Foliar Chemical Analysis, Decomposition, and Effects on Nutrient Cycling of
American Chestnut and its Hybrids

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the College of Arts and Sciences of Ohio University

In partial fulfillment
of the requirements for the degree
Master of Science

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Foliar Chemical Analysis, Decomposition, and Effects on Nutrient Cycling of American Chestnut and its Hybrids

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ABSTRACT

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Foliar Chemical Analysis, Decomposition, and Effects on Nutrient Cycling of American Chestnut and its Hybrids (57 pp.)

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The American chestnut, *Castanea dentata* (Marsh.) Borkh, was once the dominant species of many hardwood forests in eastern North America. A fungal pathogen introduced in 1904 killed mature chestnuts range-wide. Recent work has resulted in a putatively pathogen-resistant chestnut hybrid, making future reintroduction a distinct possibility. However, information on the chemistry and potential effects on nutrient cycling of the chestnut hybrid is necessary to understand the consequences of reintroduction. I predicted that litter from chestnut taxa would contain more recalcitrant compounds and subsequently decompose more slowly than the extant species *Quercus alba* L. and *Acer saccharum* Marshall. Lignin and tannin are secondary compounds which are known to inhibit decomposition, and which I predict will be relatively abundant in chestnut litter. I also predicted that there are no significant differences between the foliar nutrient content of the hybrid and American chestnut. In this experiment, I measured tannins, litter fractions, ten nutrient elements, and both decomposition rates and enzyme activity (in the Oe/A horizon) of leaves of *C. dentata*, *C. mollissima* Blume, hybrid chestnut, *Quercus alba*, *Q.prinus* L., *Acer rubrum* L., and *A. saccharum*. Nutrient resorption was also quantified in order to further understand hybrid chestnut’s potential effect on forest nutrient pools. No significant differences in chemistry or decay rate were found among any of the chestnut taxa. Chestnut contained
more tannin than non-chestnut species, and contained slightly more mass after one year of decomposition, but all metrics tested were within the ranges of co-occurring species. These data suggest that a reintroduction of hybrid chestnut will not likely alter nutrient cycles in Appalachian hardwood forests.

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INTRODUCTION

_Castanea dentata_ (Marsh.) Borkh was formerly a dominant species (and primary litter source) in late-successional hardwood forests in eastern North America, accounting for up to 45% of the mature trees in Southern Appalachia (Vandermast et al. 2002). _Castanea dentata_’s success has been attributed to its ability to self-propagate from the root crown, quick recovery after disturbance, its resistance to fire, and the ability to survive as a member of the forest understory and respond opportunistically to disturbances (Paillet 2002, McCament and McCarthy 2005). Large mast production rates and success growing in poor soil conditions are also thought to have contributed to its reproductive efficacy (Vandermast et al. 2002). The pathogen _Cryphonectria parasitica_ Murrill was introduced to America’s East coast from Asia in 1904, and resulted in the rapid mortality of most mature trees throughout the range. _Castanea dentata_’s former habitat has now largely been filled by maple, beech, hickory, and oak, and ecosystem nutrient cycling regimes may have been changed (Keever 1953, Jacobs 2007). After initial replacement by co-dominant oak species, _C. dentata_ was predominantly replaced by different hickory species at xeric sites, by _Acer saccharum_ Marshall at mesic sites, and by _A. rubrum_ in clearings (McCormick and Platt 1980). Good (1968) noted that there has not been a change towards more xeric or mesic species in the former range of _C. dentata_ in NJ, with the exception of increased relative abundance of _Betula lenta_ L. on xeric sites.

A pathogen-resistant hybrid is now a candidate for reintroduction into the former range of _C. dentata_. However, information is needed on how this reintroduction may affect ecosystem nutrient cycling. For the past 25 years, The American Chestnut Foundation (ACF) has been working to produce a blight-resistant chestnut variety, using
a back-crossing approach with a blight-resistant Chinese chestnut (*C. mollissima* Blume) variety (Burnham 1988, Griffin 2000). The resultant hybrid variety contains the gene for blight resistance and is genetically, approximately 94% American chestnut (Diskin et al. 2006).

Figure 1 – This diagram from the American Chestnut Foundation showing the backcross lineage used to produce the current “15/16” hybrid chestnut tree. This hybrid will be referred to as the “15/16 hybrid” from here forward.
A number of methods are available for the introduction of hybrid chestnut into Eastern forests. Because of *Castanea dentata*’s high tolerance of light and nutrient poor soils, afforestation is a distinct and desirable possibility (Jacobs 2007). The native range of *C. dentata* is home to an abundance of surface mining activity, and this restoration may offer an opportunity to reforest these sites with native genetic material. Afforestation at the sites of clear cuts may present an opportunity for reintroduction as well (Jacobs 2007, Rhoades et al. 2009). Because of its success in resprouting following fire, frequent burns may also enhance planted seedlings in their effort to establish canopy presence (McCament and McCarthy 2005). From these disturbed sites, hybrid trees could eventually spread into established forests. Benefits of restoration via the “15/16” hybrid include increased carbon sequestration (Jacobs et al. 2009), a regional source of high quality timber, and the restoration of an important source of wildlife fodder (Steele et al. 2005).

The introduction of a new type of litter to a forest ecosystem has the potential to alter nutrient cycling in that ecosystem. *Castanea dentata* is a member of a family (Fagaceae) known to have high foliar lignin:N ratios and abundant foliar phenolics, and tannin from *C. dentata* bark was formerly preferred in the tanning industry (Sterling 1902). Thus, there is the possibility that the 15/16 hybrid litter will increase the input of secondary compounds into the forest floor decomposition cycle and alter nutrient cycling by slowing decomposition. Decomposition is a process that is known to be controlled by the physical and chemical quality of litter, soil quality and microbial community, and by temperature and moisture (Prescott 2002). Climate tends to play a more important role in determining decay rate at large geographic scales, where litter chemical composition is of
greater importance at regional scales (Aerts and Chapin 2000). At a local scale, the quantity and state of nutrients in decaying litter varies by species and has been correlated with soil chemistry (Prescott 2002). For example, Carbon:Nitrogen (C:N) ratios in litter have been negatively correlated with N mineralization and nitrification (Wedin and Tilman 1990, Hobbie 1992, Finzi et al. 1998), which are important processes in making growth-limiting N more available. Concentrations of secondary metabolites in litter have been found to affect decay rate. In Eastern and Midwestern North American hardwood forests, lignin:N ratio has been negatively correlated with decay rate (Melillo et al. 1982, Li et al. 2009).

Polyphenolics, such as lignin and tannin, are a group of secondary metabolites prevalent in litter that have been associated with slower decomposition and mineralization, the accumulation of litter, and anti-herbivore defense (Bloomfield 1957, Feeny 1970, Horner et al. 1988, Hoorens et al. 2003, Talbot and Finzi 2008). Tannins are a sub-class of polyphenols which are thought to be the fourth most prevalent biochemical in plant tissue. They have the ability to precipitate and complex proteins and enzymes that are essential for decomposition, and in the process produce an astringency that is toxic or unpalatable to many organisms (Krauss 2003). They are also variably resistant to degradation, depending on class (condensed vs. hydrolysable), structure, and polymer size. Fungal degradation is more common than microbial degradation (Krauss 2003). Why trees produce these relatively costly metabolites has long been a subject of debate. In addition to defense compounds, polyphenolics may be a response to climatic and/or site conditions. Reactive polyphenolics, which are products of the degradation of lignin in soils, have the ability to complex proteins, nutrients, and metals, and can subsequently
prevent leaching, and alter nutrient availability and soil chemistry. Muller et al. (1987) found polyphenol levels in leaves increase as site nutrient availability decreases. Reed and McCarthy (1987) found that levels of condensed tannins in foliage were associated with site variables in some cases and population variables in others. The commonly debated carbon-nutrient balance hypothesis states that plants in abundant light and nutrient poor soils allocate more of their photosynthesis-acquired carbon (rather than growth-limiting nitrogen) to chemical defensive compounds (Bryant et al. 1983). However, this hypothesis has been called into question by several studies (Muller et al. 1987, Hamilton et al. 2001, Kraus et al. 2003). Nitao et al. (2002) suggests that tannin production may be a response to light or an adaptive response to other site conditions. It has been proposed that plants produce tannins and other polyphenolic compounds to hold nutrients in a form unavailable to competing plants and make those nutrients less vulnerable to leaching (Northup et al. 1998, Kraus et al. 2003, Wurzburger and Hendrick 2009). In this scenario, tannin rich litter would favor species that have symbionts (often fungus) to allow them to access recalcitrant nutrients (Northup et al. 1998). The concentration of tannins in litter is often reduced considerably in the first year of decomposition (Krauss 2003).

Soil microbial activity and species composition is also affected by the concentration of secondary compounds in litter (Sinsabaugh et al. 2002). Soil biota produce enzymes in response to decreases in labile nutrient pools, with the goal of freeing nutrients from complex molecules (Olander and Vitousek 2000). Negative correlations have been found between hydrolytic enzyme activity and nutrient availability (Olander and Vitousek 2000). Condensed tannins are molecules that can complex these
degrading enzymes, making both the tannin molecule and the enzyme henceforth non-reactive and recalcitrant. Reactive polyphenolics resulting from the degradation of lignin can do the same thing in the soil. Phenol oxidase and peroxidase are oxidative enzymes that can break down more complex secondary metabolites such as phenolics and lignin, and release valuable nutrients and bound carbon. Activity of oxidative enzymes is often correlated with concentrations of polyphenolic molecules (Olander and Vitousek 2000). Both hydrolytic and oxidative enzymes are important for litter decay, and changes in their activity may lead to altered nutrient release regimes in the soil.

In this study, I investigated whether an introduction of 15/16 hybrid chestnut litter into the former range of *Castanea dentata* will alter nutrient cycling regimes. I addressed this question by: 1) determining whether differences exist in the chemistry of foliage and litter among chestnut taxa, and between chestnut taxa and extant co-occurring species, 2) determining whether chestnut taxa and extant species differ in decay rate, and 3) assessing whether chestnut litter changes nutrient availability and enzyme activity in soils.
METHODS

Study Site

The litter decay and soil chemistry field experiments were conducted at the Waterloo Wildlife Research Station (WWRS; Ohio Department of Natural Resources) (N 39° 20”, W 82° 06”) in Athens and Vinton Counties, OH. The WWRS lies within the unglaciated Allegheny Plateau of southeast Ohio and is composed of a heavily dissected topography of moderately-steep to steep (20-70% grade) low hills (75-90 m) and narrow valleys. Larger ridges at WWRS typically have an east-west orientation; ridge tops and south-facing slopes are dominated by *Quercus prinus*, *Q. alba*, and *Carya* species. North-facing slopes and ravines are dominated by more mesophytic species such as *Fagus grandifolia* Ehrh., *Acer rubrum*, and *Tilia Americana* L. Floodplains and stream banks are dominated by *Ulmus Americana* L., *A. saccharinum* L., with occasional stems aged over 300 years (Small and McCarthy 2001). The soils at WWRS are mostly of the Dekalb-Westmoreland complex. Dekalb soils are loamy-skeletal, siliceous, active, mesic typic dystrudepts. Westmoreland soils are fine-loamy, mixed, active, mesic ultic
hapludalfs (characterized as well-drained, occurring on 40-70% slopes, and having a low water-holding capacity) (Small and McCarthy 2001, United States Department of Agriculture 2007). The mean annual temperature in southeastern Ohio is 10.7 °C; mean annual precipitation for is 102.5 cm, with July being the wettest month and October being the driest (NOAA 2008).

Study Species Pedigree

Pure Castanea dentata leaves came from pedigree lines (PL1op1-00 × opPL1op1-00) and (GMNewop6-00 × GMNewop6-00). Castanea mollissima leaves came from the pedigree line (CA89:mollissima12 × CA99:mollissima13). The B1 crosses, which are 7/8 C. dentata and 1/8 C. mollissima came from pedigree line (TN182[CA57:mollissima11 × WB385(RC-90 × opRC-90)] × A2158(GMNew × opGMNew-99)) and several unknown pedigree lines from the ACFs Pennsylvania 2001 orchard. These pedigrees will be referred to as 7/8 hybrid from here forward. The B3F2 crosses, which are 15/16 American chestnut and 1/16 Chinese chestnut come from pedigree lines (HB2B × CL98(LFR4T9 × Clapper)) and (WB54(RC-90 × opRC-90) × VA89(HH3K1C × Clapper)), and will be referred to as 15/16 hybrid (Hebard, F. V., Personal communication, 2008).

Field Methods

Experiment 1 - Comparing Castanea dentata and the 15/16 hybrid

Foliage and litter from four Castanea taxa were collected from American Chestnut Foundation’s Meadowview Research Farm located in Meadowview, Virginia
(N 36.760, W 81.863) during fall of 2008. Green and brown leaves were collected randomly from identical trees. Multiple genetic lines were collected for each chestnut taxon. Leaves were air-dried at approximately 40 °C in a forced air oven, ground, and stored at room temperature.

**Experiment 2 – Comparing Castanea dentata and co-occurring species**

In late September, 2009, leaves were collected from ten forest plots containing *Castanea dentata*, *Quercus prinus*, and *Acer rubrum* trees in Washington Co., VA. A 100 m transect was established parallel to the contour at mid-slope on each of two different slopes on Iron Mountain (N 36.638, W 81.771). Alternating right and left each 10 m along the transect, I walked 5 m at a perpendicular angle to the transect line and then collected leaves from the closest tree of each desired species. Leaves were stored in paper bags in a cooler and air-dried upon return to Athens, Ohio. Three mineral soil samples were collected (2 cm dia by 5 cm deep) and homogenized from each plot along the transect.

**Experiment 3 – Leaf decomposition in Castanea taxa and co-occurring species**

The litter bag method was used to analyze the decomposition rates of the leaf litter of five deciduous tree taxa: *Castanea dentata*, *C. mollissima*, 15/16 chestnut hybrid, *Quercus alba*, and *Acer saccharum*. Leaf litter from the three *Castanea* taxa was collected in large plastic bags from the Meadowview Research Farm in Meadowview, VA (N 36.760, W 81.863). *Quercus alba* and *A. rubrum* litter was collected in the areas surrounding Athens, Ohio. All leaves were collected at the time leaf senescence in early
November 2008. Leaves were air-dried at approximately 40 °C in a forced air oven. Five grams of air-dry litter was placed into 18 × 18 cm polypropylene bags (Industrial Netting, NN 1100, Minneapolis, MN) with a mesh size of approximately 2 mm (Melillo et al. 1982, Murdick et al. 1994, Sinsabaugh et al. 2002, Ishikawa et al. 2000). Litter samples from each tree species were preserved in order to determine moisture content and oven-dry mass (at 65 °C for 48 h), and for initial chemical analysis (Murdick et al. 1994, Ishikawa et al. 2007). One bag of litter from each species was transported to the field and then transported back to the lab and weighed to account for any litter lost during travel.

Five bags for each of the five species (25 total) were strung together and attached to a 30 cm long, 0.6 cm diameter piece of steel rebar using nylon wire. Four different pieces of rebar, each with an identical compliment of 25 leaf bags, were dispersed along the plot’s horizontal contour approximately 4 m from other pieces of rebar. This was done so that one rebar assembly could be collected and analyzed at each of the four collection times (every three months for one year, beginning March 2009).

**Experiment 4 – A- and O-horizon analysis**

Forest soil was experimentally covered with an organic (O) horizon of *Castanea dentata* litter, disturbed native litter, or *C. dentata* litter mixed with native litter. Available nutrients in the O horizon and mineral soil were analyzed after nine months. *Castanea dentata* litter was collected in the same manner as in the decay experiment. Six plots were established on different slopes at WWRS, each with the same relative slope position and aspect. Three replicates were placed along contours at each of the six plots.
Pieces of poultry netting (1 × 4 m) cover each of the replicates. Within each 1 × 4 m replicate, four adjacent 1 × 1 m sub-plots were designated and positioned horizontally along the slope contour, each containing a different compliment of 300 g of leaf litter.

In September of 2009, soil cores from the A, E, and/or B horizon were collected for chemical analysis using a 2 cm wide soil auger to a depth of 5 cm. Three cores were collected from within each sub-plot and homogenized. Samples were refrigerated at 4 °C until analysis. During October of 2009, three 10 cm² Oa/e horizon samples were collected from each sub-plot. Bags were labeled, placed in a cooler, and refrigerated at 4 °C upon return to the lab.

**Laboratory Methods**

Leaf samples were analyzed for C:N ratio, protein precipitation from condensed tannins, three litter fractions, and the concentration of 10 nutrients. Concentrations of total carbon and nitrogen were measured for both leaves and soil using a C/N analyzer (Elementar Vario EL II, Elementar, Hanau, Germany). Atropine standards were run at 15 sample intervals to allow for machine recalibration. Macro and micronutrients, ten in all, were quantified by combusting ground litter (500 mg) in a muffle furnace for 4 hr at 500 °C. The resulting ash was dissolved in 10.0 ml HCl (1.0 N) and brought to volume (50 ml) with deionized H₂O (Robertson et al. 1999). The elements, Ca, Mg, K, P, Al, Mn, B, Na, S, and Cu were quantified using an ICP-OES (Varian 700-ES series, Varian, Inc., Walnut Creek, CA).

Condensed tannin levels were measured using Hagerman’s (1987) radial diffusion assay, which measures tannin reactivity with proteins. This method is appropriate for this
study because it provides relative amounts of reactive tannin, which allows for comparison among multiple taxa. Non-tannin phenolics do not interfere with this assay, though this has been a problem with other quantitative methods (Hagerman 1987).

Samples were prepared by extracting 150 mg of ground litter with 1.5 ml of 70% acetone / 10 mM ascorbic acid, and were sonicated for 15 min. Samples were then refrigerated until analysis. Petri dishes were partially filled with 9.5 ml of an agar / bovine albumin solution (1 % agarose (100 ml) mixed with 0.1 g bovine albumin (Sigma Corp. # 9048-46-8)). Four wells (4 mm) were punched in solidified agar and 16 μl of leaf extract or standard was dispensed into the wells. Plates were covered and placed in an oven at 30 °C. Precipitated protein ring diameter was measured after 120 h using Image J® (Image J, NIH) software. Results are presented as Tannic Acid Equivalents (TAE’s), which is an expression of a known quantity of tannin, as compared to tannic acid (Sigma Corp. # 1401-55-4) (Hagerman 1987, Reed and McCarthy 1996).

A litter fractionation separated leaves into three categories: (1) soluble in ethanol and water, (2) acid soluble (i.e., hemicellulose), and (3) acid insoluble (i.e., lignin) (Moorhead and Reynolds 1993). Litter (0.25g) was extracted with 25 ml ethanol, incubated in a 60 °C water bath for 30 min, and centrifuged for 5 min at 2000 rpm. This process was repeated two times with ethanol, three times with water, and then one final time with ethanol. The soluble fraction was calculated as the difference between the original and residual litter weight. After measurement, 2 ml of 72% sulfuric acid was added and samples were incubated for one hour at 30 °C. Samples were brought to 30 ml with distilled H2O, transferred to 125 ml flasks, autoclaved for one hour at 120 °C, and passed through a pre-weighed filter to capture the remaining, acid-insoluble mass. Filters
were oven-dried at 60 °C for 24 h and weighed. Hemicellulose fraction weight was calculated as the difference between the pre and post acid digest sample weight. The residue was the lignin content. A mineral fraction was calculated as the ash weight of the residue after combustion in a muffle furnace (500 °C for 12 h).

Mineral soil was analyzed for treatment-level differences in N mineralization, nitrification, exchangeable acidity, cation exchange capacity, and base saturation. The fumigation / evacuation method was used to assess net N mineralization and nitrification (Zak et al. 1994). Field-fresh soil (15 g) was fumigated for 20 h in a vacuum with chloroform. Soil from an unfumigated sample (~1.0 g) was transferred into the fumigated sample. Fumigated and control samples were incubated for two weeks at 20 °C. Samples were shaken for 30 min in 30 ml of 1M KCl and filtered. Fresh soil samples were also shaken in 30 ml of 1M KCl and filtered. Solutions were analyzed colorimetrically on a Synergy HT (Biotek, Winooski, VT) microplate reader (650 nm for NH₄, 450 nm for NO₃). Exchangeable acidity and aluminum were measured by extracting 10 g of oven-dried soil with 75 ml 1M KCl. Soils were shaken for 30 min, filtered, and washed with three 25 ml aliquots of KCl. Phenolphthalein indicator was added, and solutions were titrated with 1N NaOH to assess KCl-exchangeable total acidity. Solutions were mixed with 10 ml of 1 M KF and then titrated with 1N HCl to account for KCl-exchangeable Al³⁺ (Sims 1996). To extract base cations, 75 ml of Mehlich III solution (Tran and Simard 1993) was added to 10 g of field-fresh soil and shaken for 30 min. The suspension was then filtered. Solutions were washed and filtered with three subsequent 50 ml Mehlich III aliquots, and brought to volume at 250 ml, and analyzed on an ICP-OES (Varian 700-ES series, Varian, Inc., Walnut Creek, CA).
Extracellular enzyme activity assays were conducted using fresh Oa/e-horizon samples within 24 h of collection. \( \beta-1, 4 \) glucosidase (\( \beta \text{G} \)) (depolymerizes cellulose into glucose) is an indicator of the availability of polysaccharides for recycling in chestnut littered-soils compared with other species. \( \beta \)-N-acetylglucosaminidase (NAG) depolymerizes organic N from chitin, a compound found in fungal cell walls. Acid phosphatase breaks down phosphate esters. Phosphatase indicates how much organic P is being mineralized (Olander and Vitousek 2000). Peroxidase and phenol oxidase are both involved in the break-down of lignin and other phenolic compounds (Sinsabaugh et al. 2002). Fluorescence excitation @ 365 nm, emission @ 450 nm, and absorbance @ 460 nm was measured on a Synergy HT (Biotek, Winooski, VT) microplate reader (DeForest 2007, 2009).

**Data Analysis/Statistics**

Results were analyzed using the R statistical software program (R ver. 2.8.1, R Development Core Team 2009). All data were evaluated for assumptions using the Shapiro-Wilks test for normality, followed by the Bartlett’s test for homogeneity of variance. Because of the structure of the data (i.e., non-normal) Kruskal-Wallis rank sum tests were used, in each case followed by a Kruskal-Wallis multiple comparison, to determine where significant variance in data occurred (Zar 1999). Identical statistical analyses were preformed on data from all experiments.

Mass and litter fraction loss data were applied to an exponential decay equation:

\[
M_t = M_e^{-kt}
\]
Where $M_t$ and $M_0$ represent litter fraction mass remaining at time $t$ or time 0, respectively. The variable $t$ represents time (years), and the variable $k$ represents a decay constant (years). Values for $k$ were calculated for each taxon at each collection date (Li et al. 2009).

Enzyme activity was calculated using the following equation and expressed in nmol h$^{-1}$ g$^{-1}$. The volume of the soil slurry was 125 ml and amount of MUB standard added to a well was 0.5 nmol (Deforest 2009).

\[
\text{Activity (nmol h}^{-1} \text{ g}^{-1}) = \frac{\text{Net flour.} \times 125 \text{ ml}}{\text{Emission coef.} \times 0.2 \text{ ml} \times \text{Time (h)} \times \text{Soil (g)}}
\]

where

\[
\text{Net flour.} = \left( \frac{\text{Sample assay} - \text{Soil control}}{\text{Quench coefficient}} \right) - \text{Neg. control}
\]

\[
\text{Emission coef. (flour. nmol}^{-1}) = \frac{\text{Reference standard}}{0.5 \text{ nmol}}
\]

\[
\text{Quench coefficient} = \left( \frac{\text{Quench standard} - \text{Soil control}}{\text{Reference standard}} \right)
\]
RESULTS

**Experiment 1 – Comparing Castanea dentata and the 15/16 hybrid**

There was no significant difference in foliar N among taxa for chestnut foliage or litter (Fig. 3). There was significant difference in N concentrations between foliage and litter of each chestnut taxon (Kruskal-Wallis chi-squared = 68.3, df = 9, \( P < 0.01 \)). Leaves of all four chestnut taxa resorbed N (~50.9%) prior to senescence (Fig. 4, Table 2). Foliar C, C:N ratio, and litter fractions were similar \( (P > 0.05) \) among chestnut taxa (Table 1).

Concentrations of protein precipitate tannin did not vary between *Castanea dentata* and the 15/16 hybrid (Fig. 5). In all taxa, S (27.4%), Na (36.0%), and K (35.6%) were resorbed. Conversely, Ca (27.1%) and Mn (44.4%) accumulated in litter (Table 2).
Figure 3 - Differences (%) in foliar nutrient concentrations from American chestnut in three chestnut taxa. Boxes include the middle 50% of values, lines in boxes show the median value, and whiskers enclose the lower and upper quartiles.
Figure 4 – Resorption (negative values) from leaves or accretion (positive values) to leaves of nutrients prior to senescence in four chestnut taxa.
Figure 5 – Concentrations (a), and accretion (positive values) or resorption (negative values) (b) of condensed tannin in leaves of 4 chestnut taxa. Tannic acid equivalents (TAE’s) are condensed tannin concentrations relative to a tannic acid standard curve. Boxes include the middle 50% of values, lines in boxes show the median value, and whiskers represent the lower and upper quartiles.
Table 1 – Results from the C:N analysis for leaves (both foliage and litter) of *Castanea* taxa and co-occurring species. Standard error is in parentheses.

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<thead>
<tr>
<th>Taxon</th>
<th>N (%)</th>
<th>C (%)</th>
<th>C:N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Castanea dentata</em> foliage</td>
<td>2.53 (0.05)</td>
<td>46.83 (0.46)</td>
<td>18.51 (0.27)</td>
</tr>
<tr>
<td>15/16 hybrid foliage</td>
<td>2.56 (0.05)</td>
<td>50.92 (0.76)</td>
<td>19.89 (0.22)</td>
</tr>
<tr>
<td>7/8 hybrid foliage</td>
<td>2.39 (0.13)</td>
<td>48.44 (0.38)</td>
<td>20.27 (1.24)</td>
</tr>
<tr>
<td><em>Castanea mollissima</em> foliage</td>
<td>2.75 (0.11)</td>
<td>46.14 (0.40)</td>
<td>16.78 (0.65)</td>
</tr>
<tr>
<td><em>Castanea dentata</em> litter</td>
<td>1.17 (0.07)</td>
<td>42.9 (2.17)</td>
<td>36.67 (2.29)</td>
</tr>
<tr>
<td>15/16 hybrid litter</td>
<td>1.54 (0.25)</td>
<td>45.49 (8.92)</td>
<td>29.54 (1.47)</td>
</tr>
<tr>
<td>7/8 hybrid litter</td>
<td>1.02 (0.04)</td>
<td>40.06 (0.79)</td>
<td>39.27 (1.41)</td>
</tr>
<tr>
<td><em>Castanea mollissima</em> litter</td>
<td>1.30 (0.13)</td>
<td>36.13 (0.63)</td>
<td>27.79 (2.48)</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Castanea dentata</em></td>
<td>1.59 (0.08)</td>
<td>45.24 (2.11)</td>
<td>28.95 (1.43)</td>
</tr>
<tr>
<td><em>Acer rubrum</em></td>
<td>1.40 (0.10)</td>
<td>45.84 (1.02)</td>
<td>35.32 (2.14)</td>
</tr>
<tr>
<td><em>Quercus prinus</em></td>
<td>1.63 (0.11)</td>
<td>46.41 (1.07)</td>
<td>29.01 (2.18)</td>
</tr>
</tbody>
</table>
Table 2 - Nutrient concentrations (ug g⁻¹) for foliage (f) and litter (l) of Castanea dentata (Cd), 15/16 chestnut hybrid (15/16), 7/8 chestnut hybrid (7/8), and Castanea mollissima (Cm). Standard error is in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Cd f</th>
<th>15/16 f</th>
<th>7/8 f</th>
<th>Cm f</th>
<th>Cd l</th>
<th>15/16 l</th>
<th>7/8 l</th>
<th>Cm l</th>
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<tbody>
<tr>
<td>P</td>
<td>345.96</td>
<td>350.70</td>
<td>326.14</td>
<td>383.29</td>
<td>163.09</td>
<td>126.33</td>
<td>133.51</td>
<td>207.70</td>
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<td></td>
<td>(15.79)</td>
<td>(15.20)</td>
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<td>(36.25)</td>
<td>(21.56)</td>
<td>(14.58)</td>
<td>(27.36)</td>
<td>(64.69)</td>
</tr>
<tr>
<td>K</td>
<td>1549.99</td>
<td>1341.29</td>
<td>1411.99</td>
<td>2629.66</td>
<td>1044.54</td>
<td>949.10</td>
<td>1043.90</td>
<td>1437.80</td>
</tr>
<tr>
<td></td>
<td>(158.41)</td>
<td>(52.13)</td>
<td>(218.42)</td>
<td>(297.33)</td>
<td>(142.87)</td>
<td>(128.98)</td>
<td>(97.94)</td>
<td>(350.05)</td>
</tr>
<tr>
<td>Ca</td>
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<td>2093.90</td>
<td>1632.02</td>
<td>2267.50</td>
<td>2775.81</td>
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<tr>
<td></td>
<td>(247.25)</td>
<td>(163.99)</td>
<td>(267.58)</td>
<td>(261.52)</td>
<td>(165.09)</td>
<td>(249.87)</td>
<td>(432.10)</td>
<td>(169.39)</td>
</tr>
<tr>
<td>Mg</td>
<td>757.82</td>
<td>1029.47</td>
<td>564.55</td>
<td>726.26</td>
<td>1098.39</td>
<td>996.81</td>
<td>797.63</td>
<td>632.43</td>
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<td></td>
<td>(85.51)</td>
<td>(80.48)</td>
<td>(108.58)</td>
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<td>(116.40)</td>
<td>(129.98)</td>
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<tr>
<td>Na</td>
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<td>142.13</td>
<td>164.13</td>
<td>198.14</td>
<td>107.71</td>
<td>86.79</td>
<td>104.41</td>
<td>141.42</td>
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<tr>
<td></td>
<td>(9.29)</td>
<td>(4.93)</td>
<td>(12.33)</td>
<td>(16.77)</td>
<td>(10.06)</td>
<td>(2.80)</td>
<td>(15.77)</td>
<td>(13.15)</td>
</tr>
<tr>
<td>Al</td>
<td>187.40</td>
<td>128.73</td>
<td>101.63</td>
<td>194.64</td>
<td>166.97</td>
<td>134.60</td>
<td>152.11</td>
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<td>(9.46)</td>
<td>(11.17)</td>
<td>(17.24)</td>
<td>(22.33)</td>
<td>(35.77)</td>
<td>(22.57)</td>
</tr>
<tr>
<td>Mn</td>
<td>47.17</td>
<td>38.18</td>
<td>45.61</td>
<td>68.42</td>
<td>118.10</td>
<td>71.77</td>
<td>77.19</td>
<td>97.61</td>
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<td></td>
<td>(7.66)</td>
<td>(6.41)</td>
<td>(4.72)</td>
<td>(4.70)</td>
<td>(4.26)</td>
<td>(7.35)</td>
<td>(6.79)</td>
<td>(10.84)</td>
</tr>
<tr>
<td>S</td>
<td>189.36</td>
<td>225.83</td>
<td>206.86</td>
<td>311.91</td>
<td>160.43</td>
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<td>(39.47)</td>
<td>(7.82)</td>
<td>(18.11)</td>
<td>(4.53)</td>
<td>(24.73)</td>
</tr>
<tr>
<td>Cu</td>
<td>4.10</td>
<td>4.19</td>
<td>4.28</td>
<td>4.82</td>
<td>3.69</td>
<td>4.63</td>
<td>4.12</td>
<td>4.24</td>
</tr>
<tr>
<td></td>
<td>(0.10)</td>
<td>(0.09)</td>
<td>(0.09)</td>
<td>(0.18)</td>
<td>(0.10)</td>
<td>(0.70)</td>
<td>(0.13)</td>
<td>(0.05)</td>
</tr>
<tr>
<td>B</td>
<td>175.10</td>
<td>161.04</td>
<td>179.57</td>
<td>187.30</td>
<td>124.32</td>
<td>95.51</td>
<td>125.68</td>
<td>148.98</td>
</tr>
<tr>
<td></td>
<td>(2.99)</td>
<td>(1.89)</td>
<td>(3.86)</td>
<td>(11.16)</td>
<td>(8.47)</td>
<td>(7.38)</td>
<td>(10.13)</td>
<td>(6.31)</td>
</tr>
</tbody>
</table>

**Experiment 2 – Comparing Castanea dentata and co-occurring species**

Among the three forest-grown tree species analyzed, Castanea dentata had the greatest concentration of precipitate tannin, containing 25% and 38% more than Quercus
*prinus* and *Acer rubrum*, respectively. However, only the difference in tannin between *C. dentata* and *A. rubrum* was statistically significant (Kruskal-Wallis chi-squared = 26.1, df = 2, *P* < 0.01) (Fig 6). *Acer rubrum* contained significantly more soluble material than *C. dentata* and *Q. prinus* (Kruskal-Wallis chi-squared = 16.4, df = 2, *P* < 0.01). The amount of hemicellulose was similar (*P* > 0.16) among all three species. However, *C. dentata* and *Q. prinus* contained significantly more lignin than *A. rubrum* (Kruskal-Wallis chi-squared = 16.4, df = 2, *P* < 0.01). Lignin fractions were similar (*P* > 0.05) between *C. dentata* and *Q. prinus* (Fig 7). Nutrient content among species was generally similar. *Castanea dentata* contained significantly more S than *A. rubrum* (Kruskal-Wallis chi-squared = 11.6, df = 2, *P* < 0.01), and less K than *Q. prinus* (Kruskal-Wallis chi-squared = 14.797, df = 2, *P* < 0.01). *Castanea dentata* also contained significantly more Al (Kruskal-Wallis chi-squared = 36.7, df = 2, *P* < 0.01) and Mg (Kruskal-Wallis chi-squared = 7.8, df = 2, *P* = 0.02) than *Q. prinus* or *A. rubrum* (Table 3). For further nutrient values, see Table 3.
Table 3 – Concentrations of 10 nutrients (ug g\(^{-1}\)) in *Castanea dentata* and two extant species. Standard error in parentheses.

<table>
<thead>
<tr>
<th></th>
<th><em>Castanea dentata</em></th>
<th><em>Acer rubrum</em></th>
<th><em>Quercus prinus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>319.68 (22.36)</td>
<td>345.6 (52.66)</td>
<td>341.42 (13.41)</td>
</tr>
<tr>
<td>K</td>
<td>1714.12 (131.65)</td>
<td>1563.87 (124.06)</td>
<td>2237.57 (105.38)</td>
</tr>
<tr>
<td>Ca</td>
<td>1908.34 (139.66)</td>
<td>1845.59 (185.06)</td>
<td>1747.62 (120.21)</td>
</tr>
<tr>
<td>Mg</td>
<td>488.96 (25.89)</td>
<td>413.16 (40.90)</td>
<td>398.14 (27.27)</td>
</tr>
<tr>
<td>Na</td>
<td>&lt;2.00 (4.11)</td>
<td>&lt;2.00 (3.60)</td>
<td>6.35 (4.46)</td>
</tr>
<tr>
<td>Al</td>
<td>152.74 (19.27)</td>
<td>0.31 (1.68)</td>
<td>52.11 (21.56)</td>
</tr>
<tr>
<td>Mn</td>
<td>219.38 (12.83)</td>
<td>208.21 (25.98)</td>
<td>193.53 (21.26)</td>
</tr>
<tr>
<td>S</td>
<td>186.04 (23.99)</td>
<td>129.07 (8.19)</td>
<td>175.63 (8.79)</td>
</tr>
<tr>
<td>Cu</td>
<td>&lt;2.00 (0.14)</td>
<td>&lt;2.00 (0.23)</td>
<td>&lt;2.00 (0.15)</td>
</tr>
<tr>
<td>B</td>
<td>&lt;2.00 (3.86)</td>
<td>&lt;2.00 (2.17)</td>
<td>8.78 (3.43)</td>
</tr>
</tbody>
</table>
Figure 6 - Concentrations of condensed tannins, expressed as tannin acid equivalents (TAE’s), as compared to a tannic acid standard curve, in Castanea dentata and 2 co-occurring forest species. Boxes include the middle 50% of values, lines in boxes show the median value, and whiskers enclose the lower and upper quartiles.
Figure 7 – Foliar nutrient concentrations in forest-grown *Castanea dentata* and two co-occurring species. Boxes include the middle 50% of values, lines in boxes show the median value, and whiskers enclose the lower and upper quartiles.
**Experiment 3 – Leaf decomposition in Castanea taxa and co-occurring species**

The differences in mass loss and decay constant among species varied by sampling date (90-day interval; Fig 8 a-e). After three months in the field, the three chestnut taxa lost significantly more mass (~9%) than *Quercus alba* (Kruskal-Wallis chi-squared = 50.4, df = 4, \( P < 0.01 \)), but did not differ from *Acer saccharum* (\( P > 0.05 \)).

Mass loss among chestnut taxa was similar (\( P > 0.05 \)). After six months of decomposition, chestnut taxa again contained significantly less mass than *Q. alba*, and also differed significantly from *A. saccharum* (Kruskal-Wallis chi-squared = 66.7, df = 4, \( P < 0.0001 \)). All *Castanea* taxa contained significantly less mass than *Q. alba* (Kruskal-Wallis chi-squared = 53.6, df = 4, \( P < 0.01 \)), but did not differ from *A. rubrum* after nine months. However, after one year decomposing in the field, *Castanea* taxa had significantly more mass than *Q. alba* and *A. rubrum* (Kruskal-Wallis chi-squared = 70.6, df = 4, \( P < 0.01 \)). Each of the species had lost ~50% of their original weight.
Figures 8 a-e – Litter mass remaining (LMR) and the % of that mass allocated to each of three litter fractions, 1) alcohol and water soluble (below LMR line), 2) acid soluble (hemicellulose), 3) and acid insoluble (lignin), among chestnut taxa and extant species. Error bars represent one standard error.
Precipitate tannin content decreased by more than 80% in all species over the first 90 days of decomposition (Fig 9). However, after three months, chestnut selections all contained significantly less precipitate tannin than *Quercus alba* and *Acer saccharum* (Kruskal-Wallis chi-squared = 69.5, df = 4, \( P < 0.01 \)). After six months of decomposition, chestnut selections had lost all their precipitate tannin, and again differed significantly from *Q. alba* and *A. saccharum* (Kruskal-Wallis chi-squared = 89.8, df = 4, \( P < 0.01 \)), which retained between 0-2% of their original content for another three months.

The C:N analysis was similar among species. The mean ratio among all taxa seen after 6 months, 22.1:1, changed little for the remainder of the experiment (1 year). Overall, there was very little variation in any litter fraction among chestnut selections and among co-occurring species. The only significant difference was greater lignin concentrations in the chestnut hybrid and *Quercus alba* (Kruskal-Wallis chi-squared = 88.8, df = 4, \( P < 0.01 \)) litter when compared to other taxa after 183 days. In all taxa tested, the relative percentage of alcohol soluble carbon decreased, and lignin increased, with each subsequent sampling date.
Figure 9 – Changes in concentrations of Tannin Acid Equivalents (TAE’s) over 272 days among chestnut selections and co-occurring species. Error bars represent one standard error.

Experiment 4 – *A and O-horizon analysis*

Based on Kruskal-Wallis rank-sum analysis, enzyme activity was similar among all soil treatments (Fig 10). Likewise, all measured soil variables were similar among all soil treatments (Table 4).
Table 4 – Mean mineral soil nutrient concentrations (g kg\(^{-1}\)) under four differing litter treatments after 300 days. Standard error is in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Chestnut</th>
<th>Control</th>
<th>Disturbed</th>
<th>Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>70.74 (2.73)</td>
<td>70.60 (1.74)</td>
<td>68.88 (2.96)</td>
<td>71.09 (3.86)</td>
</tr>
<tr>
<td>B</td>
<td>1.27 (0.11)</td>
<td>1.19 (0.04)</td>
<td>1.23 (0.03)</td>
<td>1.20 (0.02)</td>
</tr>
<tr>
<td>Ca</td>
<td>4.32 (1.43)</td>
<td>4.34 (0.73)</td>
<td>5.48 (1.00)</td>
<td>5.43 (1.63)</td>
</tr>
<tr>
<td>Cu</td>
<td>0.13 (0.01)</td>
<td>0.13 (0.01)</td>
<td>0.14 (0.01)</td>
<td>0.14 (0.01)</td>
</tr>
<tr>
<td>K</td>
<td>2.71 (0.38)</td>
<td>2.97 (0.26)</td>
<td>3.14 (0.18)</td>
<td>2.66 (0.26)</td>
</tr>
<tr>
<td>Mg</td>
<td>1.36 (0.53)</td>
<td>1.24 (0.29)</td>
<td>1.38 (0.30)</td>
<td>1.36 (0.36)</td>
</tr>
<tr>
<td>Mn</td>
<td>0.75 (0.15)</td>
<td>0.39 (0.05)</td>
<td>0.59 (0.04)</td>
<td>0.75 (0.04)</td>
</tr>
<tr>
<td>Na</td>
<td>1.73 (0.03)</td>
<td>1.60 (0.07)</td>
<td>1.66 (0.06)</td>
<td>1.62 (0.07)</td>
</tr>
<tr>
<td>P</td>
<td>0.46 (0.29)</td>
<td>0.56 (0.39)</td>
<td>0.57 (0.23)</td>
<td>0.55 (0.33)</td>
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</tbody>
</table>
Figure 10 – Total activity of flourimetric (beta-1, 4 glucosidase, β-N-acetylglucosaminidase (NAG), acid phosphatase, and beta-xylosidase), and oxidative (peroxidase, phenyl oxidase) enzymes in the O-horizon of forest floors underneath four different litter treatments. Boxes include the middle 50% of values, lines in boxes show the median value, and whiskers enclose the lower and upper quartiles. Hollow circles indicate outlying data points.
DISCUSSION

The larger question driving these experiments was whether or not an introduction of hybrid chestnut trees into the former range of *Castanea dentata* will have any effect on nutrient cycling. *Castanea dentata* leaf nutrient content did not differ from the 15/16 hybrid proposed for restoration. Furthermore, I only observed minor differences in leaf chemistry and decay rate among *C. dentata*, *Quercus* spp., and *Acer* spp., the latter two genera having largely replaced the former canopy dominant.

I failed to reject the null hypothesis that concentrations of precipitate tannin in *Castanea dentata* would roughly equal that of extant tree species. The historic use of tannic acid from *C. dentata* in the tanning industry due to high concentrations in bark led to the alternative hypothesis of *C. dentata* containing greater amounts of tannin. Apparently, the relative abundance of this species in the Appalachian region was a partial cause for its use in the tanning industry as well (Sterling 1902). Tannin concentration in *C. dentata* turned out to be well within the range found among other tree species typical of the Appalachian hardwood region (Reed and McCarthy 1996, Talbot and Finzi 2008).

On the other hand, I reject the null hypothesis that decay rate is similar among *Castanea dentata* and extant species; *C. dentata* decomposes more slowly than *Quercus alba* and *Acer rubrum* (Fig. 8 a-e). Greater initial precipitate tannin content in chestnut cannot explain the slower decay rate because initial orchard-grown chestnut tannin content turned out to be lower than that of *Q. alba*, and chestnut tannins leached out most quickly (Fig 9). This greater concentration of condensed tannin in *Q. alba* may be a result of differences in microsite conditions such as aspect, moisture, slope position, soil chemistry, soil biology, or light exposure. Reed and McCarthy (1996) found that *Q.*
prinus expressed different levels of tannins at different aspect and slope position. However, Reed and McCarthy (1996) also found that differences in foliar condensed tannin among Q. velutina Lamb. were most closely related to site, rather than aspect and slope position, alluding to a difference in tannin production on a population level. To add strength to the tannin results in this study, foliage samples were collected from transects in southwest Virginia, where remnant stands of Castanea dentata are abundant. In these samples, C. dentata contained greater concentrations of tannin than Q. prinus (not significant) and Acer rubrum (Kruskal-Wallis chi-squared = 26.2, df = 2, P < 0.01).

It is possible that the slower decomposition of Quercus alba was partially facilitated by the greater tannin concentrations in this species, because of the known recalcitrance of tannins (Horner et al. 1988, Northup et al. 1998). Following this line of thought, one might conclude that the disappearance of from all leaves (about 6 months into the study) led to a decay rate that was similar among all species. Correlation analysis investigating possible relationships between decay constant (k), and TAE concentration, lignin content, or nitrogen concentration did not reveal any significant relationships. There also exists the possibility, in line with the Talbot and Finzi (2008) study, that the tannins in the different species used in this study vary in composition and hence recalcitrance. This may have affected decay rates.

The early differences in decay rate found in this study are likely a result of differing response to initial nutrient content. Litter is commonly thought to decay in two stages (Berg 2000). The first of these stages is often governed by climate, concentration of the leaf’s water-soluble fraction, the leaf’s hemicellulose content, and the total concentration of major nutrients. The later stage of decomposition, which usually occurs
more slowly, is regulated more by lignin concentration. Berg (2000) notes that decay rate and mass loss will decrease asymptotically over time and may even reach a stopping point. Initially, litter from chestnut taxa had 28% more N than *Quercus alba* and 48% more N than *Acer rubrum*. This is likely due to the soil beneath the chestnut trees from which the decomposition experiment litter was collected. Soils at the Meadowview Research Farm receive periodic N fertilization. The artificially high N may have contributed to the rapid leaching of tannins as well. In the forest-collected foliage subsequently tested, *Castanea dentata* contained slightly less N than *Q. prinus* and *A. rubrum*, though not significantly so. Based on the similarity in litter fraction results between *C. dentata* and the *Quercus* species analyzed, as well as the similar nutrient content found in *C. dentata* and *Q. prinus*, I predict that had the chestnut leaves not been N-amended, their decomposition profile would more closely mirror that of *Q. alba*. In the context of this study, and despite an absence from the system or over seventy years, similar decomposition profiles imply that reintroduced hybrid chestnut litter will not alter forest nutrient cycling.

Foliar nutrient contents and resorption profiles were generally similar among all taxa analyzed. Concentrations of foliar nutrients are usually a result of available soil nutrient pools, species life history characteristics, and nutrient reserves in the plant (Cotrufo 1977, Chapin 1980). To conserve valuable nutrients, individual trees often resorb them prior to senescence, which leads to smaller pools of that nutrient in the soil. The resorbed and stored reserves of nutrients, crucial to growth, serve to jumpstart the following year’s biomass production after the dormant season. Resorption can also be considered a long-term nutrient retention strategy, as it prevents mineralization and
subsequent leaching of nutrients from decomposed leaves (Chapin 1980). A fundamental difference in leaf chemistry or resorption would have indicated that a reintroduction of chestnut may alter locations of nutrient pools, and in turn, perhaps affect competition. Whittaker et al. (1974) found that in Eastern forests, N (~66%) and P (~80%) are generally retracted from senescing leaves by trees, and concentrations of Na (~20%), Mn (~100%), and Ca (20%) in leaves tend to increase prior to leaf senescence (Chapin 1980, Killingbeck 1984, Aerts 1996). The foliar nutrient translocation results for both *Castanea dentata* and the 15/16 hybrid are consistent with the Whittaker et al. (1974) findings stated above. Nitrogen resorption proportions for chestnut taxa were also within the range of the proportions reported by Killingbeck (1984) for Burr Oak (*Quercus macrocarpa* Michx.) and Chinquapin Oak (*Quercus muehlenbergii* Engelm.), which are both also members of Fagaceae and thought to be similarly adapted to relatively nutrient-poor sites.

The fact that no variation was found among soil treatments for the several variables analyzed may be explained by the lack of difference in litter chemistry among species. N mineralization has been found to be correlated most closely with the lignin:nitrogen ratio of litter because of that ratio’s control over organic matter quality (Scott and Binkley 1997). It has also been found to be governed most closely by the quantities of acid soluble compounds and lignin (McClougherty et al. 1985). In this case, the input of litter that has higher foliar N concentrations (i.e., chestnut) did not result in a difference in enzyme activity, N mineralization, or nitrification, leading one to conclude that the similar labile fractions mediated these variables. If, in year 2 of decomposition, lignin begins to decay at differing rates in different species, one can expect to find greater
divergence in the mass loss ratio and in soil mineralization rates, and differing concentrations of reactive polyphenolics in soils (McClaugherty et al. 1985). This may in turn lead to changes in enzyme activity. At this point, though, in context of this study, one cannot expect changes in soil chemistry to occur as a result of the introduction of 15/16 hybrid chestnut litter. However, Prescott (2002) found that the effect of tree species on nutrient availability can be best predicted by analyzing the mass of litter produced and the nutrient concentrations of that litter, rather than decay rate. Species that lose mass more rapidly reach a point of slower decomposition more quickly, and at times with a greater proportion of their overall mass remaining (Berg et al. 1995). This study did not look at the overall mass produced by each of the trees used for comparison, so this variable cannot be eliminated as a possible source of future variation. Subsequent litter additions will be added in future years to continue to monitor for differences, as decomposition is a process that continues to impact soils on a timeline much greater than one year (Hector et al. 2000).
CONCLUSION

The results of these experiments give us a good idea about what impact hybrid chestnut litter may have when introduced into the former range of the native *Castanea dentata*, the American chestnut. Though this study failed to reject the null hypothesis of no difference in condensed tannin concentration between *C. dentata* and co-occurring extant species, it did discover that the tannin concentrations in chestnut litter were well within the range, found in the literature, for other congeners. This discovery eliminates the possibility of a chestnut reintroduction putting ecosystem-altering quantities of tannins into the soil. Chestnut, though native at one time, has been absent for many decades. Whilst the tannins in decomposing chestnut leaves leached out more quickly than tannins in oak and maple, all species had lost almost all of their precipitate tannin after 180 days. With this in mind, the initial variations may mean little in systems where impacts of nutrient disparities are seen on a scale of many years. For example, N has been found to be continually released from litter constituents for 5 years, with the majority of mineralization happening after year one (Laiho and Prescott 1999).

The finding that hybrid chestnut litter will be chemically similar to pure American chestnut litter can be seen as promising for the effort to reintroduce these hybrids, in that it demonstrates that a chemically “familiar” product is being introduced back into the system, even though it has been absent for over 70 years. The decomposition data and lack of change in the soil chemical and biological activity compared to native forest floors and mineral soils further confirms the notion that a reintroduction of hybrid chestnut probably will not alter nutrient cycling pathways.
CAVEATS / FUTURE DIRECTIONS

This study is subject to a couple of limitations. The chestnut leaf litter (hybrid, American, and Chinese) collected from the Meadowview Research Farm in Meadowview, VA, came from trees that grew in soil that receives annual nitrogen fertilization. Therefore, the greater nitrogen content in those leaves may have led to more rapid decomposition in a nitrogen-limited system; however, all relative comparisons among chestnut taxa remain valid. A second limitation of this study is the relatively short amount of time (10 months) that elapsed between the establishment of field decomposition sites (for the testing of underlying soils), and the soil sampling dates. Decomposition of the deposited litter was certainly not complete. However, while changes in soil chemistry can take years to appear, tannins are usually leached from leaves within one year (Parfitt and Newman 2000). I attempted to compensate for this by analyzing enzyme activity in the O-horizon, where one would expect to see relative differences in litter types fairly quickly. This limitation will be further addressed in the future through additional collections at the field sites established in this experiment. Additional annual deposits of 300 g of litter will be placed beneath the chicken-wire enclosures for each of the litter treatments. Identical soil metrics will be analyzed to look for change over time. There are also three “chains” of litter bags remaining on each of the five slopes, which will be collected at three different dates, each separated by six months. It is predicted that lignin will play a larger role in the decomposition process in the coming years, and greater variation in decay rate will be seen among species (Moorhead and Reynolds 1993).
There is an abundance of future work to be done on the effects of a chestnut reintroduction on eastern North American forests. To strengthen the findings of this study, additional litter bags containing foliage from forest-grown chestnuts could be placed on the same slopes used in this study and comparisons could be made between the decay rates of the farm-grown and forest-grown chestnut foliage.

This study has provided evidence that a reintroduction of chestnut into eastern North American forests will not significantly alter the nutrient cycling. It is my hope that this data can further the effort to restore a species that has great value to eastern forest ecosystems and to the cultures that interact with them.
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