

STUDYING THE ROLE OF WOOD-DECAY FUNGI IN CALCIUM CYCLING ON THE PENOBSCOT EXPERIMENTAL FOREST: A PROGRESS REPORT

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Abstract.—Depletion of essential mineral nutrients from the rooting zone of trees in northern forests may reduce health and productivity. Long-term field investigations coupled with detailed laboratory studies enhance understanding of the biological processes and suggest means to address potential threats. One such investigation by the U.S. Department of Agriculture, Forest Service, in cooperation with the University of Maine, involves the role of wood-decay fungi in cycling calcium (Ca) and other essential mineral nutrients. The investigation was established on the Penobscot Experimental Forest (Maine) in 1996 and 1997 and replicated in part on the Bartlett Experimental Forest (New Hampshire) in 1995. Some initial findings were: (1) A significant gain in Ca concentration in decaying wood occurred by 6 yr and that gain was sustained through 10 yr; (2) a significant gain in wood potassium was observed at 2 yr, but the gain was not sustained; and (3) observed changes in magnesium concentration in decaying wood were variable. Plans to continue this unique and important long-term study are described in this report.

INTRODUCTION

Calcium (Ca) is the fifth most abundant element in the Earth's crust. Other than nitrogen (N), Ca is considered the most important essential mineral for managing plant diseases (Rahman and Punja 2007). It is also the fifth most abundant element in trees after hydrogen (H), carbon (C), oxygen (O), and N (Shortle et al. 2008). Calcium is a structural link for wood components and regulates acidity, signals changes in various biological functions, and is needed to form protective layers in wood and bark (McLaughlin and Wimmer 1999). Therefore, living trees require a steady supply of Ca for wood formation and protection.

The depletion of root-available Ca from northern forest soils can occur over time by the processes of podzolization (Ponomareva 1969) and can be accelerated by acid deposition, the input of non-biological acidity resulting from regional emissions of sulfur (S) and N oxides (Shortle and Bondietti 1992). Declines in stem growth and mortality due to Ca depletion followed by aluminum (Al) mobilization

have been documented in spruce (*Picea* spp.) (Lawrence et al. 1995, 2005; Shortle and Smith 1988; Shortle et al. 1997) and maple (*Acer* spp.) (Johnson et al. 2008, Lawrence et al. 1999, Long et al. 2009, St.Clair et al. 2008, Zaccherio and Finzi 2007).

As trees die and woody parts are shed or broken, wood is added to the forest floor. Root-available Ca is replaced in depleted sites by the action of wood-decay fungi that both release the solar energy stored in cellulose and lignin, the two most abundant organic substances in nature, and enrich the decayed wood residue with Ca from external sources. Microcosm tests demonstrate movement of Ca into decaying wood of conifers (Connolly et al. 1999, Ostrofsky et al. 1997) and hardwoods (Clinton et al. 2009). Many of the fungi that decompose wood are large, long-lived organisms that produce extensive mycelial networks, including cords and rhizomorphs, which move essential elements for many meters through the forest floor in and out of decaying wood (Boddy and Watkinson 1995, Connolly and Jellison 1997,

Lindahl et al. 2001). Although commonly regarded as microorganisms, the dominant wood-decay fungi are anything but “micro-”.

The purposes of the studies established on the Penobscot Experimental Forest (PEF) in Maine in 1996 and 1997 and on the Bartlett Experimental Forest (BEF) in New Hampshire in 1995 were (1) to determine changes in Ca and the other two essential base cations, magnesium (Mg) and potassium (K), as well as the acid-mobilized metals manganese (Mn), Al, and iron (Fe) in wood decaying in ground contact for at least 15 yr; (2) to determine changes in these elements in the organic and underlying mineral soil contiguous with the decaying wood; and (3) to archive wood samples in progressive stages of decay that preserve features indicative of biological processes of decay and the incorporation of residues into soil. Some key features being studied are differences in decay type (brown-rot, white-rot), variations in cation solubility and exchange properties, and modifications to cell wall polymers, especially lignin.

METHODS

The tree species selected for study were red spruce (*P. rubens* Sarg.) on the PEF and BEF, red maple (*A. rubrum* L.) at both locations, eastern hemlock (*Tsuga canadensis* [L.] Carr.) on the PEF, and paper birch (*Betula papyrifera* Marsh.) on the PEF. For each combination of location and species, 3 groups of 10 trees in a dominant or co-dominant position and 15- to 45-cm diameter at 1.3 m above ground were selected and tagged to identify the tree and treatment. Treatments were assigned at random. One group was used as an uncut reference for soil samples. Trees in a second group were cut and the felled trees left in place to decay in a gap in the canopy. Trees in the third group were cut and sections of the felled trees were hauled and placed under the forest canopy to decay while the gap was left with only stumps to decay (all tops and branches were removed).

Time-zero reference disks, 5 cm thick, were cut from all felled stems at 3 m and 7 m above the stumps.

Decaying wood was subsequently collected at 2-yr intervals from the intervening 4-m bolt. Small soil pits dug next to the bolt provided samples of the O-horizon and the underlying 10 cm of mineral soil. Soil pits in the reference group and the stump-only area were taken at approximately where the 4-m bolt would have been if trees were felled. The position of each decaying bolt was mapped for future reference. Decaying wood was collected for chemical analysis by removing and discarding a 10-cm length from the lower end of each bolt followed by the removal and retention of a 5-cm-thick sample disk. Small soil pits were used to sample soil contiguous with the decaying stem at 10 and 15 yr, taking care to avoid the location of the initial sampling pits.

For chemical analysis, wood disks were air-dried at room temperature and then oven-dried at 90 °C. Rectangular prism blocks were split from the sapwood of dried disks from a position 90° around the stem from the point of soil contact. The volume of each block was calculated from the mean of four measurements of each dimension (longitudinal, radial, and tangential) and weighed (± 1 mg). Density (g cm^{-3}) was calculated as the mass to volume ratio. After density was measured, blocks were chiseled into small pieces and ground in a benchtop Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass a 1-mm mesh. One-gram portions of milled wood powder were ashed for 6 h at 550 °C, cooled, dissolved in 3 mL of 6 M HCl, and brought to a volume of 50 mL with deionized water. Concentrations of Ca, Mg, K, Mn, Al, and Fe in ash solutions, analytical standards, and blanks were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Model 750, Thermo Jarrell Ash Corp., Franklin, MA).

Element concentrations determined by ICP-OES were converted from parts per million (ppm) to mmol kg^{-1} by dividing ppm by the atomic weight. Concentrations for comparison on a constant volume basis, mol m^{-3} , were obtained as the product of the mass-based concentration, mmol kg^{-1} , and the wood density, g cm^{-3} . For each set of wood samples taken at 2-yr

intervals the mean and 95-percent confidence intervals of replicate samples were determined. Statistical analysis is presented in the initial publication of results through 6-8 yr (Smith et al. 2007).

Soil samples taken at 0 yr for each location and species and at 10 yr for spruce on the PEF, maple on the BEF and PEF, and birch on the PEF were analyzed using a suite of protocols approved by the U.S. Environmental Protection Agency (EPA) for forest soil analysis (loss on ignition, pH, acidity, cation exchange capacity, total Kjeldahl nitrogen, P, Na, Ca, K, Mg, Fe, Al, Zn) in the EPA-certified analytical laboratory at the University of Maine, Orono. Soil sampling was planned for 15 yr for each location and species. Decaying wood samples taken at 10, 12, and 15 yr are being analyzed by the same forest soil protocols applied to the O-horizon samples, in addition to the plant tissue protocol previously described, so that the nutrient status of the forest floor and wood decaying on the floor can be compared after the first decade of ground contact.

PRELIMINARY RESULTS

Preliminary results through the first 6-8 yr (Smith et al. 2007) indicated a significant accumulation of Ca in decaying wood in all tree species at both locations (30-90 percent increase after 6-8 yr). As the wood decayed and Ca was accumulated, Mg concentrations were sustained at approximately the initial concentration or had a small decrease of about 20 percent in some cases. More-detailed results for changes taking place at 2-yr intervals in decaying wood for the first 12 yr will soon be available, along with comparisons of decaying wood and organic soil at 10 and 12 yr.

Sampling decaying wood and soil at 15 yr on the BEF has been completed and chemical analyses are being performed. Sampling decaying wood of spruce and maple on the PEF was completed at 15 yr in 2011, but soil sampling was delayed until 2012 due to standing water on the plots. Soil samples were taken at the maple plots on the PEF in 2012, but standing water again delayed soil sampling at spruce plots until

2013. Sampling decaying wood and soil of hemlock and birch on the PEF has been suspended until work on spruce and maple has been completed. Subsets of archived decaying wood samples previously maintained at the University of Maine have been moved to other locations and are available to those interested in studying wood decay processes.

OUTLOOK

With our work on the Penobscot and Bartlett Experimental Forests, we have demonstrated an important dynamic of the biogeochemistry of northern forests, the translocation of essential Ca by wood-decay fungi. This work on the long-term effects of wood decay complements the existing understanding of the effects of forest management on Ca cycling. This process is driven by large, long-lived fungi in these forests and is far more dynamic than decomposition of a carbon stock—it is a unique connection between the forest floor and the atmosphere.

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