Rec'd 2/4/13

Grant Report Title: *Comparative analysis of chestnut growth and survival on Appalachian surface mine lands*
Principal Investigators: Lauren E. Bizzari and Brian C. McCarthy
Address: Porter Hall 315, Ohio University, Athens, OH 45701
Telephone: 802-279-5578

Narrative Summary

Objectives

The purpose of this research was to conduct a follow-up study on a project initiated in 2008 between TACF and the Appalachian Regional Reforestation Initiative (ARRI). Five different chestnut taxa (Chinese, American, B_1-F_3, B_2-F_3, and B_3-F_2) were planted on reclaimed surface mine lands in six different Appalachian states: PA, MD, OH, WV, KY, and TN. We sought to address the following questions:

1. After five growing seasons, do pure and hybrid *Castanea* genotypes exhibit differences in growth, survival, and form on mined lands across the Appalachian Region?
2. What specific site factors (soil properties, vegetation cover, local climate) affect the growth and survival of pure and hybrid *Castanea* genotypes on mined lands?

Methods of Monitoring and Evaluation

Field Data Collection

Travel to chestnut planting locations to collect field data began in May 2012 and concluded in August 2012. Lauren Bizzari visited four states (OH, PA, TN, and KY) and a total of six planting sites. Plantings in PA are spread out across three different sites. However, data were collected from only two of the PA sites (PA-S, PA-F). One of the original PA sites (containing biosolid amendments) had experienced complete failure by 2010 and remained a failure as of July 2012. The OH planting site is subdivided into three blocks based on slope and aspect (OH-1, OH-2, OH-3). The TN planting site is also subdivided into four blocks by slope, aspect, and FRA treatment. However, these blocks were further condensed into two blocks for our analysis (TN-A, TN-C). At each site, up to 15, 10 × 10 m plots were established (depending on size of the planting area at each site). Soil samples were collected within these plots. In addition, the plots were used to assess vegetation cover and volunteer sapling density on the sites.

In August 2012, data on chestnut growth and survival were collected from all four states. Only trees that had been protected with shelters during planting were measured. Leaf samples (for specific leaf area and chlorophyll extraction) and measurements of tree architecture were also collected at this time. Sites in OH and KY were particularly affected by the 2012 summer drought. Many trees at these sites leafed out in the spring, but died back at the peak of the drought in the summer. Some of these trees later produced a second flush a growth in July-August, while others remained apparently dead (i.e., top-killed) for the rest of the season.

Many researchers from other universities and organizations volunteered to assist with field data collection: Jennifer Franklin (University of Tennessee – Knoxville) and Chris Miller (OSM – TN) at Zeb Mountain, TN; Chris Barton (University of Kentucky) and Michael French (TACF) at Bent Mountain, KY; Doug Saylor in PA; Keith Gilland, Corey Kapolka, Alex

Anning, and Joe Moosbrugger (all of Ohio University) at Jockey Hollow Wildlife Management Area in OH. Their assistance was greatly appreciated.

Statistical Analysis

All statistical analyses were performed using the statistical software program R (R Development Core Team 2011). Survival data were analyzed using a chi-square goodness-of-fit from the "stats" package in R. Total height (2012, cm), root collar diameter (RCD, mm), and vegetation cover could not be transformed to meet the assumptions of normality (D'Agostino omnibus test, "fBasics" package in R) and heteroscedasticity (Modified Levene's test, "lawstat" package in R) to perform an analysis of variance (ANOVA). The non-parametric Kruskal-Wallis rank sum test in the "stats" package in R was used to assess these data as an alternative test (i.e., when assumptions could not be met for a parametric analysis). In addition, the soil properties pH (after conversion to concentration of hydrogen ions) and % Sand could not be transformed to meet the assumptions to perform ANOVA. These two variables were analyzed using the non-parametric Kruskal-Wallis rank sum test as well. If the Kruskal-Wallis test yielded a significant difference among sites ($P < 0.05$), we tested differences between pairs of sites using the multiple comparison test for Kruskal-Wallis (`kruskalmc`) in the "pgirmess" package in R. The remaining soil variables (% coarse fragment by volume, bulk density (g cm^{-3}), % silt, % clay, and % organic matter) met all assumptions for ANOVA and were analyzed using the `aov` function in the "stats" package in R. If ANOVA resulted in significant differences among sites, means from individual sites were compared using Tukey's Honestly Significant Differences (`TukeyHSD`) in the "stats" package.

Results and Discussion

Survival, Total 2012 Height, and Root Collar Diameter of Five Chestnut Taxa

Survival differed significantly among the five chestnut taxa across all sites (chi-square goodness-of-fit, $\chi^2 = 73$, df = 4, $P << 0.01$). Specifically, Chinese chestnuts and the earlier B_1-F_3 backcross had higher percent survival than American chestnut and later backcrosses (Figure 1). In contrast, 2012 total height and RCD did not differ among taxa (Kruskal-Wallis rank sum test, Height: $H = 4$, df = 4, $P = 0.376$; RCD: $H = 2$, df = 4, $P = 0.618$; Figure 2). This result is likely due to the large effect of site on 2012 total height ($H = 563$, df = 7, $P << 0.01$) and RCD ($H = 595$, df = 7, $P << 0.01$). Overall, the best growth occurred on sites KY and TN-A (Figure 3). In KY, growth may have been stimulated by the addition of fertilizer to the planted seeds (Chris Barton and Fred Hebard, personal communication). Survival also differed significantly among sites ($\chi^2 = 74$, df = 7, $P << 0.01$), with the highest overall percent survival in OH and PA (Figure 4).

Additionally, we explored the environmental conditions (e.g., soil properties, climate) and corresponding vegetation at the sites to identify factors that may have influenced our observed patterns in chestnut growth and survival. While analysis of these factors is ongoing, some preliminary results are available.

Vegetation Cover

Vegetation cover less than 1m in height was separated by functional group: graminoids (grasses, sedges, and rushes), legumes (nitrogen-fixing herb species), vines, ferns, shrubs (woody species < 1m in height), trees (woody species > 1m in height), and herbaceous vegetation (all other broad-leaved non-woody flowering plants). The graminoid, legume, and herbaceous groups accounted for the majority of plant species present at all sites; thus, we will focus on the results from these three functional groups.

Percent cover of herbaceous vegetation differed significantly among sites ($H = 132$, df = 7, $P << 0.01$), with sites in OH and KY having the highest percent cover of herbaceous vegetation (Figure 5). Sites also differed significantly in percent cover of graminoids ($H = 54$, df = 7, $P << 0.01$) and legumes ($H = 109$, df = 7, $P << 0.01$). Sites in PA had the highest graminoid cover (Figure 6), while sites in OH and TN-C had the highest legume cover (Figure 7). A far more interesting pattern was revealed when total vegetation cover (Σ cover of all functional groups) was examined. Sites with the highest vegetation cover (TN-C, all OH, all PA) had the least total height and RCD growth (Figures 3, 8). Two of these sites (OH-1 and PA-S) also had the highest percent survival (Figure 4).

This result is similar to that found by Gilland and McCarthy (in press) in a different chestnut planting at our OH site. American chestnuts growing on mounds classified as having "heavy" vegetation cover (>70%) showed significantly higher survival over three years than chestnuts on mounds with less vegetation cover (Gilland and McCarthy, in press). The authors suggested that heavy vegetation cover may ameliorate stress on seedlings from heat and decreased soil moisture on these mine sites. Gilland and McCarthy (in press) also discussed the potential for vegetation to provide protective cover for rodents, which could increase seedling mortality. Chestnuts on sites in PA, OH, and TN-C frequently showed signs of browse damage from either rodents or deer (L. Bizzari, personal observation). However, heavy vegetation cover and subsequent browse appears to have had a greater effect on total height and RCD than survival after five years (Figures 3, 8). Chestnuts can resprout from the root collar if the main stem dies (Paillet 2002); saplings at heavily vegetated (and browsed) sites may be reflecting this life history trait in that they were smaller (Figure 3) and multi-stemmed (L. Bizzari, personal observation) than saplings at sites with less cover.

Soil Properties

In examining soil properties, one plot in the TN-C site (TN14) was removed from the analysis. This particular plot was located in a small, very unrepresentative section of TN-C where the substrate was entirely shale.

All soil properties differed significantly among sites (Tables 1, 2). Percent coarse fragment (particles > 2mm) and bulk density were very similar among sites, though PA-F had significantly lower percent coarse fragment and bulk density than all other sites (Table 3). For soil texture, sites in OH had higher percent clay and lower percent sand than most other sites (Tables 3, 4). Sites in OH also had significantly higher percent organic matter (Table 3).

Published Works and Presentations

October 2012 - Bizzari, L. and B.C. McCarthy. *Growth and survival of five chestnut taxa on reclaimed surface mine lands across the Appalachian Region.* The American Chestnut Summit. Asheville, North Carolina. (Poster)

April 2013 – Bizzari, L.E. and B.C. McCarthy. *A large-scale experimental assessment of growth and survival of American chestnut and reforestation efforts across the central Appalachian* *range.* Association of Southeastern Biologists Annual Meeting in Charleston, WV. (Presentation)

We also expect to submit a manuscript for peer-reviewed publication in *Applied Vegetation Science* in Summer 2013.

Literature Cited

Gilland, K.E. and B.C. McCarthy. In press. Reintroduction of American chestnut (*Castanea dentata*) on reclaimed mine sites in Ohio: Microsite factors controlling establishment success. Northern Journal of Applied Forestry.

Paillet, F.L. 2002. Chestnut: history and ecology of a transformed species. Journal of Biogeography 29:1517-1530.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Figures and Tables

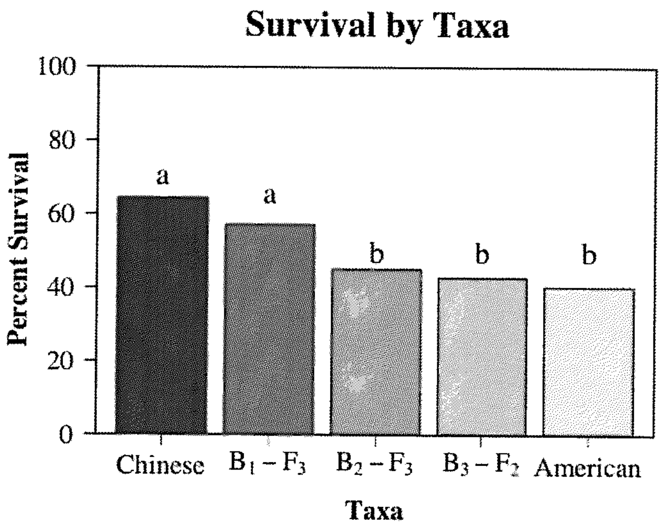

Figure 1: Percent survival of five chestnut taxa. Letters represent significant differences (P < 0.05) among taxa.

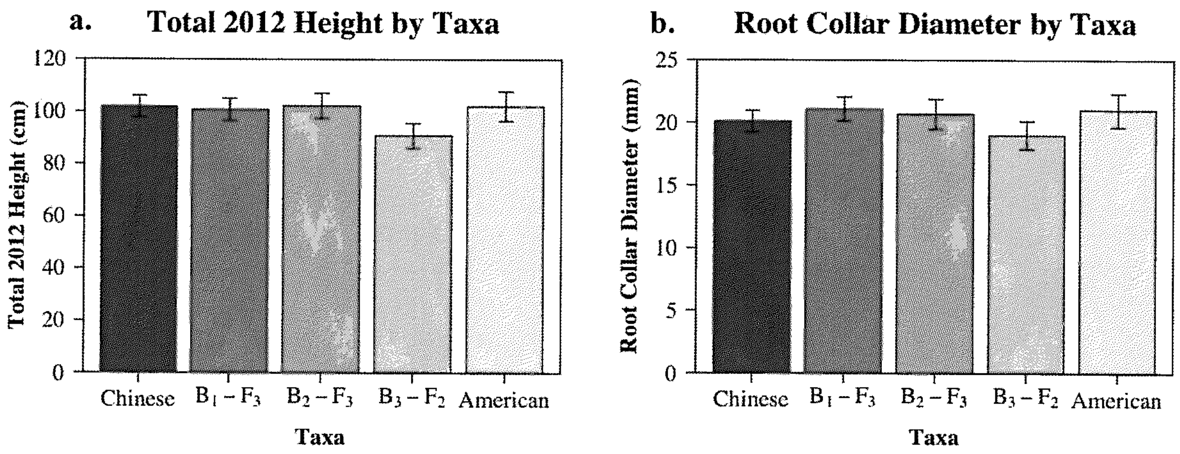

Figure 2: Mean (± SE) 2012 height (a) and root collar diameter (b) of five chestnut taxa. Taxa did not significantly differ for either measurement ($P > 0.05$*).*

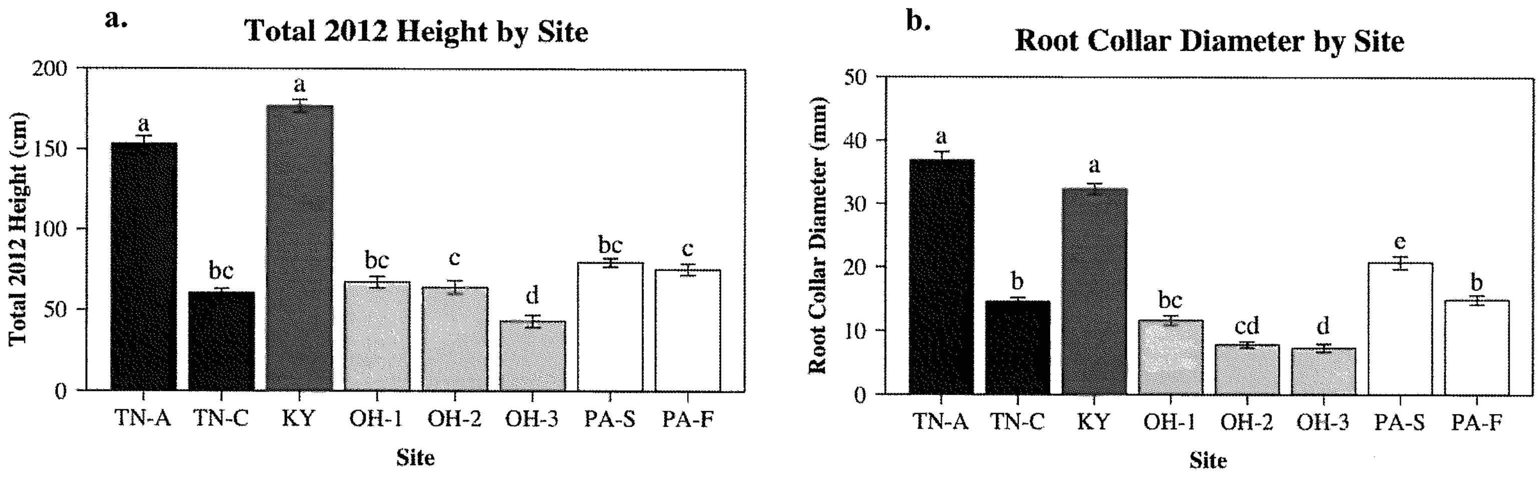

Figure 3: Mean (± SE) 2012 height (a) and root collar diameter (b) by site. Letters represent significant differences ($P < 0.05$) among sites.

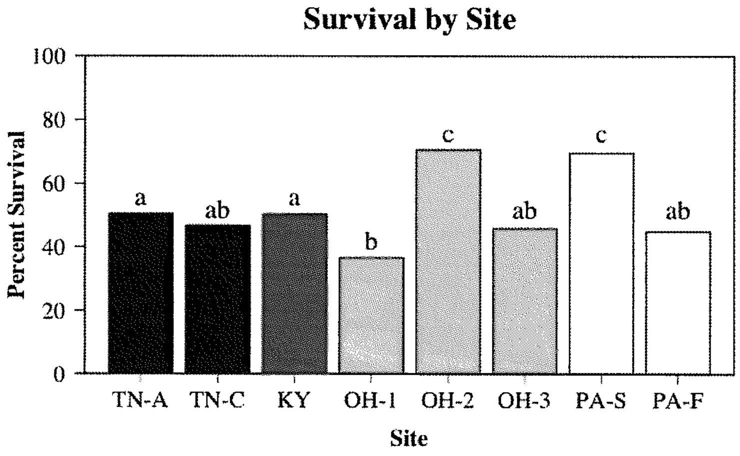

Figure 4: Percent survival across eight reclaimed mine sites. Letters represent significant differences ($P < 0.05$) among sites.

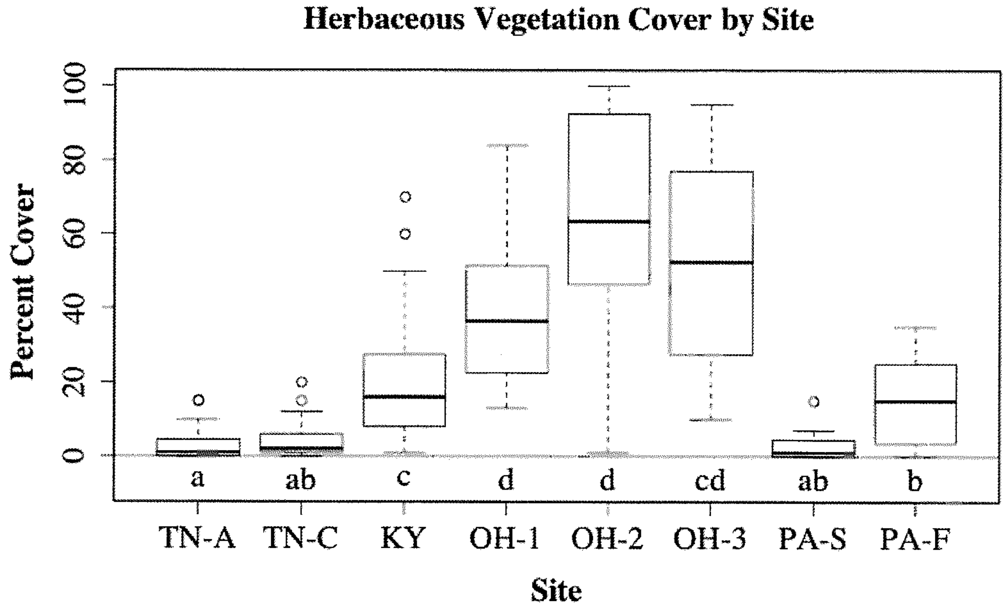

Figure 5. Boxplot of herbaceous vegetation cover (%) by site. Box represents Interquartile Range (IQR, 25th-75th percentile) and bold centerline represents the median. Whiskers represent largest and smallest values within 1.5 × IQR from top or bottom of box. Circles represent outliers (values < or > 1.5 × IQR from top or bottom of box). Letters represent significant differences (P < 0.05) among sites.

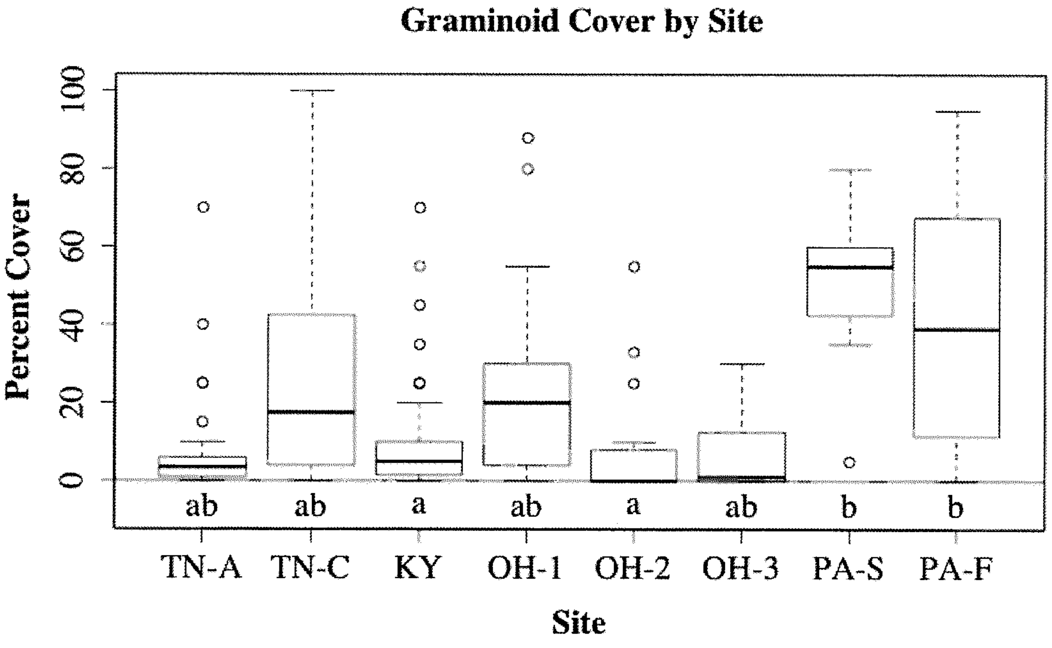

Figure 6. Boxplot of graminoid vegetation cover (%) by site. Letters represent significant differences (P < 0.05) among sites.

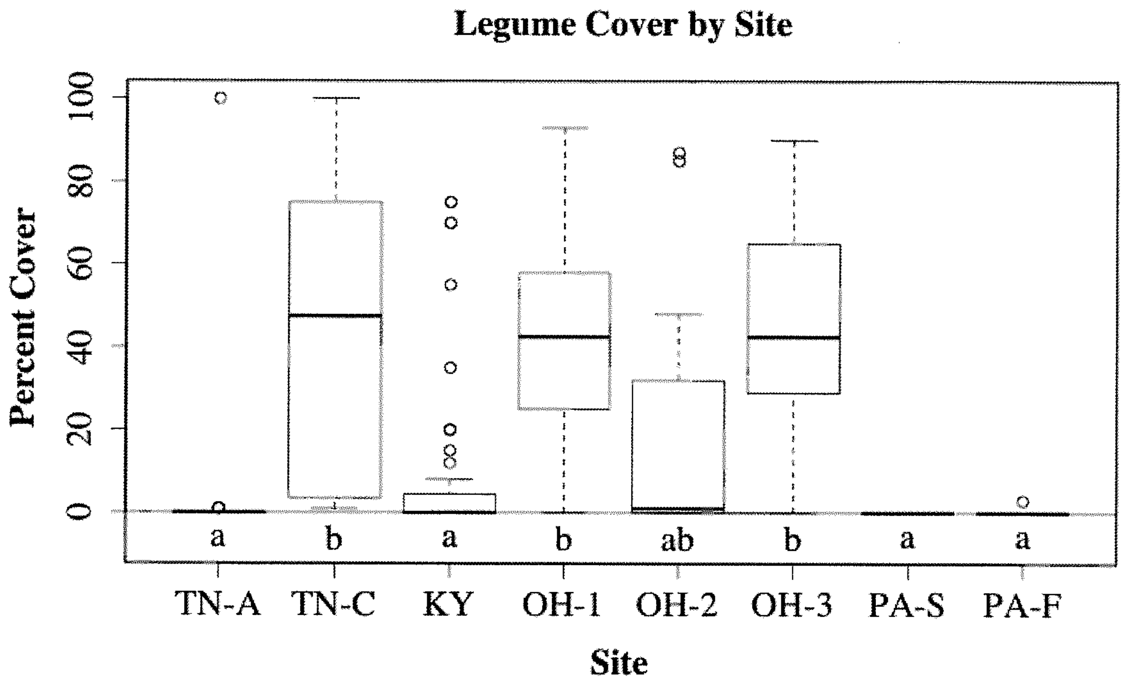

Figure 7. Boxplot of legume vegetation cover (%) by site. Letters represent significant differences (P < 0.05) among sites.

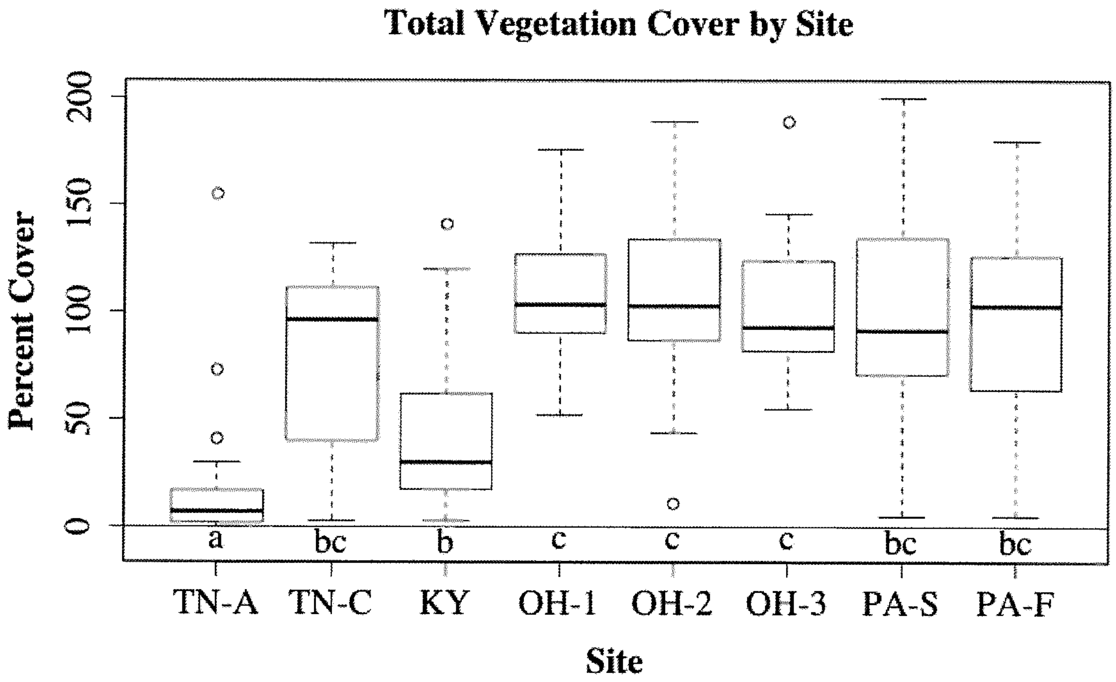

Figure 8. Boxplot of total vegetation cover (%) by site. Letters represent significant differences (P < 0.05) among sites.

Table 1. ANOVA table for soil properties: % coarse fragment, bulk density, % silt, % clay, and % organic matter.

Soil Properties	***df***	**MS**	***F***	***P***
% Coarse Fragment	7	134.41	7.624	<< 0.01
Bulk Density (g cm^{-3})	7	0.1184	8.3	<< 0.01
% Silt	7	206.61	11.04	<< 0.01
% Clay	7	121.6	12.04	<< 0.01
% Organic Matter	7	44.22	101.8	<< 0.01

Table 2. Kruskal-Wallis rank sum test results for pH and % Sand

Soil Properties	***df***	***H***	***P***
pH	7	35.6	<< 0.01
% Sand	7	36.1	<< 0.01

*Table 3. Mean (± SE) of soil properties by site: percent coarse fragment by volume (particles >2mm); bulk density (g cm^{-3}); percent silt and clay (by weight); percent organic matter (by weight). Letters represent significant differences (*P *< 0.05) among sites using Tukey's HSD multiple comparisons.*

	Site							
Soil Properties	**TN-A**	**TN-C**	**KY**	**OH-1**	**OH-2**	**OH-3**	**PA-S**	**PA-F**
% Coarse Fragment	24.0 ± 1.5^{a}	26.4 ± 3.1^{a}	25.9 ± 0.9^{a}	25.7 ± 1.6^{a}	22.9 ± 2.4^{a}	26.6 ± 0.8^{a}	24.2 ± 0.5^{a}	12.3 ± 2.0^{b}
Bulk Density (g cm^{-3})	1.28 ± 0.06^{a}	1.25 ± 0.06^{ab}	1.35 ± 0.03^{ab}	1.45 ± 0.03^{ab}	1.39 ± 0.04^{ab}	1.52 ± 0.06^{b}	1.31 ± 0.01^{ab}	1.04 ± 0.03^{c}
% Silt	41.7 ± 1.4^{a}	28.0 ± 0.8^{c}	43.0 ± 1.6^{ab}	49.1 ± 1.2^{b}	47.8 ± 1.5^{ab}	47.3 ± 0.3^{ab}	40.0 ± 2.0^{ab}	39.0 ± 1.7^{a}
% Clay	16.1 ± 1.0^{ad}	10.3 ± 0.8^{d}	16.5 ± 0.8^{a}	24.1 ± 1.5^{b}	22.3 ± 1.0^{bc}	24.0 ± 0.7^{b}	14.0 ± 0.0^{acd}	15.7 ± 2.2^{ad}
% Organic Matter	3.6 ± 0.2^{a}	3.0 ± 0.1^{a}	4.9 ± 0.2^{b}	7.5 ± 0.2^{c}	7.9 ± 0.3^{c}	6.8 ± 0.4^{c}	15.2 ± 0.8^{d}	4.8 ± 0.3^{b}

*Table 4. Median (with IQR) of soil properties by site: pH and sand (by weight). Letters represent significant differences (*P *< 0.05) among sites using Kruskal-Wallis multiple comparisons.*

	Site							
Soil Properties	**TN-A**	**TN-C**	**KY**	**OH-1**	**OH-2**	**OH-3**	**PA-S**	**PA-F**
% Sand	42 *(39-44)*^{ab}	61 *(60-63)*^{b}	40 *(33-46)*^{ab}	27 *(25-29)*^{c}	30 *(28-32)*^{ac}	29 *(28-29)*^{ac}	46 *(45-47)*^{abc}	46 *(40-51)*^{ab}
pH	3.84 *(3.64-3.97)*^{a}	5.30 *(5.01-6.02)*^{ab}	5.45 *(4.86-6.63)*^{b}	6.97 *(5.30-7.66)*^{b}	7.61 *(7.55-7.64)*^{b}	7.57 *(7.51-7.67)*^{b}	4.15 *(4.09-4.21)*^{ab}	4.52 *(4.46-4.87)*^{ab}