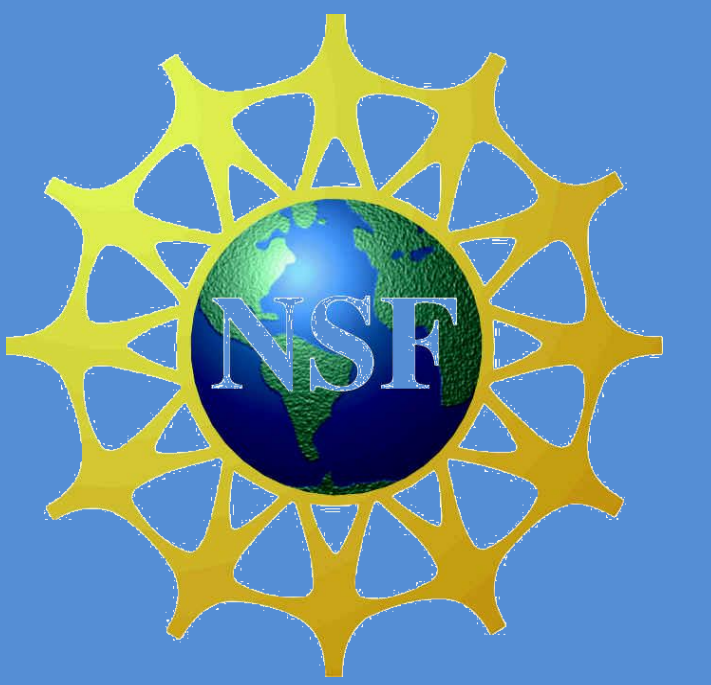


Defining Whole Watershed Nitrogen Dynamics with ^{15}N



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INTRODUCTION

Long-term study of N dynamics in the northeastern US

The northeastern U.S. experienced changes in air quality over recent decades, with significant decreases in atmospheric depositions of nitrogen (N) and sulfur (S) since the 1990 Clean Air Act Reauthorization. Nevertheless, a warming climate, continued but improved atmospheric S and N deposition, and a growing human population suggest ongoing increase and influence of reactive N in the environment in the future. These changes threaten ecosystem services, including water quality and plant diversity. Long-term studies of the biogeochemical response of ecosystems to chronic perturbations are essential to better understand the effects of a changing physical and chemical climate on northeastern U.S. forests.

OBJECTIVE

Investigate changing N dynamics as a result of ambient and experimental N additions and a changing climate using a paired forested watershed approach.

STUDY SITE and METHODS

The Bear Brook Watershed in Maine (BBWM)

The BBWM (44°52' N. lat. and 68°06' W. long.) is a long-term, paired watershed experiment situated approximately 60 km northeast of Acadia National Park (Fig. 1). The watersheds occupy the upper 210 m of the southeast slope of Lead Mountain (elev. 475 m). The research site comprises a treated (West Bear) and a reference (East Bear) watersheds (10.3 and 11.0 ha, respectively, Fig. 1). Since November 1989 West Bear has been treated with ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$ fertilizer applied bi-monthly, experimentally increasing N and S deposition by 25.2 kg N $\text{ha}^{-1}\text{yr}^{-1}$ and 28.8 kg S $\text{ha}^{-1}\text{yr}^{-1}$, respectively.

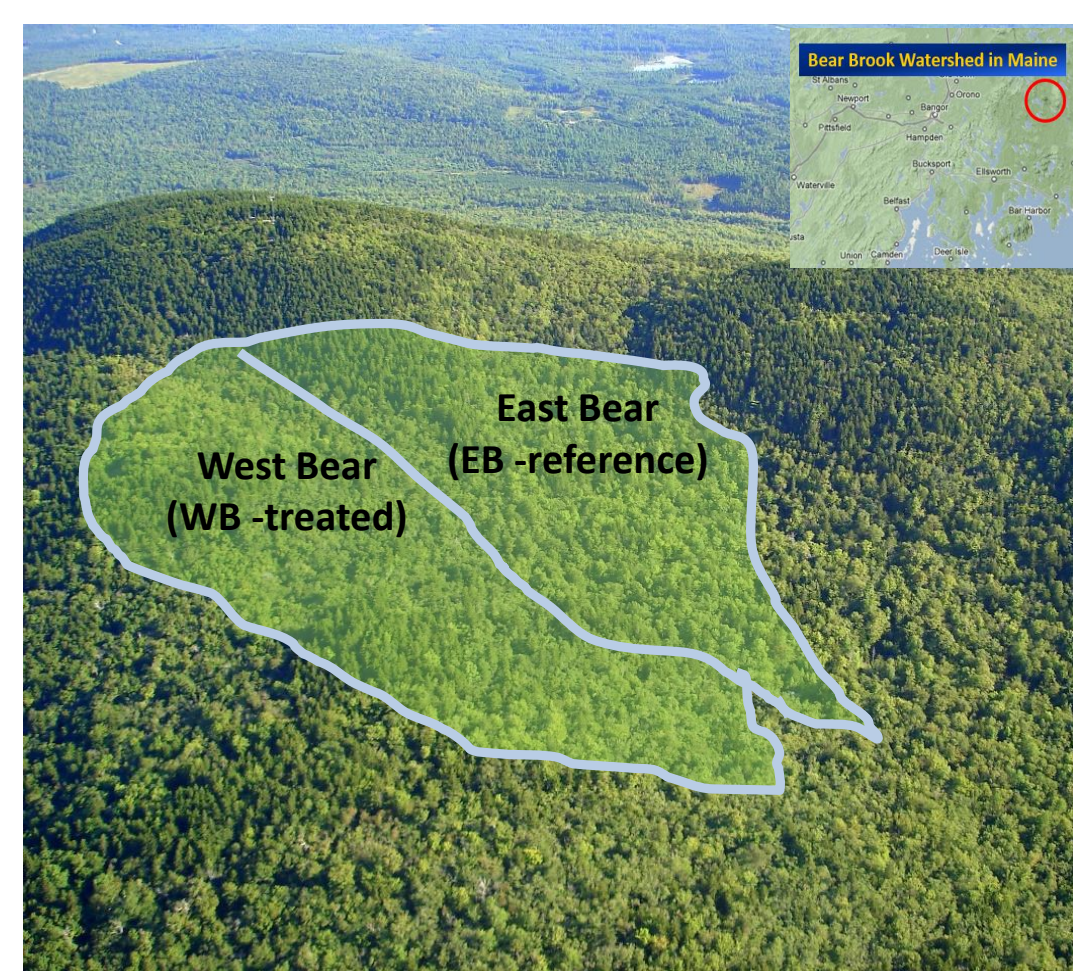


Figure 1. Location and set up of the Bear Brook Watershed in Maine at Lead Mountain.

Lower elevations are dominated by hardwoods (*Fagus grandifolia* Ehrh., *Acer rubrum* L., *Acer saccharum* Marsh., *Betula alleghaniensis* Britton, *Betula papyrifera* Marsh., and *Acer pensylvanicum* Marsh.) and the higher elevations by softwoods (*Picea rubens* Sarg., *Abies balsamea* Mill., and *Tsuga canadensis* (L.) Carr.). Soils are predominantly coarse, loamy, mixed, frigid Typic Haplorthods, with Typic Haplhumods and Lithic Cryofolists, all derived from till averaging 1 m in thickness. The bedrock is dominantly quartzite and phyllite with granite intrusions.

Stable Isotopes of N

Stable isotopes of N (i.e., the common ^{14}N and heavy but rare ^{15}N) are used in this study to detect subtle changes in ^{14}N and ^{15}N ratios of plants, soils, and stream water, indicating changes in rates of N transformations due to the long-term N+S manipulations. Stable isotopes of N are expressed using the δ notation as follows:

$$\delta^{15}\text{N} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

where R = $^{15}\text{N}/^{14}\text{N}$ of the sample and the standard (atmospheric N_2 , 0.0036765), respectively.

Since the ^{14}N and ^{15}N ratios of plants, soils, and stream water change with N transformation types and their rates, $\delta^{15}\text{N}$ in forest components reflect net changes in N cycling (multiple transformations).

In addition to ^{15}N natural abundance determination, ^{15}N tracers are used to study the redistribution of ^{15}N among forest components as a pulse-chase experiment. On June 5, 2012, a ^{15}N tracer (i.e., $(^{15}\text{NH}_4)_2\text{SO}_4$, 98 atom-%, 0.4 kg ha^{-1}) was applied by backpack sprayers (Fig. 2) over both East and West Bear watersheds. Subsequently, forest components were sampled several times over the 2012 and 2013 growing seasons.



Figure 2. Application of the ^{15}N tracer by backpack sprayer.

Natural abundances

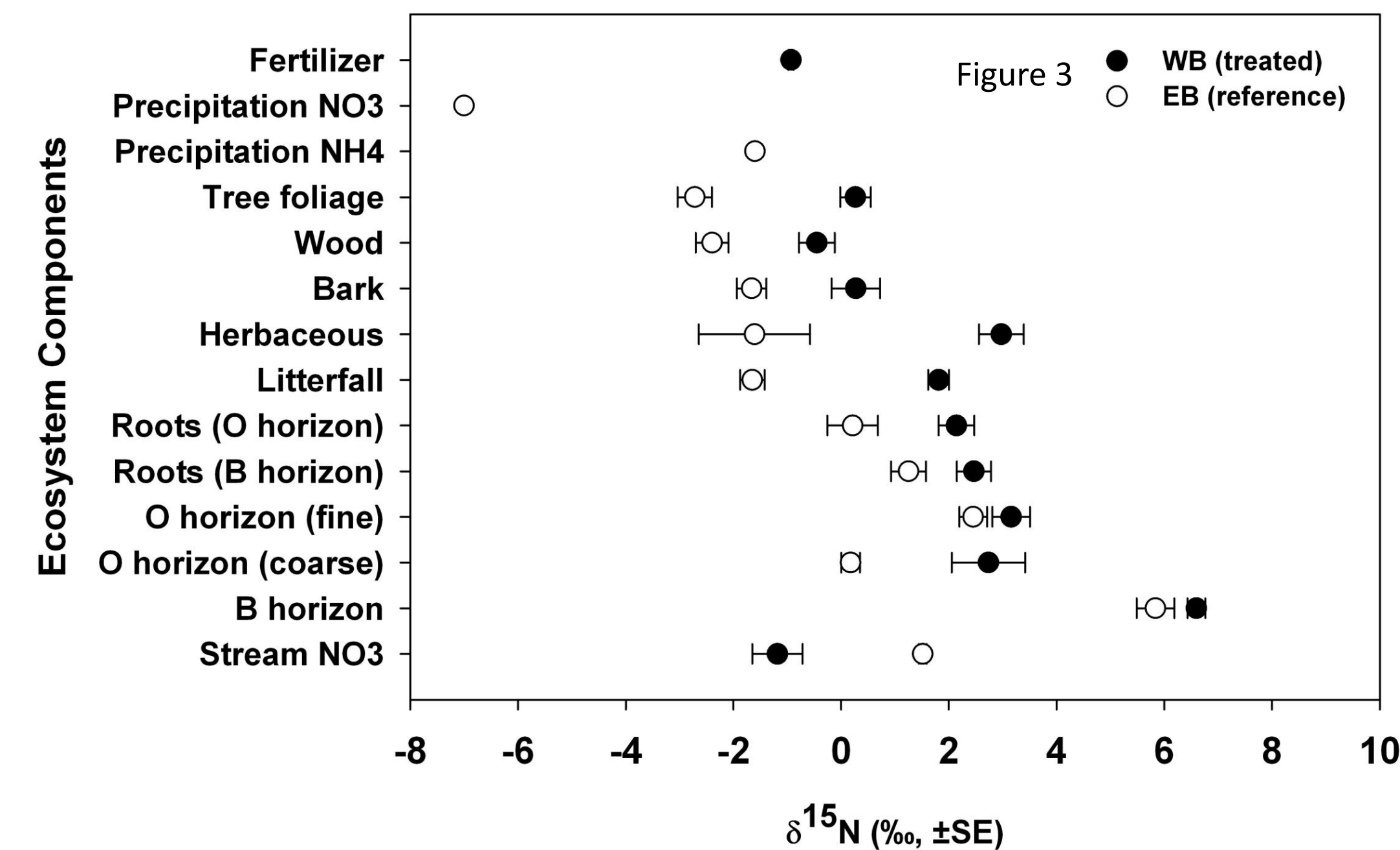


Figure 3. ^{15}N natural abundances (‰, mean $\pm$ SE) across ecosystem components in East Bear and West Bear watersheds. For fertilizer and precipitation samples $n = 1$.

- The long-term N additions and acidification in West Bear significantly increased $\delta^{15}\text{N}$ natural abundances in plant tissues, forest floor, and mineral soil compared to East Bear (reference).
- This was observed in all ecosystem components from tree canopy to the mineral soil (B horizon).
- The exception to this pattern was found in stream water with reverse trends: Stream nitrate (NO_3^-) was more enriched in ^{15}N in the East Bear than in the West Bear stream.

Tracer enrichments

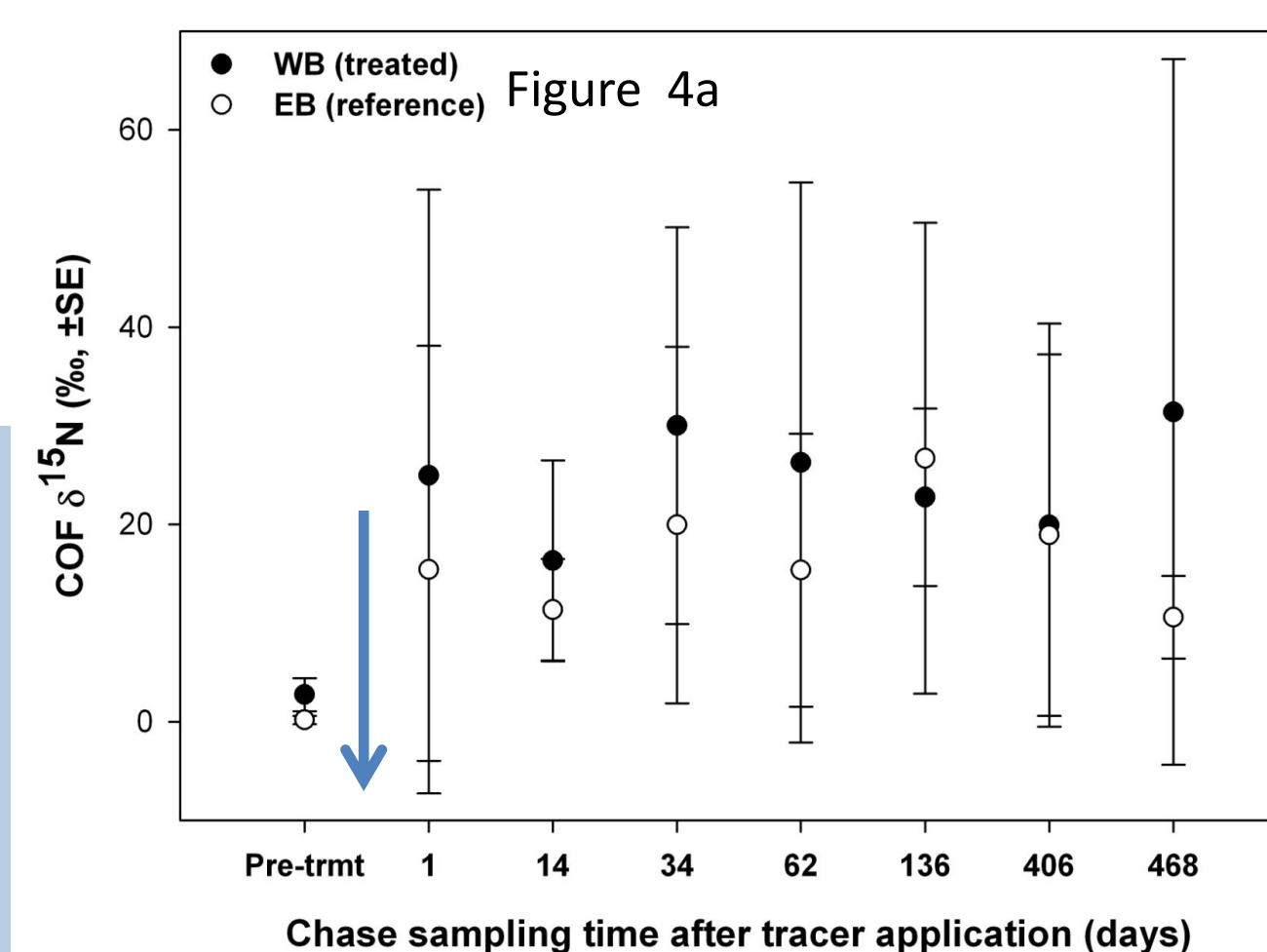


Figure 4. ^{15}N tracer enrichment (‰, mean $\pm$ SE) in the coarse organic fraction (COF) (4a) and the fine fraction (4b) of the forest floor in East Bear and West Bear watersheds during 2012 and 2013. Note the different scales on the Y axis. The arrow indicates the tracer application.

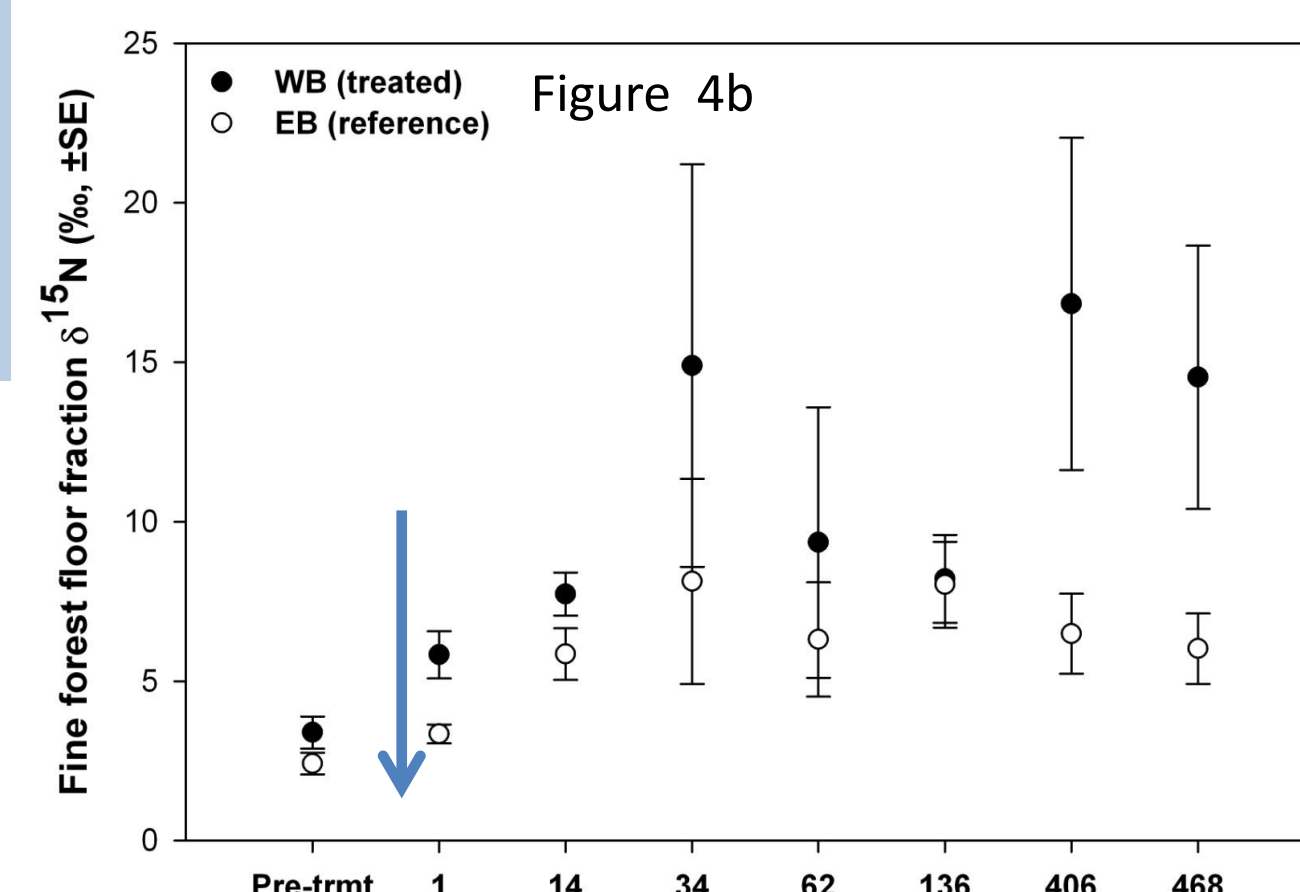
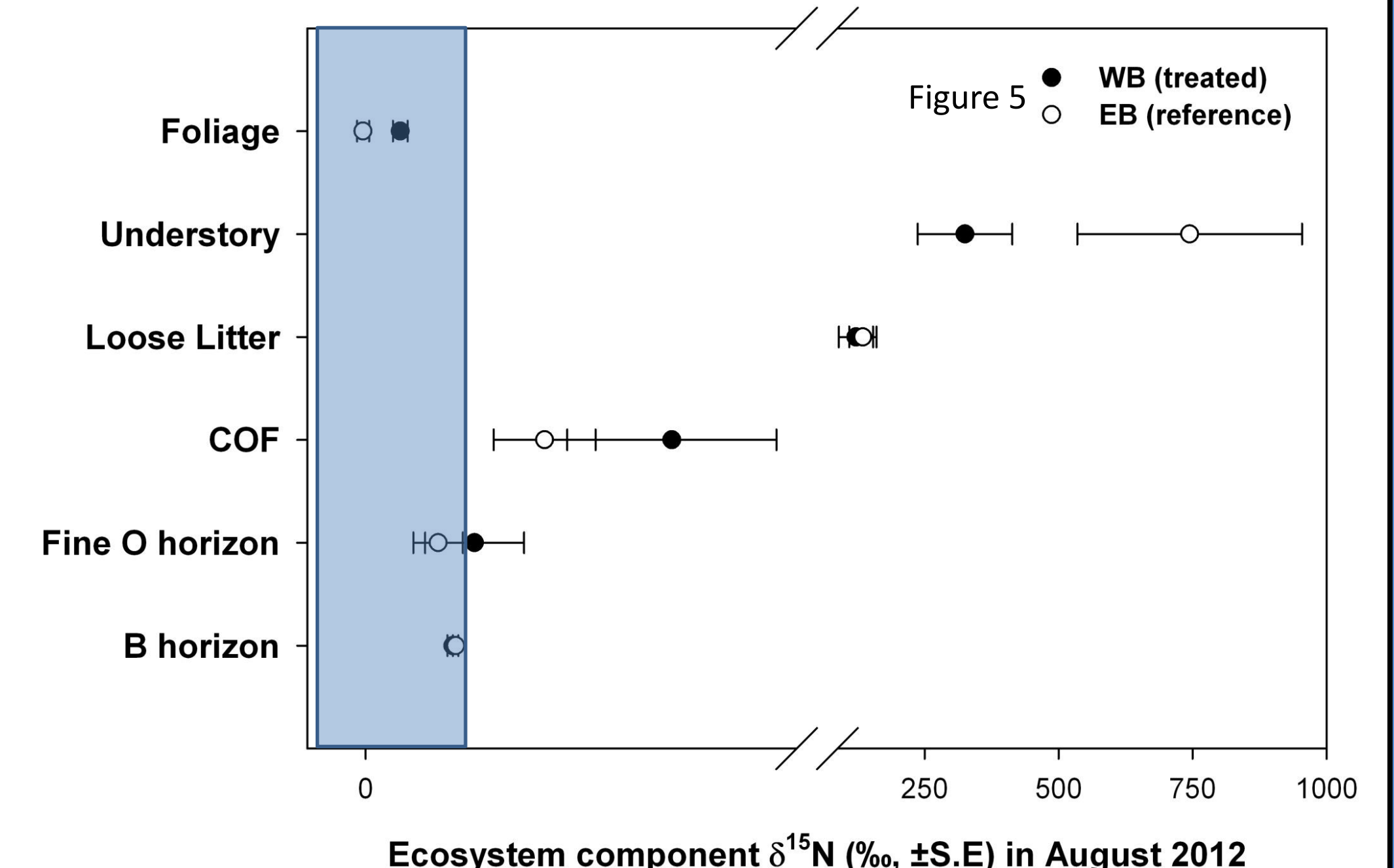


Figure 5. ^{15}N tracer enrichment (‰, mean $\pm$ SE) across ecosystem components in East Bear and West Bear watersheds two months after the tracer application. The shading indicates the ^{15}N natural abundances range.



- The ^{15}N pulse labeling significantly increased ^{15}N in plant tissues, forest floor, and mineral soil over natural abundance levels in both watersheds (Fig. 4a, 4b, and 5).
- The ^{15}N enrichment in the coarse and fine fractions (Fig. 4a and 4b) of the forest floor was variable but tended to be higher in West Bear than East Bear. This trend persisted across the 2012 and 2013 growing seasons.
- Understory vegetation showed the greatest enrichment with the ^{15}N tracer two months after application (Fig. 5) indicating a high N demand for understory growth. This was more evident in East Bear.

DISCUSSION

- The **natural abundance** results show that the whole ecosystem shift towards higher $\delta^{15}\text{N}$ in West Bear is due to accelerated N cycling (especially nitrification) resulting from the long-term input of fertilizer N.
- Evidence supporting accelerated N cycling in West Bear, compared to East Bear, include increased tree foliar N concentrations (Elvir et al. 2005), reduced fine root biomass (Fernandez and Rustad, unpublished data), increased NO_3^- in soil solutions (Fatemi et al. 2012), increased net nitrification rates (Jefts et al. 2004), and increased NO_3^- stream export (Fernandez et al. 2010).
- The whole watershed ^{15}N pulse labeling successfully increased ^{15}N over natural abundances in both watersheds.
- The **tracer pulse-chase** results show that the understory vegetation exhibits a strong demand for N in both watersheds, especially in East Bear, reflecting the role of N as a growth limiting nutrient.
- The **tracer pulse-chase** results also show that the top layer of the forest floor (loose litter) is more enriched than deeper soil layers, indicating high microbial N demand necessary for the decomposition of C-rich organic matter.

CONCLUSIONS

- This research demonstrates the utility of: (1) N stable isotope natural abundances as indicators of multi-decadal whole forested ecosystem shifts in N dynamics, and (2) whole ecosystem stable isotope tracer additions to study N transformations in the ecosystem.
- Analysis of ^{15}N natural abundances across ecosystem components showed increases in $\delta^{15}\text{N}$, reflecting accelerated N cycling consistent with earlier literature.
- The determination of natural abundances is biologically meaningful, simple, and relatively inexpensive. The addition of a ^{15}N tracer is an expensive but powerful technique to identify forest components and ecosystem processes involved in shifting N dynamics.

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