrations did not change through time despite 25 yr of N amendments (Patel and Fernandez, 2018). The minimum detectable change (for 95% confidence intervals) for these data was  $\sim 380 \text{ kg N ha}^{-1}$  for organic horizons,  $1175 \text{ kg N ha}^{-1}$  for mineral horizons, and  $1234 \text{ kg N ha}^{-1}$  for the total pedon. This suggests that even if all the N retained in West Bear ( $582 \text{ kg N ha}^{-1}$ ) were stored in the soil, we would not be able to statistically detect the change in total soil N from the treatment in West Bear soils based on the sampling intensity in this study.

In the softwood stands, West Bear organic horizons had significantly more total N than East Bear (Table 2), because West Bear soils had a significantly greater soil mass (Supplemental Table S2). Nitrogen enrichment suppresses organic matter decomposition, particularly for high-lignin litter (Edwards et al., 2011; Frey et al., 2014; Morrison et al., 2018; Van Diepen et al., 2015). Slower decomposition of high-lignin softwood litter in West Bear could contribute to the thicker horizons in these stands. This is consistent with findings by Tatariw et al. (2018) that N additions increased C-limitation for microbes in West Bear organic horizons, indicating that the N additions had altered the decomposer community. In contrast, neither organic horizon thickness nor total N differed between watersheds in the hardwood stands, suggesting that if N additions were suppressing rates of organic matter decomposition in softwood stands, hardwood stands did not reflect a similar response. Experimental N additions also increased litter-fall mass in softwood stands but not in hardwood stands at the Harvard Forest (Frey et al., 2014). It is possible that increased litter inputs could also have contributed to the thicker organic horizons in the West Bear softwood stands at BBWM.

Our results highlight the importance of forest type on net ecosystem N retention, consistent with previous studies that demonstrated species-specific effects on N cycling (Booth et al., 2005; Oulehle et al., 2018; Templer et al., 2005). The  $\sim 45\%$  greater biomass N increment (Table 3) in West Bear hardwood compared to East Bear hardwood stands suggests N additions reduced N limitations resulting in accelerated biomass and N annual increment in the treated watershed. In contrast, biomass N increment was similar between West Bear softwoods and East Bear softwoods (Table 3). It is possible that experimental N additions in West Bear, combined with the relatively low rates of historical atmospheric deposition compared to more highly impacted sites farther southwest (McNulty et al., 2007; Pardo et al., 2011) were not sufficient to increase soil N availability to a level that resulted in a detectable increase in softwood growth. Another possible reason for the lack of softwood response is limitation by other nutrients. Elvir et al. (2010) attributed the apparent lack of tree growth response to added N at BBWM to the lower base cation availability in the West Bear softwood soils, caused by the N and S additions. Indeed, studies previously conducted at BBWM provide evidence that softwoods had approximately half the soil exchangeable Ca and Mg of hardwood soils (Fernandez et al., 2003; SanClements et al., 2010), suggesting other limiting nutrients in softwood stands may be limiting the growth response to N additions. While the focus of this study is on the effects of long-term N additions at BBWM, the treatment also added S which played an important role in altering conditions in West Bear such as soil base cations and Al. The effects of the S additions on the responses in ecosystem N dynamics reported here are poorly understood and are not explored in this analysis.

Despite no significant increase in biomass N accumulation in the softwoods (Table 3), the softwood stands in West Bear did show significantly greater biomass N pools in 2014 (Table 2). This suggests that there may have been a growth response in the softwood stands in the earlier years of the experiment that are no longer occurring, although we do not have empirical data to quantify this pattern over time. The lack of growth response in the softwood stands suggests that if the added N was not taken up by the trees, it was either retained in the soil or lost via leaching. Studies conducted using ion-exchange resins in soils (Szillery et al., 2006) and lysimeters (Fatemi et al., 2012) demonstrated that  $\text{NO}_3^-$  leaching was greater in West Bear softwoods than in West Bear hardwoods reflecting the consequence of the lower N uptake in softwoods and the relative absence of ground vegetation compared with the hardwood stands.

Other studies reporting increased foliar and/or total biomass N accumulation in conifers [e.g., Harvard Forest, USA (Magill et al., 2000) and Gårdsjön, Sweden (Kjonaas and Stuanes, 2008)] added significantly more N to their ecosystems ( $\geq 50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , compared to  $25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  at BBWM). In contrast to these studies, Lovett et al. (2013) found that additions of  $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  did not alter aboveground biomass growth in the Catskill Mountains of New York, USA, likely because of the high historical N deposition, the advanced age of the forest, and/or limitation by some other nutrient.

## Nitrogen Saturation and Critical Loads Exceedance

Substantial research has been conducted over more than three decades to improve our understanding of forest ecosystem effects of N enrichment, including the development of the concept of N saturation, and the identification of important thresholds of ecological and biogeochemical responses. The conceptual model of Aber et al. (1989, 1998) described N saturation in terms of N mineralization and plant productivity. According to this framework, the reference East Bear watershed was in Stage 0 (N limitation of plant growth and decomposition), and the treated West Bear was in Stage 1 (increased foliar N concentrations and increased biomass) during the third decade of treatment. The conceptual model of Stoddard (1994) defined N saturation in terms of ecosystem N retention and  $\text{NO}_3^-$  export. At the start of this study (1988–1999), both East Bear and West Bear were in Stage 1 (higher spring  $\text{NO}_3^-$  concentrations compared to stage 0), reflecting the transient winter frost effects discussed earlier during the initial years of this study. Subsequent declines in East Bear stream  $\text{NO}_3^-$  resulted in East Bear transitioning back to a Stage 0 status (low  $\text{NO}_3^-$  concentrations during base flow, with higher concentrations during snowmelt and spring runoff) by the mid-1990s, which persisted to at least 2014. In West Bear, N additions increased  $\text{NO}_3^-$  exports almost immediately, and the watershed has remained at Stage 2 (increased baseflow  $\text{NO}_3^-$  concentrations) through 2014. Despite chronic multi-decadal elevated N inputs to West Bear, the watershed has not progressed over time to more advanced stages of N enrichment, and has not shown increases in tree mortality or increasing stream  $\text{NO}_3^-$  export.

Critical loads have been used to describe thresholds of N deposition above which detrimental ecological effects are reported (Pardo et al., 2011). Critical loads of 8 to 10 kg N ha<sup>-1</sup> yr<sup>-1</sup> are generally recognized for significant  $\text{NO}_3^-$  leaching in temperate forests of North America and Europe (Campbell et al., 2004; Dise et al., 2009; Pardo et al., 2011; Watmough et al., 2005). Our treatment of 25.2 kg N ha<sup>-1</sup> yr<sup>-1</sup> was more than double this threshold and, as expected, we saw increased stream  $\text{NO}_3^-$  exports in West Bear (Fig. 3 and 4). The low critical loads for mycorrhizae and lichens (<7 kg N ha<sup>-1</sup> yr<sup>-1</sup>; Pardo et al., 2011) suggest that the community structure and functions of these indicators have likely been altered in West Bear. Studies are currently underway to examine mycorrhizal communities at BBWM.

Over the past three decades, improvements in air quality have reduced N deposition in the northeastern United States, potentially altering the N status of temperate forest ecosystems (Groffman et al., 2018; Patel and Fernandez, 2018). The concomitant increases in air temperatures and atmospheric carbon dioxide concentrations (Wuebbles et al., 2017) have stimulated biomass growth (Kosiba et al., 2018), potentially increasing plant N demand. There is also evidence of increased C flow to soils increasing soil C availability and potentially stimulating microbial N immobilization in temperate forests (Groffman et al., 2018). The increased biological N uptake, combined with reduced atmospheric N inputs, have reduced the available N in soils, and there is accumulating evidence for the oligotrophication of these

temperate forests (McLauchlan et al., 2017; Craine et al., 2018; Groffman et al., 2018). The results reported here also suggest that these forests may be transitioning to greater N limitation over time, and long-term studies like BBWM are essential to understand how these changes are altering forest productivity and forest health.

## Missing Nitrogen Fluxes

Our calculations of net ecosystem N retention in East Bear ( $94\% \pm \text{SE } 2.5$ ) and West Bear ( $81\% \pm \text{SE } 1.4$ ) used the input–output approach commonly seen in the literature that relies on inorganic N inputs in atmospheric deposition and exports via surface waters in recently unharvested ecosystems. A number of components of the N cycle are commonly not included in these analyses because the data are unavailable and measurements are often difficult to quantify. For the BBWM N mass balance, N inputs represent above-canopy atmospheric inputs of inorganic N and do not reflect the interaction of the canopy with deposition processes (Decina et al., 2018; Fernandez et al., 1999; Lovett and Lindberg, 1993; Weathers et al., 2006). Throughfall measurements at BBWM and elsewhere in Maine have highlighted the significant contribution of fog deposition and forest canopy interception to ecosystem N inputs (Fernandez et al., 1999; Norton et al., 1999; Weathers et al., 2006). At Acadia National Park in coastal Maine, total deposition inputs of N were found to be ~4-fold the bulk precipitation inputs (Weathers et al., 2006). If a similar “scaling factor” of 4 were to hold true for BBWM, it would suggest an important additional N input to the budget (~6 to 8 kg ha<sup>-1</sup>). Biological N fixation can also contribute small additional inputs of atmospheric N in these temperate forest ecosystems. Studies in northern hardwood forests at the Hubbard Brook Experimental Forest in nearby New Hampshire reported ~0.5 kg N ha<sup>-1</sup> yr<sup>-1</sup> fixed in 40- to 60-yr old stands (Roskoski, 1980). Recent studies have reported that rock weathering may contribute as much as 15% to ecosystem N inputs (Houlton et al., 2018) in temperate ecosystems. However, this is likely not a significant source of N at BBWM because of the low N concentration of the bedrock and till rock components (<0.1% by weight, unpublished data), and low weathering rates of the till and bedrock at BBWM (Lawrence et al., 1997). We did not include dissolved organic N (DON) inputs in our calculations, but DON likely did not contribute significantly to the N inputs at BBWM. At Hubbard Brook, DON was ~10% of bulk N precipitation (Yanai et al., 2013), and limited collections at BBWM in the 1990s showed that DON was below-detection in precipitation at BBWM (unpublished data).

Similarly, our estimates of total N export do not include measurements of DON and denitrification. Limited measurements of DON conducted in East Bear and West Bear streams during the study period (data not shown) indicated that DON concentrations were similar in both streams, approximately double the inorganic N concentrations in East Bear. But the limited period of DON measurements precludes our quantitative estimate of DON export over the full time series presented



in this study. Denitrification fluxes were measured at BBWM during 2000 to 2001 (Venterea et al., 2004) and 2010 to 2011 (Morse et al., 2015). Venterea et al. (2004) found that nitric oxide fluxes were negligible in East Bear and  $<0.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in West Bear ( $\sim 1.6\%$  of the annual inputs), while Morse et al. (2015) found that  $(\text{N}_2 + \text{N}_2\text{O})$  flux was  $<1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in East Bear and West Bear and did not differ between watersheds. Both studies found that denitrification was a relatively small export of N from our ecosystem. Isotopic analyses of groundwater at Hubbard Brook suggest that traditional estimates of denitrification may underestimate total denitrification fluxes from these northern temperate forested ecosystems (Wexler et al., 2014).

This discussion of missing N fluxes in this study suggests that missing N inputs, driven primarily by a deposition scaling factor, are larger than the missing exports, and therefore the realized N retention in these ecosystems is actually greater than the calculated retention reported here.

### Implications for Nitrogen Cycling

Our understanding of ecosystem processes at BBWM assumes that East Bear and West Bear were similar in terms of soil (mass and chemistry), vegetation (biomass and chemistry), and stream (discharge and nutrient export) characteristics prior to treatment. The streams were the only ecosystem component monitored prior to the beginning of the West Bear manipulation, and the site was chosen because of the comparable hydrology and chemistry of the streams, including N export, and general similarity in aspect, topography, watershed size, soils, and vegetation (Fig. 4; Norton et al., 2010). We added  $25.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  to West Bear, significantly more than the mean ambient deposition of  $3.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  during the 25-yr study period, making this chemical perturbation the likely primary factor driving ecosystem responses reported here, rather than pre-treatment differences between the watersheds. The ecosystem pools (Fig. 5) indicate that West Bear had  $\sim 700 \text{ kg N ha}^{-1}$  more than East Bear, reasonably similar to the net excess retention of  $\sim 20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  over 25 yr. While it is unlikely that these rates of accumulation were consistent over this time period, the agreement of these estimates lends support for the dominant role of the experimental N additions being responsible for altered patterns of N cycling in West Bear.

The contrasting responses between hardwood and softwood stands at BBWM highlight the critical role of forest composition in determining the response of forests to elevated N inputs. The lack of a clear biomass N accumulation response and the higher N leaching response in softwood stands, compared to hardwoods, suggests a greater likelihood for associated surface water impacts in softwood dominated landscapes. Twenty-five years of chronic whole watershed N additions had a positive effect on forest growth in hardwoods, a negative consequence in stream water chemistry, and a relatively rapid emergence of these trends early in the experiment that have persisted in a dynamic equilibrium for 25 yr. We suggest this dynamic equilibrium will persist until stochastic disturbance events (e.g., ice storms, wind,

insect and disease, fire) or the emergence of a later successional stage reduces the aboveground biomass uptake of soil N.

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### SUPPLEMENTAL MATERIAL

Supplemental material is available with the online version of this article. The supplemental document contains four tables: Table S1, Coefficients for allometric equations; Table S2, Tree biomass and biomass accumulation values, and oven-dried soil mass values for the two watersheds; Table S3, Tissue N concentration in vegetation at BBWM (measured for the period 2010–2013); and Table S4, Total N concentration by soil increment for each compartment at BBWM (for the 2010 quantitative pits).

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