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NEAR-SURFACE SOIL NITROGEN AND VEGETATION RESPONSE TO INVASIVE EMERALD ASH BORER IN FORESTED BLACK ASH WETLANDS OF THE WESTERN UPPER PENINSULA, MICHIGAN, USA

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By

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Abstract

Invasive emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) poses an imminent threat to the structure and function of North American hardwood forests, particularly black ash (*Fraxinus nigra* Marshall), and alters the hydrologic and ecological services of their wetlands. Black ash trees regularly grow in seasonally saturated soils and are responsible for hydrologic regulation and nutrient cycling. In this study, a gradient of black ash wetlands impacted by EAB were monitored to assess vegetation changes and near-surface soil nitrogen availability. Vegetation community changes were intertwined with nitrogen cycle disturbances following EAB infestation. As black ash died and fell to the wetland, more total organic nitrogen was returned to the environment and promptly incorporated into the growing shrub and sapling layers. Assessing vegetation and biogeochemical changes along an EAB gradient in the environment improves our understanding of the ecological ramifications for a future landscape without black ash wetlands as they presently exist.

Vegetation Composition and Site Characteristic Response to a Gradient of EAB Infestation

1.1 Abstract

Invasive Emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) poses an imminent threat to the structure and function of North American hardwood forests. Paramount, the fate of black ash (*Fraxinus nigra* Marshall) will have large ecosystem impacts. Black ash trees regularly grow in seasonally saturated soils and are responsible for hydrologic regulation, nutrient cycling, and frequently dominate the canopy in black ash wetlands. To research future impacts caused by EAB, a gradient of black ash wetlands impacted by EAB was monitored to assess vegetation changes. The wetlands were characterized by small, riparian forested areas with black ash as the dominant canopy species. Infestation severity varied as a result of the length of time EAB has been in the sites and created the gradient of site treatments. In the near future, the sites heavily impacted by EAB will see continued ash mortality and then complete removal from the forest landscape. When ash is removed, a novel ecosystem will begin. A gradient exists in site parameters, with greater deviation from an unimpacted site as EAB infestation progresses on the landscape A new vegetation community has developed as forested black ash wetlands are slowly being converted into a post-EAB ecosystem, characterized by large amounts of coarse woody debris from dead ash, rising water levels from a lack of transpiration, and an absence of ash trees recruiting to the canopy where they were once dominant. Other tree saplings occurring in the wetlands will be recruited to the canopy, and a new shrub layer has emerged as the EAB infestation progressed.

1.2 Introduction

Black ash (*Fraxinus nigra* Marshall) wetlands deliver invaluable ecosystem services to our society such as water retention, streamflow regulation, and habitat diversity (Zedler and Kercher 2005). Emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) raises a deep concern on wetland ecosystem health because of its severe impacts on ash trees (Herms and McCullough 2014). Since its original identification in Detroit, MI in 2002 (Siegert et al. 2014), EAB has spread to 35 states and 5 Canadian providences (EAB Information Network 2023). EAB can cause 100% ash mortality within 3-6 years (Klooster et al. 2014). Previous studies have focused on pest biology (Prasad et al. 2010), ash reaction (Smitley, Davis, and Rebek 2008), and management methods for control (Liu and Bauer 2008). However, less attention has been dedicated to the effects of EAB on vegetation dynamics in black ash forested wetlands (Kolka et al. 2018).

Forested black ash wetlands in the western Great Lakes region (MI, MN, WI) occupy approximately 1,000,000 ha with 1,778,000,000 individual black ash trees (USDA 2023) and are a significant landscape component within the region’s ecological communities. Black ash trees dominate the wetland canopy ranging from 40% to nearly 100% in some areas (Looney et al. 2015; M. Van Grinsven et al. 2017) and help regulate forest hydrology, nutrient cycling, and biodiversity in these sensitive wetlands. Black ash plays an important role in the creation and maintenance of forested black ash wetlands, since they are responsible for regulating the environment for themselves and other co-occurring species (Kolka et al. 2018; Shannon et al. 2018).

Black ash wetlands occupy a unique ecological niche, which will be adversely affected by the invasion of EAB. When EAB larvae first hatch within the bark of an ash tree, they begin girdling the tree and feed on the phloem (Burr and McCullough 2014). EAB larvae are phloem feeders that girdle the tree from within. The initial impact of EAB is to disrupt the carbohydrate flow from the top of the tree (Mercader et al. 2016; Smith et al. 2015). With this disruption of photosynthate being sent to the roots, ash tree health declines as the roots begin to die (Matthes et al. 2018). The loss of root health, nutrient acquisition, and upward nutrient transfer cause the top of the tree to become stressed (Jennings, Duan, and Shrewsbury 2015).

The first physiological response of stressed trees is to produce epicormic branches in an attempt to maintain the supply of photosynthate sent to the root system from the canopy. (Burr and McCullough 2014; Kappler et al. 2018). It is easier for adult EAB to lay eggs towards the top of the tree, since the outer bark is thinner and easier for their ovipositor to penetrate. As the infestation progresses and more beetles lay eggs within the same tree, the larvae are deposited further and further down the tree (Pureswaran and Poland 2009). As the newly acquired carbohydrate transfer from epicormic branches higher towards the canopy are disrupted, new epicormic branches are created lower on the tree. These new epicormic branches sprout below the height of greater EAB larvae infestation as a mechanism to continue the transfer of photosynthate to the roots.

Bark blonding follows a similar top-down progression as epicormic branching. It is important to note bark blonding is not directly caused by EAB, but woodpecker activity (Jennings et al. 2016). Bark blonding is a sign of advanced EAB infestation (Marshall 2020). Woodpeckers regularly prey on EAB larvae that are just under the outer bark. Woodpecker feeding activity removes flakes of outer bark, revealing the lighter, inner bark. The inner bark has a blonde appearance, which contrasts the gray color of ash outer bark. Since larvae are initially deposited up high in the ash trees, woodpeckers first prey on EAB up high in trees, causing bark blonding up high. As the infestation moves down the ash trees, so do the woodpecker bark blonding signs.

The entire forest community structure and composition will change following EAB infestation. After an EAB infestation, forest inventories showed nearly all ash trees greater than 10 cm diameter at breast height (DBH) were dead (Siegert, Engelken, and McCullough 2021). Black ash is the most susceptible to mortality following an EAB infestation (Rebek, Herms, and Smitley 2008). The degree of EAB impact, time span, and forest changes are all dependent on preexisting site vegetation conditions (Marshall 2020). Species composition, hydrology, and site location all influence the newly developed vegetative community (Burr and McCullough 2014).

The current impact severity of an EAB infestation does not affect the future forest community composition. Ash in all types of forest communities are susceptible, and ash met their demise in the same abrupt and swift manner regardless of existing forest community composition (Smith et al. 2015). Herbaceous cover frequently increased at sites disturbed by actual EAB (Knight, Brown, and Long 2013) and in EAB simulated treatment sites (Davis et al. 2017). Woody species regeneration is variable by species and is strongly influenced by location of the seedling. Species performed better on hummocks than in hollows (Looney, D'Amato, Palik, and Slesak 2017). However, with observed EAB dynamics, it is unlikely that ash will reach canopy status as they have in the past (Kashian and Witter 2011). EAB has the ability to revisit and kill emerging ash understory that have reached 2.5 cm diameter, effectively limiting the species to seedling and understory status (Davis et al. 2017).

Expanding research into newly infested black ash wetland sites will further contribute to understanding of the impacts of EAB to black ash wetlands, while informing future land management practices and leading to new management activities. Determining the effects of EAB infestation on black ash wetlands will have vast economic, cultural, and ecological ramifications (Toczydlowski et al. 2020). This multiyear observational study was conducted in naturally occurring EAB infested black ash wetlands to understand the vegetation community structure of forested wetlands along an impact gradient. While simulated EAB invasion sites provided a sound foundation, implementing testing in a gradient of time since EAB invasion will assess the validity of data generated by simulated treatment responses and allow careful monitoring of EAB infestation in black ash wetland ecosystems. Specifically, this chapter focuses on the vegetation composition and changes in an actual EAB infested area to build on a decade of black ash and EAB research in the Ottawa National Forest, Michigan, USA.

1.3 Hypotheses

From a review of previous studies relating to EAB impacts on ash and forest communities around original infestation sites and other interactions between insect pests and host tree species, I hypothesize that a thinning canopy from dying and dead black ash trees will invite an invasion of herbaceous species and a transformation from a forested wetland to a scrub/shrub wetland, as a result of canopy closure changes. I predict that impacts to black ash forests will be more severe than those observed in other ash forests such as lowland hardwood forests with green ash as a major component, since they are the dominant canopy tree species, are tied heavily to hydrologic control, and there is a lack of co-occurring tree species present at treatment sites. Black ash has been very susceptible to EAB in past studies, and I believe this will result in more severe forest impacts when this keystone species is lost when compared to the loss of other ash species.

I predict vegetation data will align with the EAB infestation gradient. When impacts of EAB are affecting site characteristics, I predict the vegetation response at higher EAB impact (HighEAB) sites will be more severe and altered than the no EAB impact

(NoEAB) sites. Since NoEAB sites serve as the control with little to no EAB impacts, these sites should capture the ecosystem normal for black ash wetlands in this part of their range. Over time and with continued data collection, I predict the lower EAB impact to intermediate EAB impact (LowEAB to IntEAB) sites will progress towards the state of the HighEAB sites. Since EAB is in the area, it is only a matter of time before the impacts on site characteristics match those currently observed at the HighEAB sites. However, what is unknown is the progression towards complete black ash mortality along the infestation gradient, illustrated by the treatment sites. Continued observation and revisiting the sites will be necessary to determine the fate of forested black ash wetlands.

I hypothesize water tables, microsite changes, and the degree of EAB site infestation will explain the differences in vegetation observations. As explained above, the advance of EAB will have dire forest repercussions and forever alter forest composition on the landscape. Additionally, sampling years (2021 and 2022) had different weather patterns heavily influencing wetland characteristics and function with 2021 much drier than 2022, which affected the amount of water passing through these ecosystems and the timing of annual water table drawdown.

1.4 Methods

1.4.1 Research Sites

Research sites center in a gradient of black ash wetlands that were infected with EAB starting in 2020 in the Ottawa National Forest in the western Upper Peninsula of Michigan, USA (Figure 1.1). Sites are characterized by small, riparian forested wetlands ranging from 6 to 40 meters wide and 1.2 to 2.4 kilometers long, with black ash as the dominant canopy species. These wetlands serve as headwaters for the Silver River, which originates in Houghton County, MI, and drains to the Sturgeon River which empties into Keweenaw Bay of Lake Superior (note that this Silver River is not related to Silver River near L'Anse, MI). Situated in a slight landscape depression left by glacial retreat, these sites are among several parallel drains situated in the larger watershed area.

Water levels increase following precipitation, peak in spring with snowmelt, and rise again in the fall with late season rains (Davis et al. 2019). It is common for the drains to dry completely in summer, particularly during periods of drought (M. J. Van Grinsven 2015). Watershed streamflow and transpiration predominantly drive the changes in hydrology. During periods of snowmelt or high volumes of precipitation, the wetland drains will collect water and become more saturated (Shannon et al. 2018). Pools of water and surface flow are common in spring following snowmelt and in fall following periods of high precipitation and low evapotranspiration.

Mean annual precipitation was 82.0 cm and mean annual temperature was 5.0 ^{o}C from 1991-2020. The January average precipitation from 1991-2020 was 4.3 cm and January average temperature was -9.6 ^{o}C. For a comparison, July average precipitation from

1991-2020 was 9.3 cm and July average temperature was 18.9 ^{o}C. Water table, temperature, and precipitation trends throughout an average year in a black ash wetland are displayed in Figure 1.2 (see also Appendix V). The degree of seasonal drying and wetting is directly related to precipitation (including snowmelt) and transpiration. Intensity of seasonal changes varies with temperature, precipitation, and seasonal weather patterns for a given year.

There is a distinct transition from the wetland to the upland forest ecosystem. Adjacent to the forested black ash wetland, the ground slopes upward at 2-5 percent. Wetland soils consist of Gay-Leafriver complex, 0 to 2 percent slopes (NRCS Soil Map classification 8125), while adjacent upland ecosystems consist of Nunica silt loam, 1 to 6 percent slopes (NRCS Soil Map classification 8126B). The distribution of these soil types is consistent across all treatment sites.

The Leafriver soil series is characterized by a shallow organic layer atop a poorly draining mineral layer, with a soil taxonomic classification of sandy, mixed, frigid Histic Humaquepts. The mineral layer is comprised of loamy sand or sandy loam, left in depressions created by glacial activity. Depth to water level remains between 0 and 0.5 m below the surface during dry periods. Surface water is regularly present throughout the year reaching a depth of up to 15 cm during peak snowmelt, except for very dry years when the level of ponding is lower. This soil series regularly supports a wetland plant community of sedges, reeds, and willow. Tree species are less common, but black ash, quaking aspen (*Populus tremuloides* Michaux), balm of gilead (*Populus balsamifera* L.), tamarack (*Larix laricina* (Du Roi) K.Koch), and/or black spruce (*Picea mariana* (Miller) Britton, Sterns & Poggenburg) regularly grow in these soils.

The Nunica soil series is characterized by hardwood litter atop silty loam or silt loam mineral soil, with soil taxonomic class fine-silty, mixed, superactive, frigid Haplic Glossudalfs. Following glacial melt, these soils formed on lacustrine deposits. This soil is well drained, with moderate to rapid surface runoff and does not routinely support ponding surface water. When water does flow across the surface, it regularly collects in adjacent wetlands composed of Leafriver soils. Vegetation communities are composed of northern hardwood species including sugar maple (*Acer saccharum* Marshall), basswood (*Tilia americana* L.), eastern hemlock (*Tsuga canadensis* L.), and yellow birch (*Betula alleghaniensis* Britton).

1.4.2 Site Treatments

The treatment sites were selected along a gradient of EAB infestation severity. The EAB severity ranges from: high infestation severity (trees showing advanced signs of EAB infection and complete tree mortality), moderate (canopy dieback is beginning), low (signs of declining tree health), and no EAB present (healthy ash trees and intact wetland ecosystem function) (Figure 1.3). All vegetation and environmental monitoring data was collected at all gradient treatment sites unless otherwise noted. In total, twelve plots were established, with three in each EAB infestation gradient site.

1.4.3 Vegetation Plots

Vegetation surveys were conducted in the summer of 2021, such that the timing of observations occurred within full leaf out. At each EAB treatment site. A 16 m radius plot was established from a wetland center post, and all overstory (trees greater than 10 cm DBH) and sapling (woody vegetation less than 10 cm DBH) species were documented (Figure 1.4). Tree diameters were measured to the nearest millimeter and saplings were counted in 2 cm DBH ranges (0-1.99 cm, 2-3.99 cm, 4-5.99 cm, 6-7.99 cm, and 8-9.99 cm). DBH was measured from ground height, to standardize measures as water was frequently standing on the surface. Overstory trees were tagged with metal medallions to enable remeasurement in the future. Tree tag number, species, DBH, azimuth, distance from plot center, and Alive/Dead status were noted for all overstory trees.

DBH measurements were used to calculate individual tree basal area, which was then used to calculate total basal area (m^2) for each species present. After plot level basal area was determined, the seven most numerous tree species had density, frequency, and dominance calculated to determine a species importance value. Tables 1.1 and 1.2 summarize vegetation data measurements.

We calculated relative density, relative frequency, and relative dominance following the methods described in Table 1.3. With these three values, they were summed to calculate a relative importance value (RIV). When RIV is standardized out of 100%, an importance value (IV%) is derived. The IV% shows which species are the greatest component in the landscape, based on species number, abundance compared to other species, and total basal area occupied.

1.4.4 Ash Health and Signs of EAB Invasion

During vegetation data collection, a bark indicator of ash health was noted with the following four categories: epicormic branching high, epicormic branching low, bark blonding high, and bark blonding low. It was assumed that if an individual tree displayed either epicormic branching or bark blonding low, it also displayed the same symptoms up high. Trees were not limited to either epicormic branching or bark blonding, since both signs of EAB often co-occur in the same individual trees.

When the presence of bark blonding and/or epicormic branching was observed, its location on the tree was recorded and totaled to confirm the EAB gradient that progresses from healthy (alive) to dead ash (Figure 1.5).

1.4.5 Canopy Closure

Canopy cover percentage at each treatment site was measured to document changes in ash coverage. As EAB infestation progresses, canopy dieback is common before ultimate ash mortality. A spherical crown densiometer was used prior to leaf off (early September) each year to capture canopy changes and differences between treatment sites and annual variation. Four measurements were taken facing the four cardinal directions (north, east, south, west) at each vegetation plot center, for a total of twelve sampling points. To capture more information related to canopy cover, categories of closure were used instead of the standard canopy/no canopy.

Canopy cover classes ranged from no canopy to full canopy with increments of 0%, 1-25%, 26-50%, 51-75%, 76-99%, and 100%. After using the densiometer to categorize canopy into the six categories, total plot cover was calculated. Corresponding approximate median values used in calculating total canopy closure were 0%, 12.5%, 37.5%, 62.5%, 87.5%, 100%, respectively. Calculations for a plot canopy closure was equal to:

$$\frac{((\#*0)+(\#*0.125)+(*0.375)+(\#*0.625)+(\#*0.875)+(\#*1))}{96}$$

Where # is the number of occurrences of a canopy closure category occurring in a plot and 96 is the total number of samples taken in each plot, based on boxes within the densiometer.

After calculating canopy closure for all treatment plots in the two sampling years, values were compared across the gradient of EAB treatments. Also, differences in canopy closure from 2022-2021 were calculated. Since 2021 was significantly drier, there was more stress on vegetation and less resources to allocate to leaf production. Finally, all sites along the same EAB treatment gradient were averaged, to calculate the annual change in canopy closure for each EAB infestation gradient site.

1.4.6 Soil Cores

Soil cores were taken at all EAB treatment sites. Three 30 cm soil cores and O horizons were taken at 0°, 120°, and 240° at 6 m, 12 m, and 16 m from wetland center post, respectively at all EAB treatment sites and replicates (Figure 1.4). Each core was further broken into three depths from surface, ranging from 0-10 cm, 10-20 cm, and 20-30 cm. This design allowed a near surface and deeper depths to be analyzed. Sampling techniques varied between a gauge auger and AMS slide hammer, depending on sampling needs at the specific site. When soil cores were returned to the lab, they were processed to calculate weight of fine roots, rock, and soil in each core. Soil analysis also produced the soil percent carbon and percent nitrogen. The soil carbon to nitrogen (C:N) ratio was calculated for each EAB treatment site.

1.4.7 Groundwater Monitoring Wells

Monitoring wells were installed to measure changes in the water table. Each treatment intensity had one well installed, at the middle plot within each forested wetland drain. Measurements were taken seasonally and corresponded with IERC retrievals timed with phenological events (i.e., leaf on, onset of senescence, and leaf off). In taking measurements, depth was recorded as distance from the top of tube to the water surface. To convert the measurements to a depth from the surface of the soil, the height of the tube extended above the soil level was also recorded.

1.4.8 Statistical Analysis

Data was grouped by treatment site for analysis purposes. Statistical analysis was performed in Microsoft Excel. Significant values were tested using analysis of variance tests (ANOVA) for comparison of means across collections of parameters (i.e., EAB treatment, season, depth, nutrient species). When comparing means of two groupings (i.e., between seasons for a given EAB treatment), a t-test of paired two sample for means was used. Significance was recorded as $p < 0.10$, with notations when significance was recorded as $p < 0.05$, $p < 0.01$, $p < 0.001$.

1.5 Results

1.5.1 Vegetation Plots

A total of 982 trees were measured and tagged in summer 2021 across 12 plots in 4 EAB gradient black ash wetlands. Black ash, red maple (*Acer rubrum* L), sugar maple, northern white cedar (*Thuja occidentalis* L.), yellow birch, eastern hemlock, white ash (*Fraxinus americana* L.), and green ash (*Fraxinus pennsylvanica* Marshall) were the most common (Tables 1.1 Table 1.2). Other co-occurring species included American elm (*Ulmus americana* L.), white spruce (*Picea glauca* (Moench) Voss), basswood, balsam fir (*Abies balsamea* (L.) Miller), and ironwood (*Ostrya virginiana* Miller); however, they occurred less numerously than the common species mentioned above. Black ash was the dominant species across all plots, in terms of count and basal area. In reviewing importance value (IV%), black ash far exceeds other species when comparing species presence at the sites ($p < 0.001$ and between species within treatment groups $p < 0.001$).

Sapling regeneration was primarily composed of maple and ash seedlings (Tables 1.4 and 1.5), with an overall greater number of stems per hectare present at higher levels of EAB infestation (Table 1.6). ANOVA analysis of number of saplings regenerating in each size class across infestation gradient was statistically significant ($p = 0.01$). Across sites, 304, 279, 191, and 151 saplings were recorded at HighEAB, IntEAB, LowEAB, and NoEAB sites, respectively (Figure 1.6). A greater amount of sapling size class regeneration was observed for all sizes, excluding advanced regeneration. More advanced sapling regeneration (8-10 cm) was observed at NoEAB sites (n = 38), compared to HighEAB (n = 24), IntEAB (n = 29), and LowEAB (n = 16) sites (Figure 1.7).

There were no recorded hazelnut (*Corylus cornuta* Marshall), musclewood (*Carpinus caroliniana* Walter), alder (*Alnus incana* (L.) Moench), or Michigan holly (*Ilex verticillata* (L.) Gray) saplings present at the NoEAB site (Figure 1.8). These shrubs are not common in intact black ash wetlands or the adjacent upland northern hardwood ecosystem. There were a greater total number of shrub saplings in HighEAB, compared to other infestation severities.

1.5.2 Ash Health and EAB Signs

Signs of declining ash health increased with progressing EAB infestation severity (Figure 1.8). The number of healthy, live ash decreased with more intense EAB infestation, while the number of dead ash trees increased (Table 1.7). When infestation severity crosses into the advanced stages, as seen in the HighEAB sites, both epicormic branching and bark blonding are seen low on the tree bole (Figure 1.8). The shift in ash health signifies the beginning of ash mortality and ecosystem change. Signs and symptoms of EAB infestation varied across treatment sites and were statistically significant ($p < 0.001$).

1.5.3 Canopy Closure

Sites heavily impacted by EAB displayed a less intact canopy than those not affected by EAB (Figure 1.9). Since environmental conditions influenced resource availability and the intactness of the canopy, a more noteworthy metric of canopy change is the degree of change between each site (Figure 1.10).

From 2021 to 2022, specific sites within each EAB treatment displayed a variety of canopy cover percent changes (Figure 1.11). HighEAB and IntEAB sites displayed a negative change in canopy cover while LowEAB and NoEAB showed an increase in canopy cover (Figure 1.12). Even the LowEAB site, which showed an increase in canopy cover from 2021 to 2022, still gained 9.8% less canopy than the NoEAB reference site (Figure 1.12). Changes in growing condition between 2021 and 2022 can explain the difference in canopy observation between years, as 2022 had a greater amount of precipitation and less negative impacts from drought stress. Site canopy change differences were not significant ($p = 0.23$), likely due to the small sample size (n = 3). Despite improved growing conditions resulted from more preciptiation, impacted sites were not able to produce the same canopy vigor as NoEAB sites. Ash mortality and canopy dieback from EAB were primary factors affecting negative changes in ash canopy cover.

1.5.4 Soil Cores

Percent carbon and percent nitrogen in soils were highest in the 0-10 cm depth (Figure 1.13 and Figure 1.14). The least percent carbon and percent nitrogen was found in the 20-30 cm depth. Differences in values of percent carbon ($p = 0.009$) and percent nitrogen ($p = 0.011$) were significantly different between treatment sites. Also, differences in values between specific depths ranges were statistically significant for near-surface depths, but not for 20-30 cm depths (Table 1.9). Average values of C:N ratio across treatment sites is displayed in Figure 1.15. Mean value of C:N ratio across treatment sites ranged from 10-15.5. Difference in mean C:N ratio between all sites was statistically significant based on ANOVA ($p = 0.002$).

There is more percent carbon and percent nitrogen in soil samples taken from IntEAB and LowEAB sites (Figure 1.16). These treatment sites are more organic in nature, as illustrated by the greater amount of nitrogen in the soil. Mean percent carbon and percent nitrogen at various depth intervals is included in Table 1.10. Across all depths, approximately two times the percent carbon and percent nitrogen were available at IntEAB and LowEAB sites. Individual sites follow depth trends, with increasing percent carbon and percent nitrogen available closer to the surface.

Soil C:N ratios were highest closer to the soil surface. Statistically significant differences in C:N ratio between treatment sites were recorded for average C:N ratios and ratios between specific soil depths based on ANOVA ($p = 0.042$ for 0-10 cm depth, $p = 0.005$ for 10-20 cm depth, and $p = 0.056$ for 20-30 cm depth) (Table 1.11). IntEAB and LowEAB sites had more organic soils than the other sites, as indicated by their elevated C:N ratio (Figure 1.17).

1.5.5 Groundwater Monitoring Wells

Seasonal observations of groundwater well levels correspond with precipitation and drought data for 2021 and 2022. With more annual and seasonal precipitation, 2022 showed a higher water table across gradient sites. Monthly precipitations in several months of 2021 fell below those captured in the previous 30 years. These drought conditions had a great influence on severely lowering the water table. The drought conditions resulted in the lowest well readings for all sites across all seasons in summer 2021 (Figure 1.18). All treatment sites had a water table closer to the soil surface for summer 2022 than for summer 2021, likely a result of increased precipitation in 2022 (Appendix V). The NoEAB treatment site consistently had a lower water table level than other treatment sites. Since these sites are tied so closely to hydrology, changes in snowpack melt and timing along with decreased growing season rainfall totals will influence vegetation characteristics.

1.6 Discussion

Black ash regularly grows in homogenous stands. While these research sites were not pure black ash, they were the dominant component across all sites. Black ash were the most important species across all treatment sites (Table 1.1 and 1.2). Impacts to black ash are more severe than those to other ash species, and the consequential ecological changes exacerbate the impacts of EAB (Siegert, Engelken, and McCullough 2021). When other species are co-occurring at the treatment sites, there is still the possibility to maintain canopy cover as other advanced regenerating trees have additional resources to reach the canopy. The dominance of black ash in these wetlands and its demonstrated susceptibility to EAB impacts does not create a promising future for maintaining these forested wetland habitats (Mercader et al. 2016).

In the short period of this study, vegetation response from other co-occurring species has not been observed to counter canopy losses from black ash mortality at individual sites. Since a two-year period is short in the span of hardwood forest lifecycles, additional recruitment into the canopy has not yet been observed. Canopy variations at sites were related to changes in growing season and the loss of ash canopy following EAB induced mortality. Continued study will indicate if co-occurring species are sufficiently abundant to maintain canopy closure.

Despite having other species co-occurring in these wetlands, many species currently co-occurring are not equipped to replace black ash in these forested wetlands. As these vegetation composition changes, one consequence is a rising water table. Water tables recorded in sites impacted by EAB were regularly higher than the NoEAB control. Both white ash and green ash meet the same demise as black ash following EAB outbreak (Kappler et al. 2020). Species like sugar maple are not adapted for wetland conditions, and predominantly occur along the transition where the wetland meets the upland northern hardwood ecosystem. Sugar maple will not likely replace black ash following EAB induced mortality (Engelken, Benbow, and McCullough 2020).

Other species like red maple and yellow birch, which commonly occurred in treatment sites are not as adapted to growing in wetland conditions when compared to black ash (Davis et al. 2017). Therefore, with predicted increases in water level, these species will struggle to reach canopy dominance and regenerate saplings following black ash loss (Klooster et al. 2014). The role black ash plays to draw down the water table during the growing season allows these other species to establish and thrive (Robertson, Robinett, and McCullough 2018). The loss of ash coupled by an increase in the water table will likely prevent other forest species from recolonizing the former sites of black ash and they will lose their forested nature following EAB infestation (Looney et al. 2017; Youngquist et al. 2017).

While some tree species like northern white cedar, American elm, and tamarack may appear to be quality candidates for a forested wetland tree species since many are present at the wetlands impacted by EAB, many factors negatively impact their growth and establishment (Youngquist et al. 2017). Northern white cedar is prone to deer herbivory and its slow growth habit will likely prohibit it from replacing black ash at the rate ash die out from EAB infestation (Villemaire-Cote, Ruel, and Tremblay 2022). American elm is prone to Dutch elm disease, which currently limits its presence on the landscape (Hale, Alsum, and Adams 2008) and will continue to inhibit it from moving into the vacant canopy created by the loss of black ash. Tamarack was not detected in any vegetation plots and is prone to infestation by Eastern larch beetle where it occurs in great numbers (Crocker et al. 2016). As black ash is lost from these wetland sites due to EAB, it is not likely other tree species present will be able to move into the wetlands and retain the canopy. No quality facultative wetland tree species exist on the landscape presently to replace black ash when it dies (Marshall 2020).

Ash saplings are not likely to grow past the 10 cm DBH category (Ellison et al. 2016). EAB has been documented in ash as small as 3 cm (Nisbet et al. 2015). Since ash saplings had a lower count in the HighEAB site, despite there being more saplings regenerating, ash saplings may have already begun to be impacted by the presence of EAB. Since EAB infestation severity is not as intense in the IntEAB sites, adult beetles will favor larger, mature trees instead of infesting sapling size ash. This may account for the increased abundance of ash saplings in IntEAB sites when compared to HighEAB sites (Figure 1.7). Ash saplings are also a favorite browse for deer and moose (Kolka et al. 2018). The high concentration of nitrogen in the buds and leaves led black ash to be more palatable for herbivores, further decreasing the recruitment of black ash to the canopy (Ferrari 1999; Pastor and Post 1986).

As black ash weaken and die, canopy gaps are created that usher in the invasion of a new shrub class (Marshall 2020). EAB is enabling this ecosystem transformation as more resources (space, light, nutrients) are available to increase seedling regeneration (Burr and McCullough 2014). Herbaceous cover is expected to increase as EAB infestation progresses in black ash wetlands (Davis et al. 2017). Canopy changes are directly related to EAB impacts on ash (Knight, Brown, and Long 2013). Measurements with the densiometer corroborate predicted outcomes and observations from individual ash trees, as an average canopy loss was present at HighEAB and IntEAB sites while LowEAB and NoEAB sites gained additional canopy from 2021 to 2022. The changes and canopy measures between HighEAB and NoEAB are already impacting the landscape. Despite canopy increases from 2021 to 2022 across the forest landscape due to more water availability, the HighEAB site lost canopy closure, while the NoEAB site gained additional canopy closure (Figure 1.12). This result implies that invasion and expansion of herbaceous covers most likely occur following an EAB infestation, at least a few years after the infestation.

Following ash mortality in other forest ecosystems, similar shifts in vegetation were observed creating a new demand on available nitrogen (Looney et al. 2017; Kappler et al. 2020). These herbaceous species tolerate more saturated soil and take advantage of the additional sunlight (Engelken, Benbow, and McCullough 2020). Shrubs have had longer time to invade in HighEAB sites where impacts have been ongoing for a greater length of time. The IntEAB and LowEAB sites will likely follow behind with an increasing number of shrubs. The intact canopy of the NoEAB site limits the number of regenerating samplings. Sites impacted by EAB show much smaller diameter saplings than the NoEAB site. Since the NoEAB site is least impacted by canopy mortality, there are less resources available for saplings to regenerate (Kashian 2016).

Black ash vigor and overall health impacts are similar to those observed in other ash species in other forest types impacted by EAB (Nisbet et al. 2015; Kreutzweiser et al. 2019). With continued presence on the landscape, EAB is increasingly deadly to ash trees. The presence of low epicormic branching and low bark blonding at the HighEAB site is indicative of declining ash health and the forthcoming removal of canopy black ash (Figure 1.7). The NoEAB site has a greater number of healthy ash trees, unimpacted by EAB. The ash health observations as well as the rate and severity observed throughout these black ash treatment wetlands follow the fate of green ash ecosystems and riparian forested areas with ash as a component (Kappler et al. 2020; Klooster et al. 2014; Engelken, Benbow, and McCullough 2020).

When ash trees display epicormic branching low and bark blonding, rapid ash decline follows. Epicormic branching is a sign of tree stress and a last attempt to gain vital nutrients and resources for survival, while other parts of the canopy die (Sibley et al. 2020). This physiological change is a result of EAB restricting photosynthate movement throughout ash trees as larvae feed within trees. Bark blonding is another sign EAB larvae have hatched within ash bark, resulting in a girdling effect that is detrimental to ash survival. As woodpeckers strip away the outer bark to feed on the inner bark, the blonding pattern is indicative of advanced stages of EAB infestation. Observed signs of EAB were greater at more impacted sites. While not exclusively a one to one, each fleck of blonde bark is the result of a woodpecker feeding upon an EAB larvae (Jennings et al. 2016).

Soil C:N ratios measured in treatment sites were below those reported at sites in northern Minnesota in mineral soils documented in unpublished data from Slesak (2015). Black ash wetland soil C:N ratios are typically between 14-15 at 0-30 cm depth, with over 75% of the total nitrogen found in the top 15 cm (Kolka et al. 2018). The infestation gradient sites had an average C:N ratio between 9-14 at 0-30 cm depth, as measured from soil cores in 2021. Decreasing soil C:N ratios have been documented following spruce dieback due to progressing bark beetle infestation (Spielvogel, Prietzel, and Kögel-Knabner 2006). Additional vegetation added to forest soil following tree mortality increased percent nitrogen in the environment which consequently decreased C:N ratios (Christenson et al. 2002). Observing changes in soil C:N ratios may not be measurable on this short timescale, due to the slow decline of black ash over several years. Individual treatment site variability is likely a product of differences in the environment and not tied to EAB impacts over this two year time period (Garten Jr and Ashwood 2002). Long-term monitoring of changes in site C:N ratios will help illustrate the ecosystem changes observed following an EAB impact.

During simulated forest disturbances, more intense disturbances (i.e. whole tree removal) resulted in greater changes to soil percent carbon and percent nitrogen than moderate harvest activities due to nitrogen leaching and C removal from the environment (Mroz, Jurgensen, and Frederick 1985; Zummo and Friedland 2011). EAB intensity mimics these simulated disturbances, with HighEAB sites corresponding to more intense disturbances while moderate disturbances are similar to IntEAB/LowEAB sites. In

HighEAB sites, there is less carbon and nitrogen available in the soil. Studies on hemlock wooly adelgid indicated the C:N ratio dropped following a multiyear study of insect pest impacts (Orwig et al. 2008). It may be that the initially higher C:N ratio observed in IntEAB/LowEAB sites was a short-term response of the stressed environment before the ratio decreases as infestation progresses.

1.7 Conclusions

Vegetation and site data collected throughout this chapter help to illustrate the context for the emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) infestation gradient in forested black ash (*Fraxinus nigra* Marshall) wetlands identified on the Ottawa National Forest. As EAB progresses, sites will continue to change and display increasingly negative effects from EAB. As we move forward, IntEAB/LowEAB will show ever more severe impacts and ultimately reach the stage of HighEAB now. In the near future, the HighEAB treatment site will see continued ash mortality and then complete removal from the forest landscape. When ash is removed, a novel ecosystem will develop, and a new vegetation community will exist in its place.

The HighEAB site is characterized by advanced signs of EAB infestation. Ash mortality has begun, and nearly all ash trees are showing signs of canopy dieback related to EAB. EAB exit holes and feeding galleries are apparent throughout the site. As ash trees are impacted, an increased presence of shrubs and understory trees is beginning to develop. Sapling regeneration is greatest at the HighEAB site. As ash trees die and environmental changes continue to take place, the forested black ash wetlands are slowly being converted into a post-EAB ecosystem, characterized by large amounts of coarse woody debris, rising water levels, and an absence of ash trees where they were once dominant.

IntEAB and LowEAB are showing signs of EAB infestation. Declining ash health is present, and many trees are showing signs of canopy dieback. While these sites are still showing ecosystem function as a black ash forested wetland, it is only a matter of time until EAB infestation changes these ecosystems. Sapling regeneration is increasing as mature ash consume less resources as they are succumbing to EAB. IntEAB and LowEAB represent a middle ground between NoEAB and HighEAB infestation sites. Due to their geographic proximity and similarity in EAB signs, some measures along the EAB infestation for the IntEAB and Low EAB gradient reverse. Generally, and in terms of geographic location, IntEAB displays more advanced signs of EAB than LowEAB; however, that is not always the case due their geographic proximity and time since initial EAB infestation.

In the NoEAB site, little sapling recruitment is occurring as the canopy trees are excluding the growth of other individuals. Given enough time, EAB will inevitably find this wetland and cause the same ecosystem changes that are being observed in other treatment sites. The widespread and sweeping changes brought by EAB to forested black ash wetlands will forever alter the vegetation composition and ecosystem function of these unique and important habitats.

1.8 Tables

Table 1.1. A total of 982 trees were measured across all plots, with the seven most numerous tree species being reported for HighEAB and IntEAB sites. Other cooccurring species included in order of frequency: hemlock, elm, white pine, basswood, balsam fir, ironwood. Black ash topped the list for all treatment sites.

Treatment	**Species**	**Number**	**DBH±SE (cm)**	**Total Basal Area±SE (m^2)**	**Basal Area±SE ($m^2 \cdot ha^{-1}$)**	**Relative density**	**Relative frequency**	**Relative dominance**	**RIV**	**IV%**
High	Black Ash	94	22.64±0.99	1.49±0.04	18.50±0.49	38.06	15.79	43.18	97.03	32.34%
High	Red Maple	62	19.59±1.11	0.74±0.29	9.25±3.55	25.10	15.79	21.60	62.49	20.83%
High	Sugar Maple	58	16.61±1.21	0.55±0.17	6.78±2.16	23.48	15.79	15.82	55.09	18.36%
High	N W Cedar	9	29.07±2.90	0.21±0.06	2.67±0.80	3.64	15.79	6.24	25.67	8.56%
High	Yellow Birch	10	23.21±3.11	0.16±0.04	2.04±0.46	4.05	15.79	4.75	24.59	8.20%
High	Green Ash	1	11.00±0.00	0.003±0.003	0.04±0.04	0.40	5.26	0.09	5.76	1.92%
High	White Ash	0	0	0	0	0.00	0.00	0.00	0.00	0.00%
Int	Black Ash	137	18.84±0.69	1.51±0.15	18.75±1.89	54.80	11.54	42.07	108.41	36.14%
Int	N W Cedar	23	30.04±1.97	0.59±0.25	7.40±3.13	9.20	11.54	16.60	37.33	12.44%
Int	Red Maple	33	19.90±1.45	0.40±0.05	4.98±0.62	13.20	11.54	11.17	35.91	11.97%
Int	Yellow Birch	11	33.73±4.41	0.38±0.07	4.77±0.88	4.40	11.54	10.70	26.64	8.88%
Int	Sugar Maple	18	12.92±0.91	0.10±0.07	1.22±0.90	7.20	7.69	2.73	17.63	5.88%
Int	Green Ash	3	25.97±1.43	0.05±0.05	0.66±0.66	1.20	3.85	1.49	6.53	2.18%
Int	White Ash	0	0	0	0	0.00	0.00	0.00	0.00	0.00%

Table 1.2. A total of 982 trees were measured across all plots, with the seven most numerous tree species being reported for LowEAB and NoEAB sites. Other cooccurring species included in order of frequency: hemlock, elm, white pine, basswood, balsam fir, ironwood. Black ash topped the list for all treatment sites.

Treatment	**Species**	**Number**	**DBH±SE (cm)**	**Total Basal Area±SE (m^2)**	**Basal Area±SE ($m^2 \cdot ha^{-1}$)**	**Relative density**	**Relative frequency**	**Relative dominance**	**RIV**	**IV%**
Low	Black Ash	114	19.67±0.74	1.34±0.11	16.67±1.39	48.93	12.00	38.00	98.93	32.98%
Low	Red Maple	52	22.40±1.06	0.76±0.12	9.45±1.52	22.32	12.00	21.55	55.87	18.62%
Low	N W Cedar	17	27.06±1.76	0.35±0.16	4.33±1.93	7.30	12.00	9.86	29.16	9.72%
Low	Yellow Birch	10	29.05±1.93	0.23±0.15	2.86±1.84	4.29	12.00	6.51	22.80	7.60%
Low	Sugar Maple	16	14.86±1.56	0.11±0.03	1.34±0.39	6.87	12.00	3.06	21.93	7.31%
Low	White Ash	0	0	0	0	0.00	0.00	0.00	0.00	0.00%
Low	Green Ash	0	0	0	0	0.00	0.00	0.00	0.00	0.00%
No	Black Ash	108	20.52±0.79	1.38±0.16	17.18±1.99	42.86	11.11	38.75	92.72	30.91%
No	Sugar Maple	64	17.68±1.01	0.63±0.05	7.85±0.66	25.40	11.11	17.69	54.20	18.07%
No	Red Maple	32	24.89±2.63	0.70±0.23	8.68±2.86	12.70	11.11	19.58	43.39	14.46%
No	White Ash	12	28.57±2.46	0.28±0.13	3.45±1.58	4.76	11.11	7.77	23.65	7.88%
No	Yellow Birch	10	22.56±2.43	0.15±0.08	1.83±0.94	3.97	7.41	4.13	15.50	5.17%
No	Green Ash	2	25.00±9.10	0.04±0.04	0.46±0.46	0.79	3.70	1.04	5.54	1.85%
No	N W Cedar	0	0	0	0	0.00	0.00	0.00	0.00	0.00%

Table 1.3. Description of column titles and calculations performed to obtain values in columns in Tables 1.1 and 1.2.

Column Title	Calculation of Value
Relative density	Number species / total trees * 100
Relative frequency	number plots with a species / total number trees in plots * 100
Relative dominance	total basal area species / total basal area plot
Relative Importance Value	Relative Density + Relative Frequency + Relative Dominance
IV%	Average of RIV, out of 100%

Table 1.4. Species observed at each treatment site (n = 3) and total species count in each DBH class for HighEAB and IntEAB sites. Stems/hectare is included to display abundance of saplings on the landscape. A total count row is included to capture all the species in each size class.

HighEAB	<2 cm	2-4 cm	4-6 cm	6-8 cm	8-10 cm	Total N	Stems/ha
Total	126	86	30	38	24	304	3779.930
Sugar Maple	22	50	19	28	18	137	1703.455
Musclewood	48	12	2	0	0	62	770.907
Black Ash	17	10	0	1	3	31	385.453
Hazelnut	22	0	0	0	0	22	273.548
Red Maple	3	4	4	6	1	18	223.812
White Spruce	7	2	1	0	0	10	124.340
Elm	0	5	4	0	1	10	124.340
Yellow Birch	3	0	0	2	0	5	62.170
Basswood	1	3	0	0	1	5	62.170
Balsam Fir	1	0	0	1	0	2	24.868
Green Ash	2	0	0	0	0	2	24.868

IntEAB	<2 cm	2-4 cm	4-6 cm	6-8 cm	8-10 cm	Total N	Stems/ha
Total	93	78	46	33	29	279	3469.080
Black Ash	1	26	17	19	18	81	1007.152
Sugar Maple	12	20	15	10	5	62	770.907
Red Maple	10	18	7	2	1	38	472.491
White Spruce	12	10	6	0	3	31	385.453
Michigan Holly	22	0	0	0	0	22	273.548
Alder	18	0	0	0	0	18	223.812
Hazelnut	17	0	0	0	0	17	211.378
Yellow Birch	0	2	1	1	2	6	74.604
Mountain Maple	0	1	0	0	0	1	12.434
White Pine	1	0	0	0	0	1	12.434
Black Cherry	0	1	0	0	0	1	12.434
Elm	0	0	0	1	0	1	12.434

Table 1.5. Species observed at each treatment site (n = 3) and total species count in each DBH class for LowEAB and NoEAB sites. Stems/hectare is included to display abundance of saplings on the landscape. A total count row is included to capture all the species in each size class.

LowEAB	**<2 cm**	**2-4 cm**	**4-6 cm**	**6-8 cm**	**8-10 cm**	**Total N**	**Stems/ha**
Total	93	52	18	12	16	191	2374.890
Sugar Maple	18	24	9	2	4	57	708.737
Hazelnut	40	0	0	0	0	40	497.359
White Spruce	11	17	3	1	1	33	410.321
Black Ash	1	8	4	7	9	29	360.585
Michigan Holly	18	0	0	0	0	18	223.812
Red Maple	3	2	1	1	1	8	99.472
Yellow Birch	1	0	0	1	1	3	37.302
Ironwood	1	1	0	0	0	2	24.868
White Cedar	0	0	1	0	0	1	12.434

NoEAB	**<2 cm**	**2-4 cm**	**4-6 cm**	**6-8 cm**	**8-10 cm**	**Total N**	**Stems/ha**
Total	16	46	31	20	38	151	1877.531
Sugar Maple	14	39	26	17	31	127	1579.115
Elm	0	0	4	0	3	7	87.038
Ironwood	2	3	1	0	0	6	74.604
Red Maple	0	1	0	2	1	4	49.736
Yellow Birch	0	0	0	0	2	2	24.868
White Ash	0	2	0	0	0	2	24.868
Black Ash	0	0	0	0	1	1	12.434
White Spruce	0	1	0	0	0	1	12.434
Basswood	0	0	0	1	0	1	12.434

Table 1.6. Summary table for total saplings in each treatment site. These values are displayed separately in Table 1.4 and Table 1.5 in the Total row.

	<2 cm	**2-4 cm**	**4-6 cm**	**6-8 cm**	**8-10 cm**	**Total N**	**Stems/ha**
HighEAB Total	126	86	30	38	24	304	3779.930
IntEAB Total	93	78	46	33	29	279	3469.080
LowEAB Total	93	52	18	12	16	191	2374.890
NoEAB Total	16	46	31	20	38	151	1877.531

Table 1.7. Number of ash trees with the most extreme values of ash health, summarizing number of healthy ash trees without sign of EAB and the total dead ash trees at each site.

	Ash Health	HighEAB	IntEAB	LowEAB	NoEAB
	No Sign, Alive	3	16	18	34
	Dead	9	6	6	6

Table 1.8. Symbology for Figure 1.6. Colors advance from light blue to black as EAB infestation intensifies and trees ultimately die.

	Alive	No EAB Sign
	EH	Epicormic (High)
	EL	Epicormic (Low)
	BH	Blonding (High)
	EH/BH	Epicormic (High)/Blonding (High)
	BL	Blonding (Low)
	EH/BL	Epicormic (High)/Blonding (Low)
	EL/BH	Epicormic (Low)/Blonding (High)
	EL/BL	Epicormic (Low)/Blonding (Low)
	Dead	Ash Dead, EAB Sign

Table 1.9. ANOVA *p* values, when grouping all values of percent carbon and percent nitrogen at treatment sites (HighEAB, IntEAB, LowEAB, NoEAB) for individual soil core depths (0-10 cm, 10-20 cm, and 20-30 cm). The All Depths row considers all depths for a given treatment site. Significant values ($p < 0.10$) are shown in green boxes.

	Percent Carbon	Percent Nitrogen
0-10 cm	0.0183	0.0060
10-20 cm	0.0265	0.6807
20-30 cm	0.1028	0.1036
All Depths	0.0091	0.0024

Table 1.10. Values of soil percent carbon and percent nitrogen by depth range (0-10 cm, 10-20 cm, and 20-30 cm) at each treatment stie (HighEAB, IntEAB, LowEAB, NoEAB). All Depths row is an average percent carbon and percent nitrogen across depths for a given treatment site.

Means	Percent Carbon	Percent Carbon	Percent Carbon	Percent Carbon
	HighEAB	IntEAB	LowEAB	NoEAB
0-10 cm	10.95	18.11	20.04	9.70
10-20 cm	2.82	5.90	7.97	1.49
20-30 cm	0.51	3.51	2.28	0.55
All Depths	4.76	9.17	10.10	4.01

Means	Percent Nitrogen	Percent Nitrogen	Percent Nitrogen	Percent Nitrogen
	HighEAB	IntEAB	LowEAB	NoEAB
0-10 cm	0.66	1.28	1.30	0.74
10-20 cm	0.20	0.42	0.47	0.46
20-30 cm	0.06	0.27	0.15	0.07
All Depths	0.31	0.66	0.64	0.33

Table 1.11. Values of soil C:N Ratios by depth range (0-10 cm, 10-20 cm, and 20-30 cm) at each treatment stie (HighEAB, IntEAB, LowEAB, NoEAB). All Depths row is an average C:N Ratio across depths for a given treatment site.

Means	C:N Ratio	C:N Ratio	C:N Ratio	C:N Ratio
	HighEAB	IntEAB	LowEAB	NoEAB
0-10 cm	16.655247	14.339542	15.120071	13.078202
10-20 cm	13.194863	12.538137	15.938691	9.851218
20-30 cm	7.738103	9.468574	12.321822	7.066835
All Depths	10.133753	10.791996	13.391008	8.532793

1.9 Figures

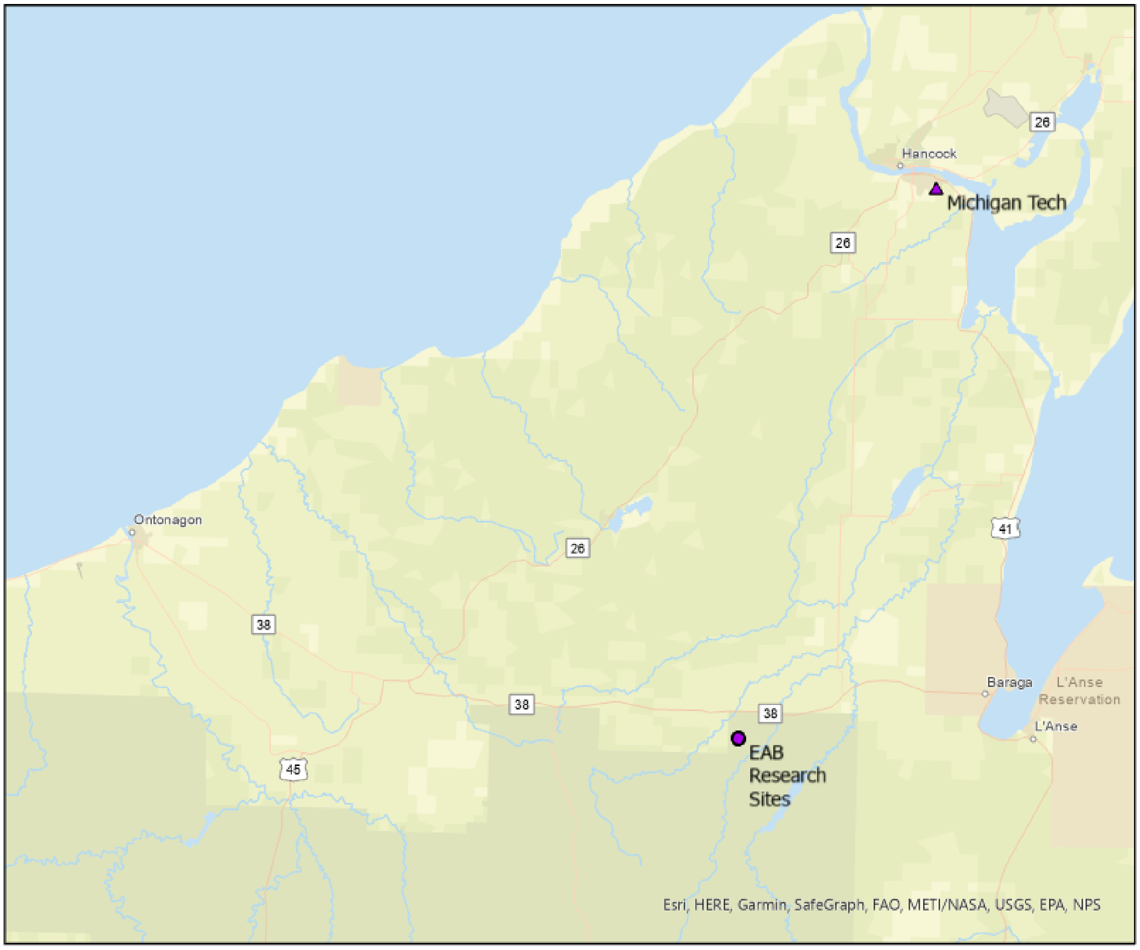

Figure 1.1. Overview map showing location of EAB research sites in western Upper Peninsula, Michigan, USA. Sites are approximately 27 miles south of Michigan Tech (Houghton, Michigan, USA), 12 miles west of Baraga (Michigan, USA), and 28 miles east of Ontonagon (Michigan, USA).

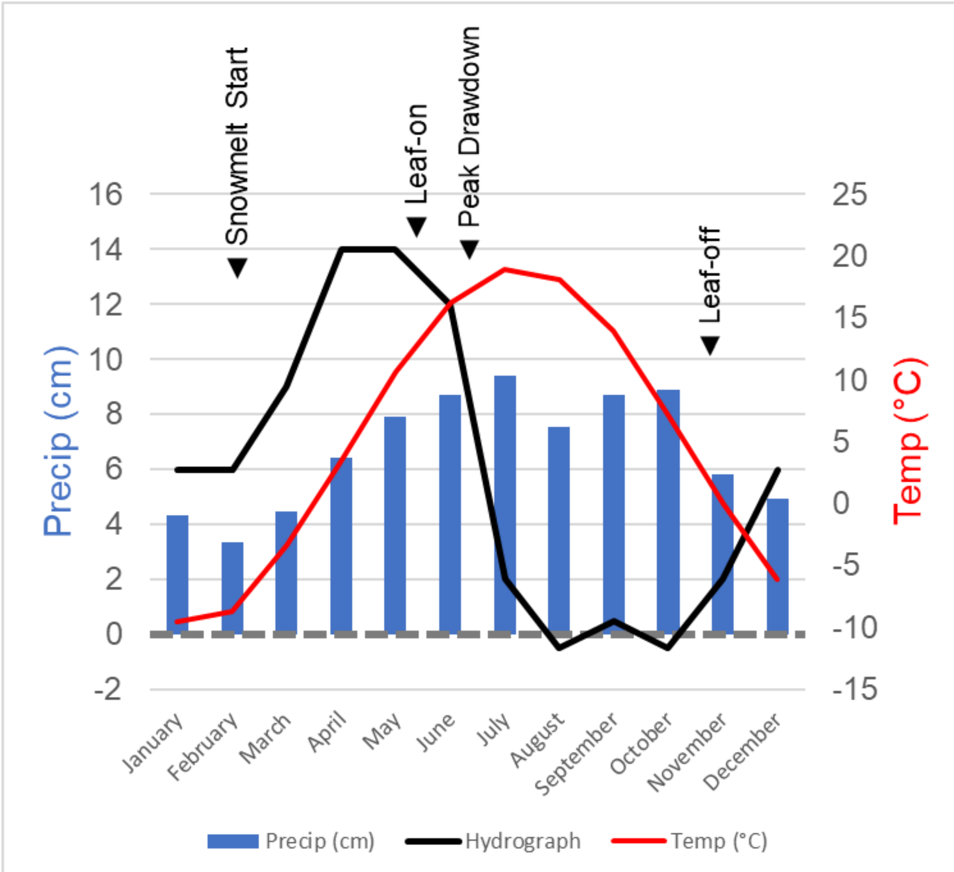

Figure 1.2. Seasonal hydroperiod of a black ash wetland (black line) relative to ground surface symbolized at Precip = 0 cm with the black dashed horizontal line. Precipitation (cm) is shown with blue bars based on 30-year normal from 1991-2020. Temperature (°C) is shown with red line based on 30-year normals.

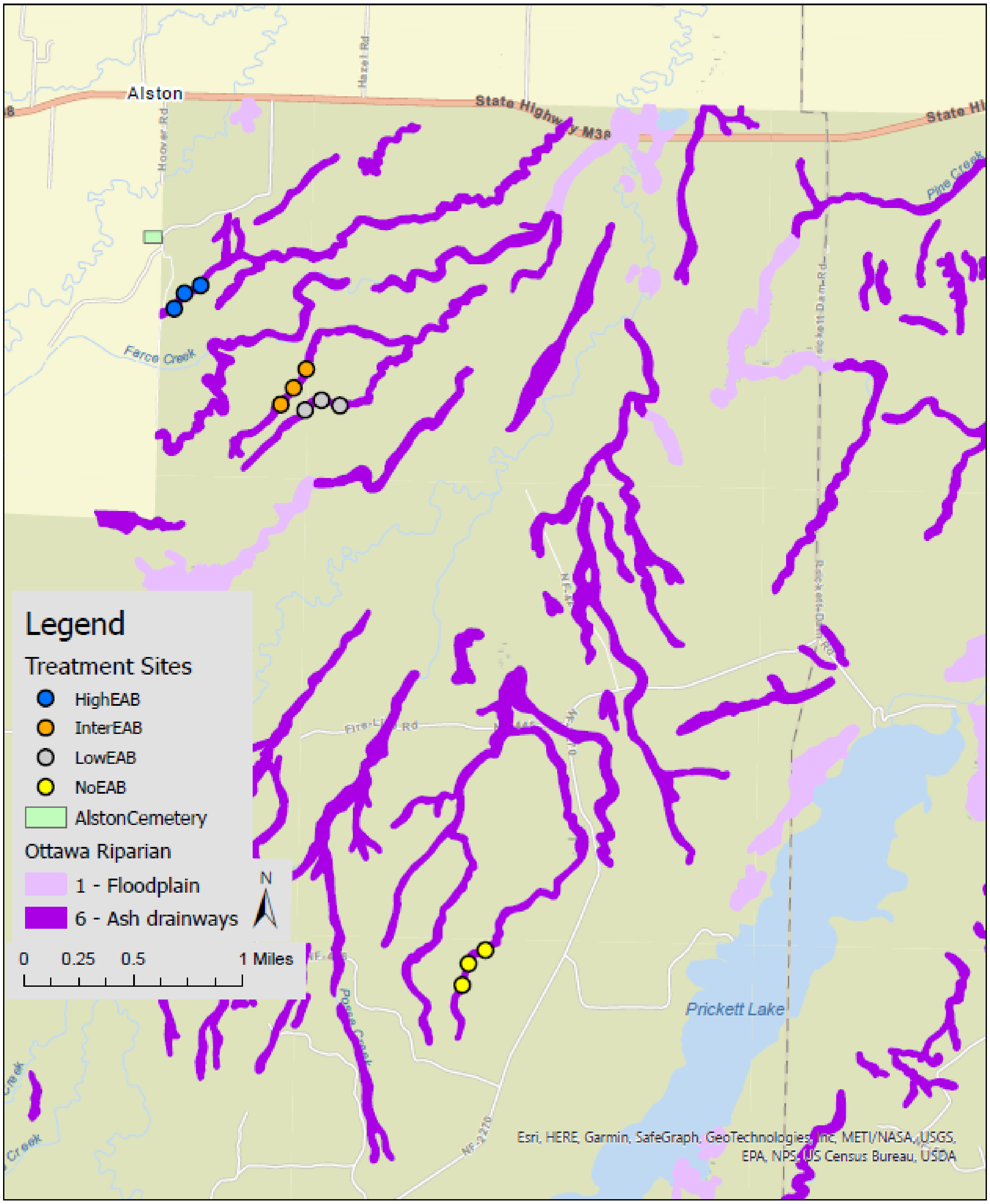

Figure 1.3. Research treatment sites map near Alston, Michigan, USA. Sites are located in the SWSW, Sec. 6; NE, NWNW, Sec. 7; SWSE, Sec. 20; NWNE, Sec. 29, T. 50 N., R. 35 W., Houghton County, MICHIGAN MERIDIAN. EAB gradient moves from high EAB impact (HighEAB) in the northwest to intermediate EAB impact (IntEAB), low EAB impact (Low EAB), and finally no EAB impact (NoEAB) in the southeast.

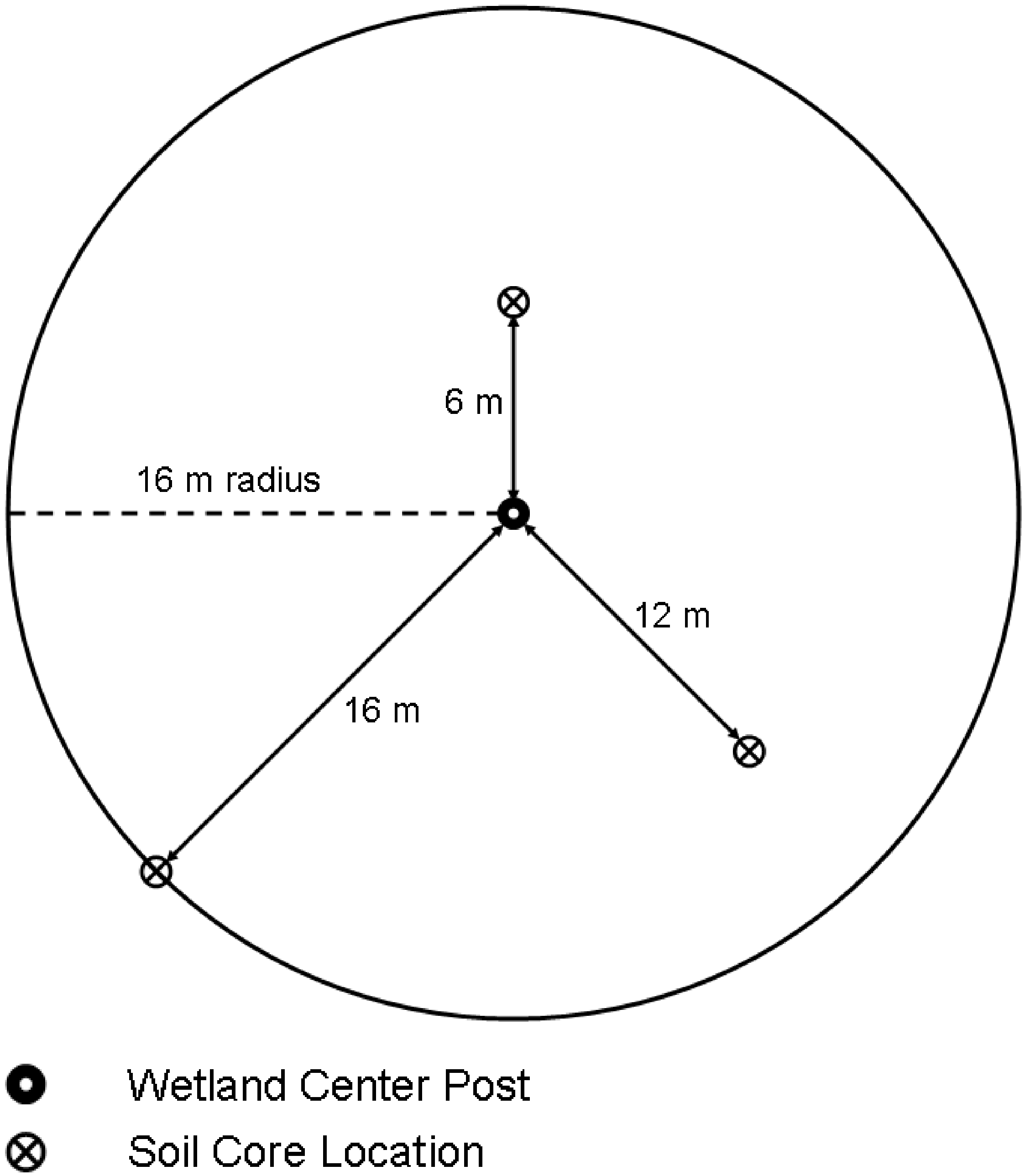

Figure 1.4. Treatment site sampling layout map. All overstory trees (>10 cm DBH) and woody shrubs(<10 cm DBH) within the 16 m radius plot were measured and counted. Soil cores were taken at 0°, 120°, and 240° at 6 m, 12 m, and 16 m distance from wetland center post, respectively, to 30 cm depth. Soil cores were separated into three depth ranges (0-10 cm, 10-20 cm, 20-30 cm).

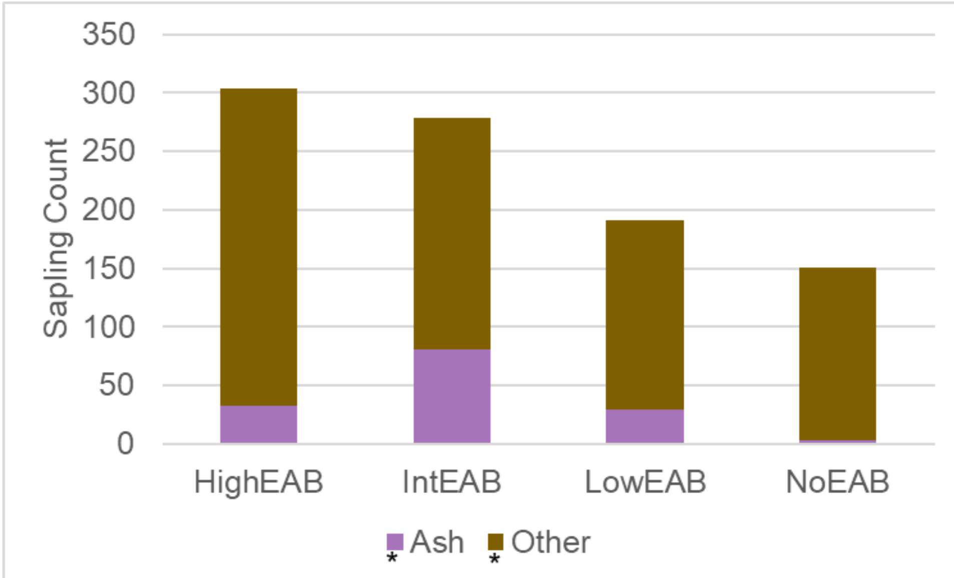

Figure 1.5. Portion of Trees Regenerating as Ash. Total number of saplings per treatment sites (n = 3) with portion of ash separated. Ash regeneration was dominant across treatment sites disturbed by EAB (HighEAB, IntEAB, and LowEAB sites). Legend labels marked with an asterisk are statistically significant from one another across EAB infestation site.

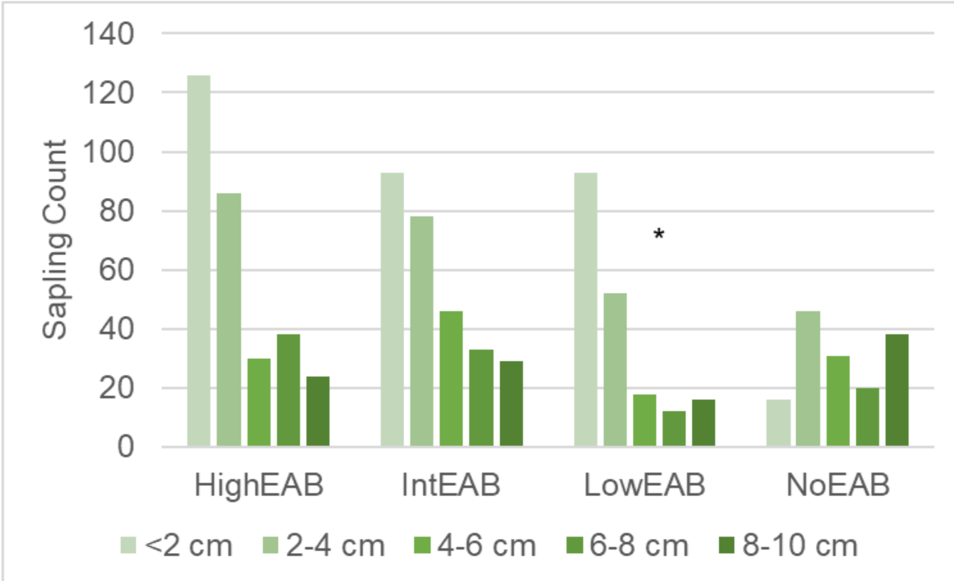

Figure 1.6. Number of saplings (0-10 cm DBH) regenerating in each size class per treatment sites (n = 3). Smaller saplings were more common in disturbed environments, while a more even distribution of sapling size classes was observed at the NoEAB control site. Sapling species are listed in Tables 1.4 and 1.5. EAB infestation sites marked with an asterisk have sapling regeneration that is statistically different from one another in size-class comparison.

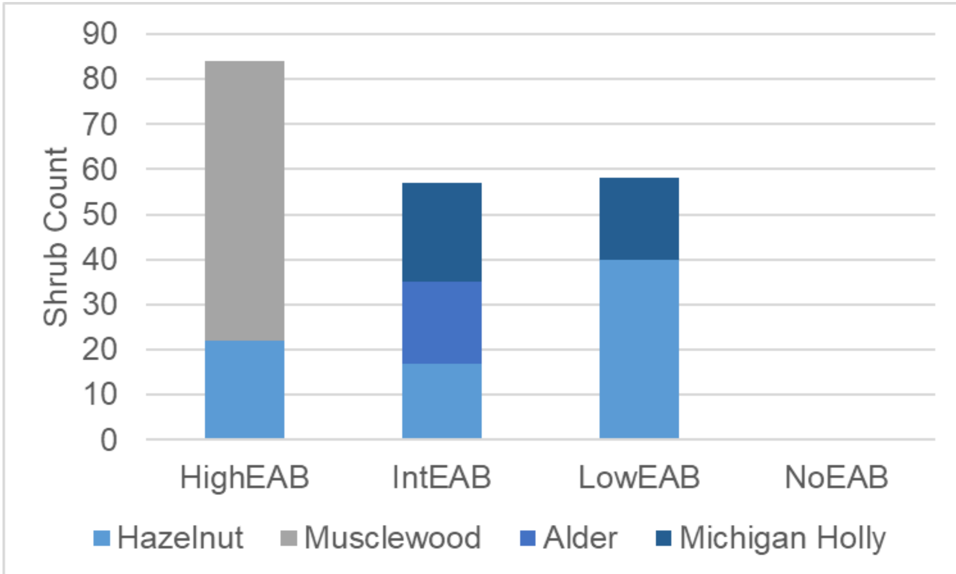

Figure 1.7. Shrub count by EAB site. Non-tree shrubs counted in each EAB treatment site. No hazelnut, musclewood, alder, or Michigan holly were counted in the NoEAB black ash wetland.

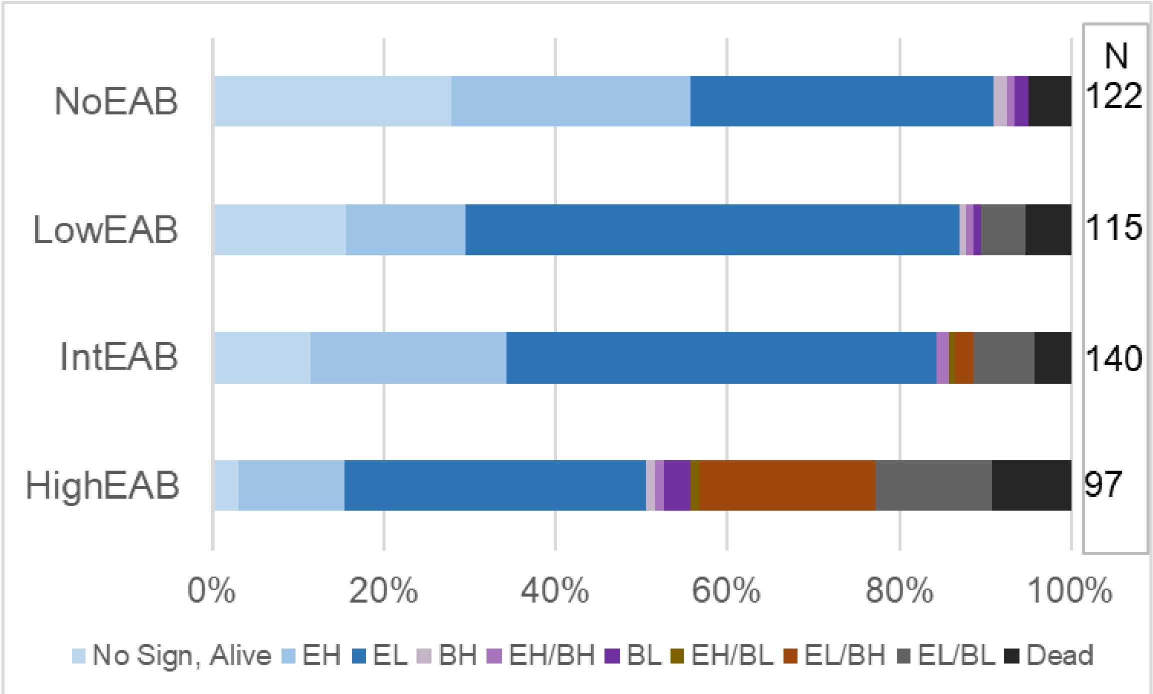

Figure 1.8. Ash tree health indicator broken down by treatment site. The total number of ash present in each site is represented by the N column. As ash trees continue to decline, trees move from left (No Sign, Alive) to right (Dead) through a series of epicormic branching and bark blonding.

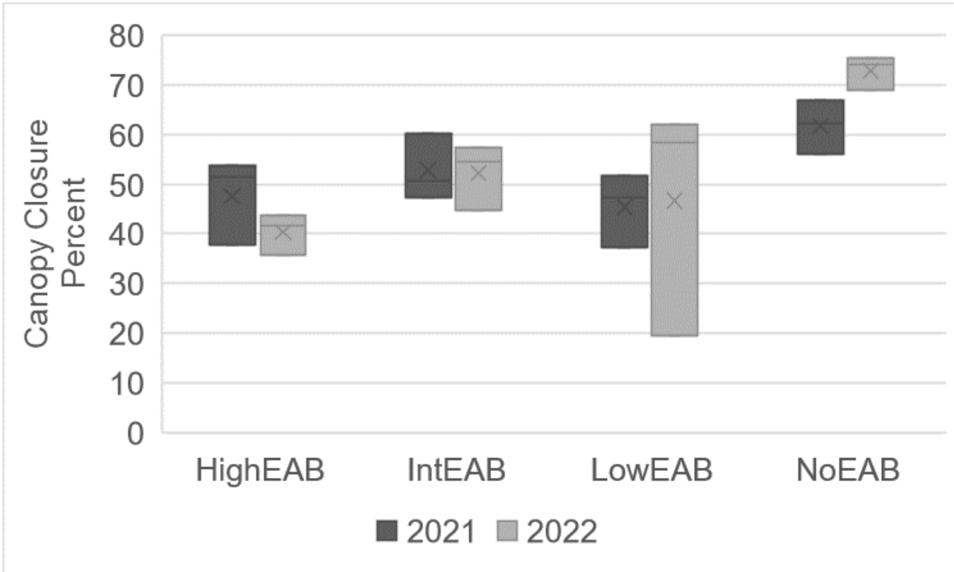

Figure 1.9. Percent canopy closure of EAB treatment sites in 2021 and 2022. Individual treatment sites are combined by intensity. A canopy closure percent of 0 would indicate no leaves present and an open canopy, while a canopy closure percent of 100 would indicate a complete canopy, with little sunlight reaching for forest floor. The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value.

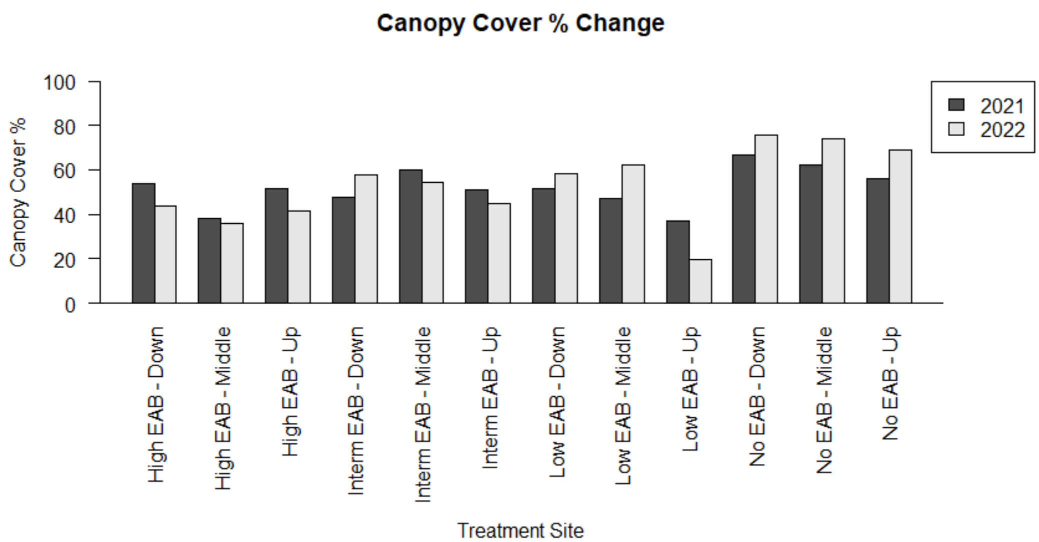

Figure 1.10. Percent canopy closure of EAB treatment sites in 2021 and 2022. Individual treatment sites are separated by intensity and drain location. Drain location (down, middle, up) corresponds to individual gradient site replicates, as they relate to one another along a drainflow course.

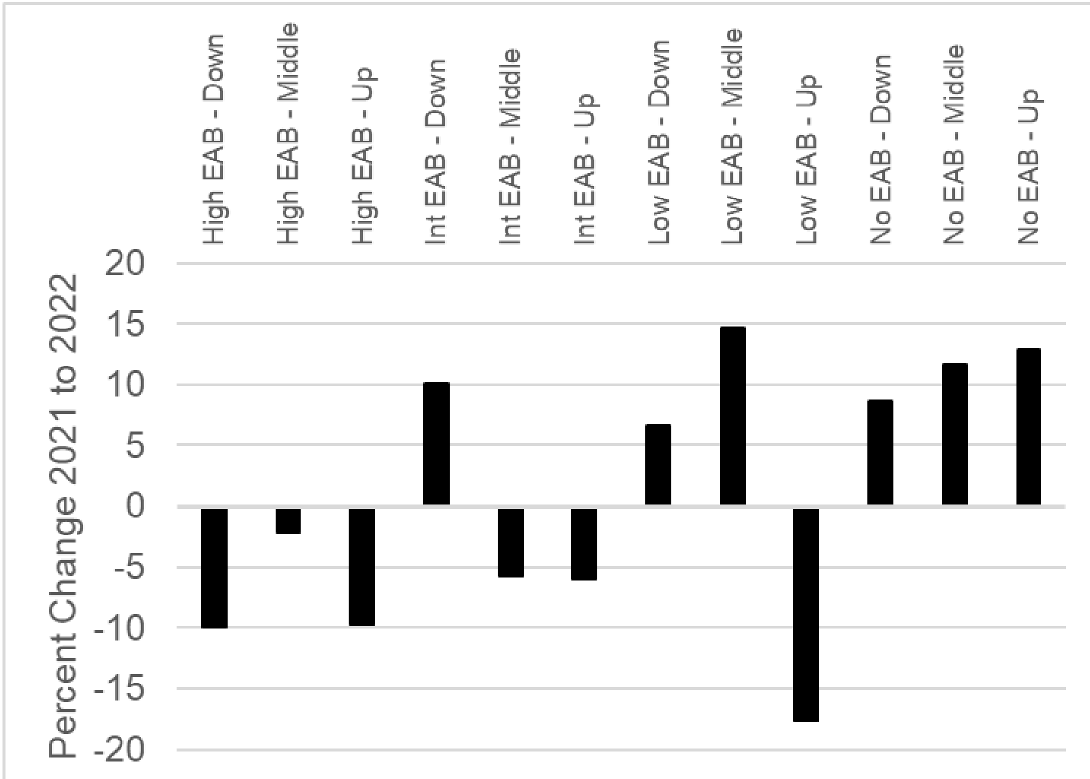

Figure 1.11. Canopy Cover Percent Change by plot, calculated as the difference between 2022 and 2021. A negative values indicates less canopy cover in 2022 than 2021, while a positive value indicates more canopy cover in 2022.

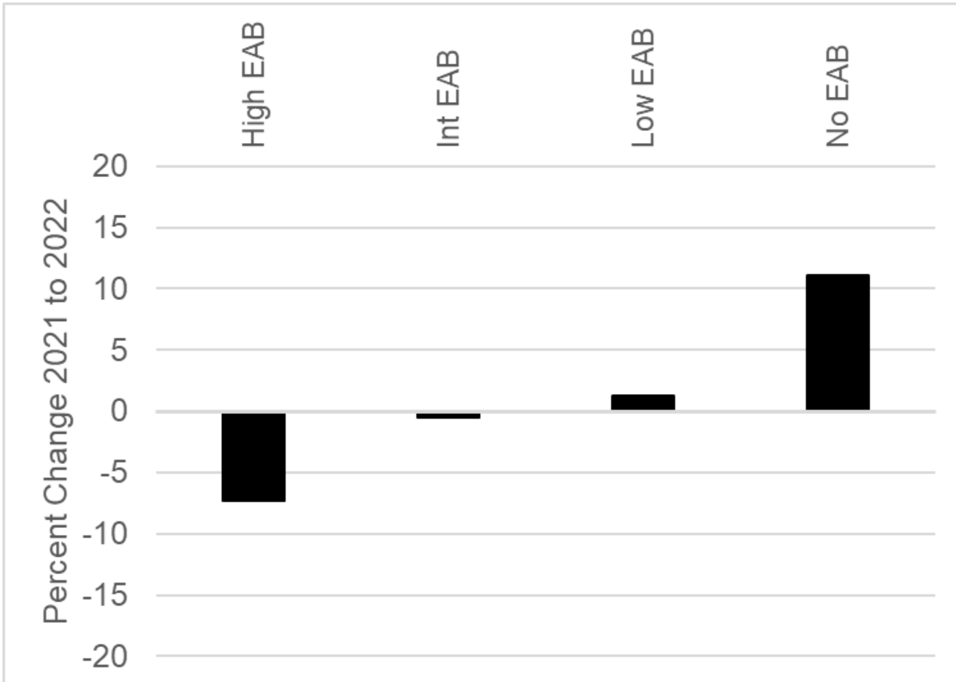

Figure 1.12. Average canopy cover percent change for each treatment site (n = 3), calculated by 2022 average minus 2021 average for each site. Individual sites are displayed in Figure 1.10.

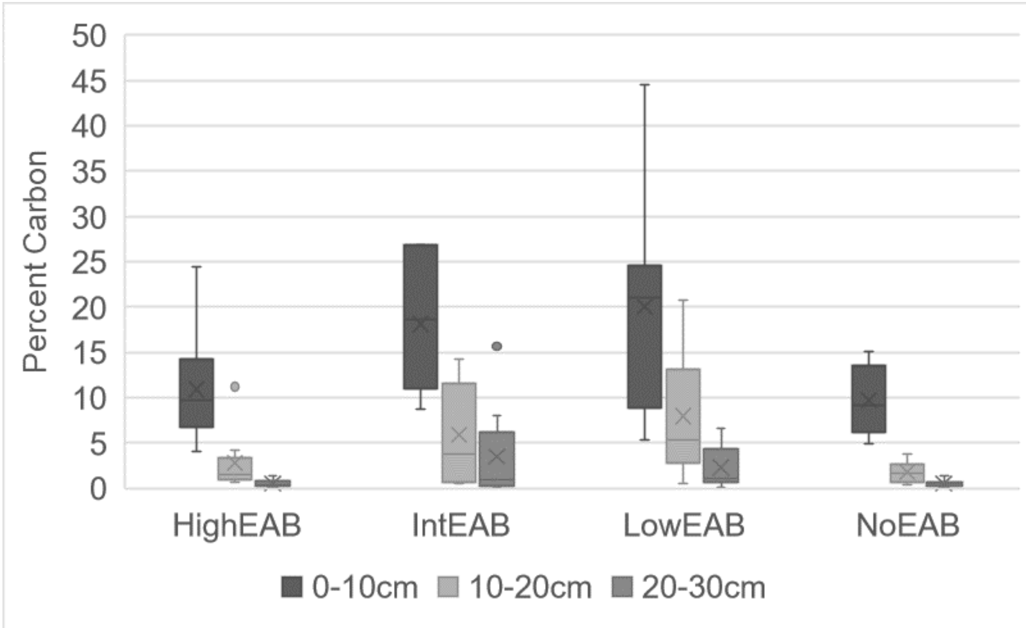

Figure 1.13. Variation of values of percent carbon across treatment sites (HighEAB, IntEAB, LowEAB, and NoEAB) and soil core depths (0-10 cm, 10-20 cm, and 20-30 cm). The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value.

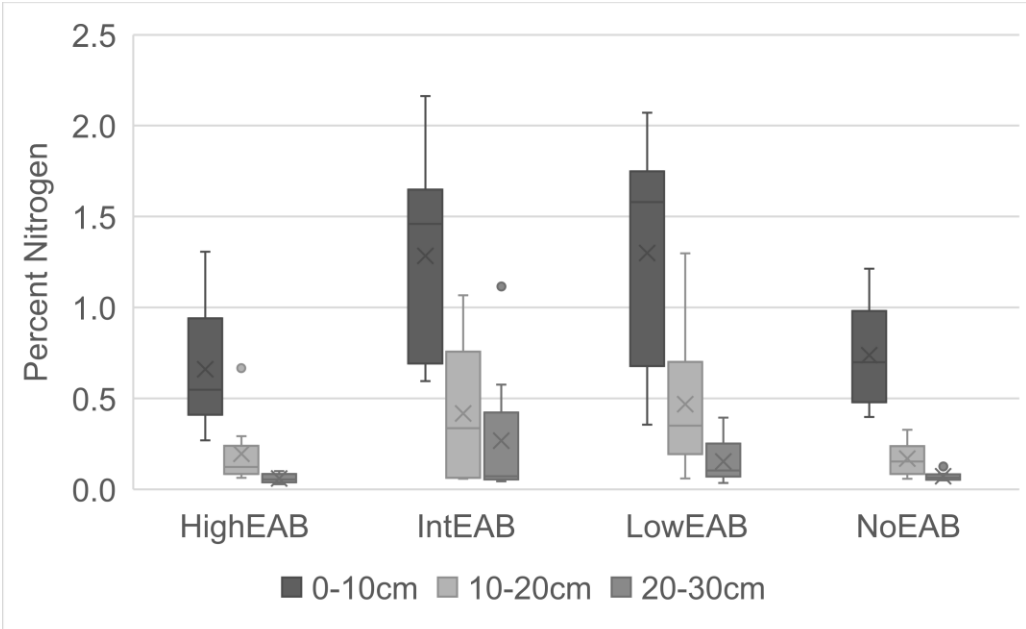

Figure 1.14. Variation of values of percent nitrogen across treatment sites (HighEAB, IntEAB, LowEAB, and NoEAB) and soil core depths (0-10 cm, 10-20 cm, and 20-30 cm). The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value.

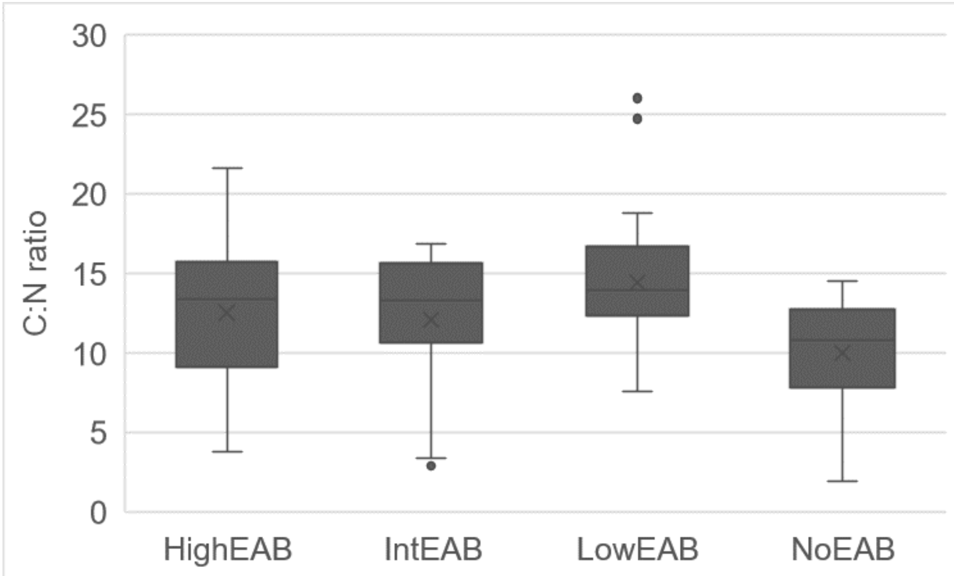

Figure 1.15. Variation of values of C:N ratios from all sample depths across treatment sites (HighEAB, IntEAB, LowEAB, and NoEAB). The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value.

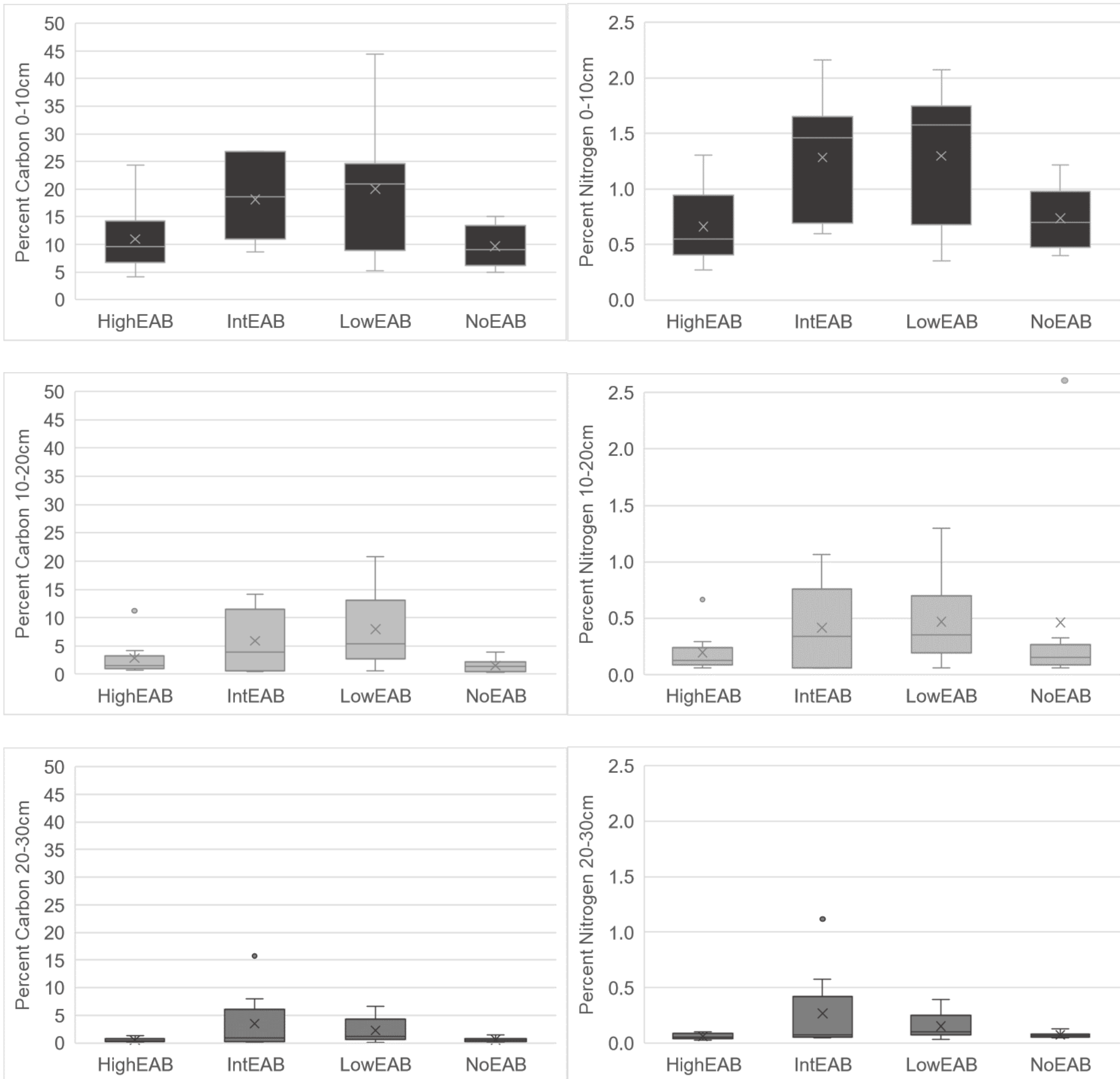

Figure 1.16. Soil percent carbon (left column) and percent nitrogen (right column) by depth range (0-10 cm, 10-20 cm, and 20-30 cm, respectively, corresponding to top, middle, and bottom rows) at each treatment stie (HighEAB, IntEAB, LowEAB, NoEAB). The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value.

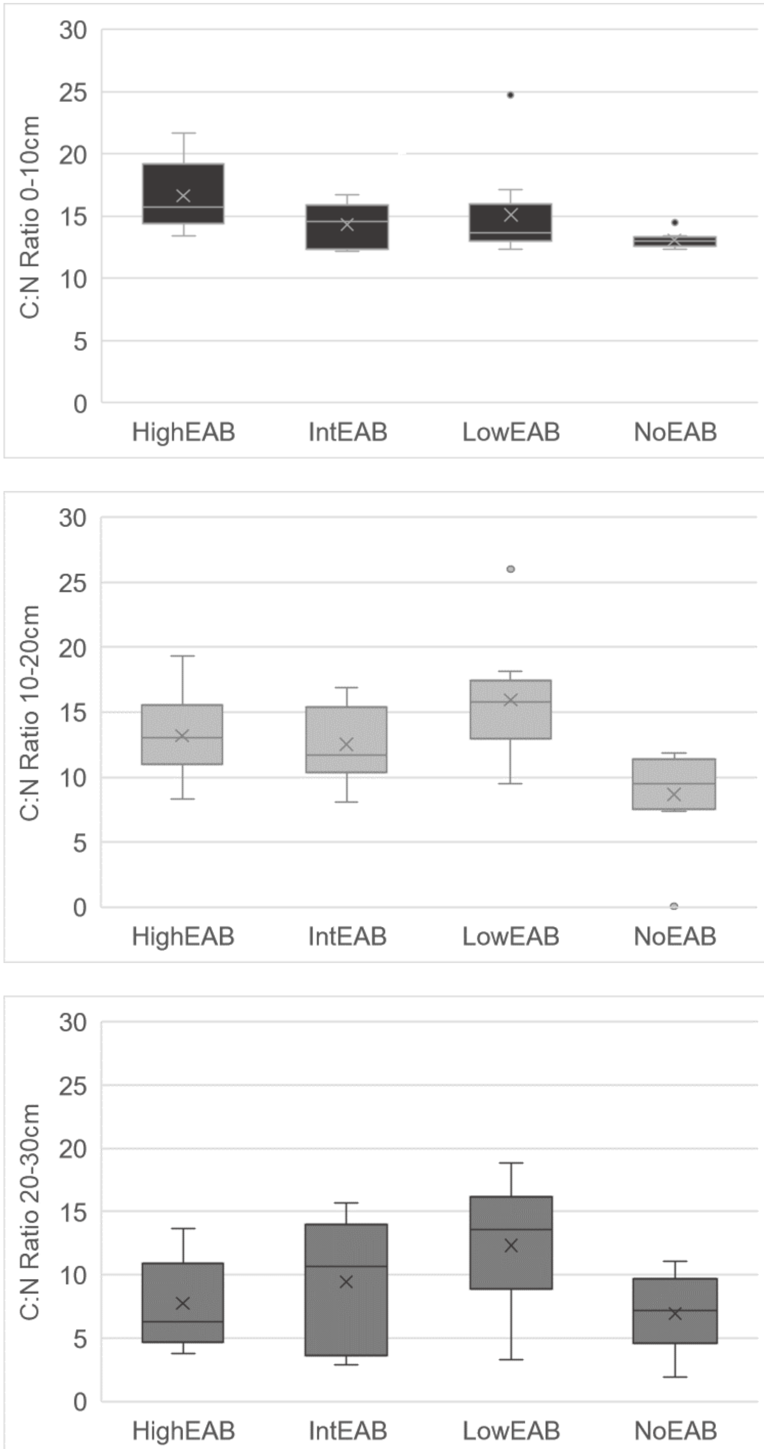

Figure 1.17. Soil C:N Ratios by depth range (0-10 cm, 10-20 cm, and 20-30 cm) at each treatment stie (HighEAB, IntEAB, LowEAB, NoEAB). The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value.

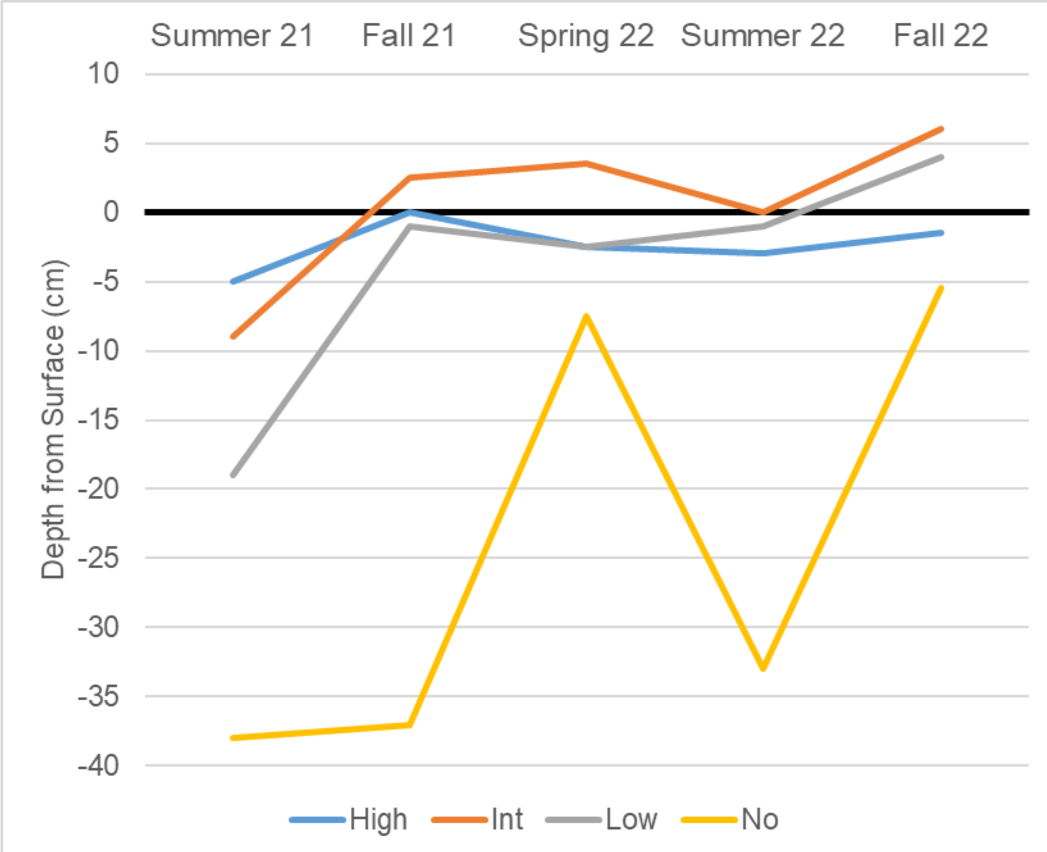

Figure 1.18. Water table measurements during collection seasons. One measurement was made seasonally in each treatment site.

2 Near-Surface Soil Nitrogen Response to a Gradient of EAB Infestation

2.1 Abstract

Invasive emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) poses an imminent threat to the structure and function of North American hardwood forests, particularly black ash (*Fraxinus nigra* Marshall), and alters the hydrologic and ecological services of their wetlands. Black ash trees regularly grow in seasonally saturated soils, are responsible for hydrologic regulation and nutrient cycling, and frequently dominate the canopy in these areas. To study future impacts caused by EAB, a gradient of black ash wetlands impacted by EAB was monitored to assess near-surface soil nitrogen availability using ion-exchange resin capsules. More total nitrogen (TN) was available at more impacted sites, increasing from a mean of 4.51 ppm at the control (NoEAB) site to a mean of 8.30 ppm at the highly impacted (HighEAB) site. Due to the aquatic nature of these forested wetlands, NH_4^+-N was the primary component of resin TN at all sites, accounting for up to 94% TN. $NO_3^-.NO_2^-$-N was far less abundant since the anoxic environment quickly facilitated its use by the microbial community. As black ash died and fell to the wetlands, more total organic nitrogen (TON) was returned to the environment and potentially incorporated into the growing shrub and sapling layers. Measurements of Ca, Mg, Na, and P from ion exchange resin capsules also showed greater amounts of major elements available in sites more impacted by EAB. Oxygen was precluded from the soil by rising water levels, further reducing denitrification and preventing microbes from converting NH_4^+-N via oxidation. Assessing biogeochemical changes along an EAB gradient in the environment improves our understanding of the ecological ramifications for a future landscape without black ash wetlands as they presently exist.

2.2 Introduction

Black ash (*Fraxinus nigra* Marshall) wetlands deliver invaluable ecosystem services to our society such as water retention, streamflow regulation, and biodiverse habitats (Zedler and Kercher 2005). Emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) raises a deep concern on wetland ecosystem health because of its severe impacts on ash trees (Herms and McCullough 2014). Since its original identification in Detroit, MI in 2002 (Siegert et al. 2014), EAB has spread to 35 states and 5 Canadian providences (EAB Information Network 2023). EAB can cause 100% ash mortality within 3-6 years (Klooster et al. 2014). Previous studies have focused on pest biology (Prasad et al. 2010), ash reaction (Smitley, Davis, and Rebek 2008), and management methods for control (Liu and Bauer 2008). However, less attention has been dedicated to the effects of EAB on nutrient cycling, particularly in black ash wetlands (Kolka et al. 2018). Several simulated EAB infestations have been implemented in black ash wetlands. While simulated sites provided a sound foundation, implementing testing in an EAB infested ecosystem will assess the validity of data generated by simulated treatments and allow careful monitoring of EAB infestation in black ash wetland ecosystems.

Forested black ash wetlands in the western Great Lakes region (MI, MN, WI) occupy approximately 1,000,000 ha with 1,778,000,000 individual black ash trees (USDA 2023) and are a significant landscape component within the region's ecological communities. Black ash trees dominate the wetland canopy ranging from 40% to nearly 100% in some areas (Looney et al. 2015; M. Van Grinsven et al. 2017) and help regulate forest hydrology, nutrient cycling, and biodiversity in these sensitive wetlands. Black ash trees play an important role in nitrogen cycling as they are the largest demander of nitrogen as a species and their leaflitter provides a substantial return of nitrogen in these forested wetlands (Ferrari 1999; Pastor and Post 1986). This substantial return of nitrogen serves as a natural fertilizer and an environment high in nitrogen. This site preparation helps to increase sapling recruitment and growth for young ash saplings.

Black ash wetlands occupy a unique ecological niche. Nitrogen mineralization rates in black ash wetlands are lower than surrounding upland forests and species competition for nitrogen is greater (Zak et al. 1990). Black ash trees exert a major strain on available nitrogen in the environment, while black ash leaves had the greatest percent nitrogen of trees occurring in research sites based on leaf-level percent nitrogen analysis (Ferrari 1999). Black ash are dominant consumers of nitrogen across northern hardwood forests (Pastor and Post 1986). This research will shed light on how forested wetlands will change as EAB infests and kills most, if not all, of the black ash in these forested wetlands.

The consequences of losing black ash to EAB and the impacts on nutrient cycling in the years following are largely unknown. Prior research has been limited to artificial treatments used to simulate EAB infestation as black ash dominated wetlands have been largely out of the range of EAB. Other insect pests regularly disrupt nitrogen cycling and alter forest composition (Jenkins, Aber, and Canham 1999; Cessna and Nielsen 2012; Griffin, Turner, and Simard 2011; Keville, Reed, and Cleveland 2013). These results provide valuable insights into EAB impacts on black ash wetlands (Orwig et al. 2008). A noteworthy difference to black ash is their aquatic nature, as most research into the effects of biotic forest disturbances on nitrogen cycling has been conducted in upland ecosystems. When research extends into a wetland ecosystem, it regularly occurs in managed or industrial wetland systems (Nakagawa et al. 2012). Expanding research into new sites with the presence of EAB will broaden our understanding of the EAB impacts on black ash wetlands, while informing future land management practices and lead to new science questions.

Determining the effects of EAB infestation on black ash wetlands will have vast economic, cultural, and ecological ramifications (Toczydlowski et al. 2020). We have conducted a multiyear observational study in naturally occurring EAB infested black ash wetlands. Specifically, this chapter focuses on the changes of nutrient availability in near-surface soils in an actual EAB infested area to build on a decade of black ash and EAB research in the Ottawa National Forest, Michigan, USA.

2.3 Hypotheses

From a review of previous studies into nitrogen cycling in black ash wetlands, nitrogen cycling in disturbed forests, and nitrogen cycling in aquatic systems, I hypothesize that near-surface nitrogen availability will increase following ash mortality. Since there will be a greater input of nitrogen sources from dying ash (leaf matter, fine branching, course woody debris, root volume), microbial activity will be able to return the organic nitrogen stored to the environment. Also, without ash on the landscape following mortality, there will be less nitrogen uptake demand from ash on nitrogen sources available in the environment. This will leave the greater nitrogen available in the environment, stored in largely inorganic forms.

Also, I hypothesize that most available nitrogen will be in NH_4^+-N form. The NH_4^+-N availability generally increases in North American forests following insect pest mortality (Lovett et al. 2006). Forests displayed greater NH_4^+-N availability following tree mortality and subsequent ecosystem change in both hemlock wooly adelgid (Jenkins, Aber, and Canham 1999; Cessna and Nielsen 2012) and mountain pine beetle (Griffin, Turner, and Simard 2011; Keville, Reed, and Cleveland 2013) affected forests. Also, $NO_3^-.NO_2^-$-N is not frequently available in saturated soils (Jicha et al. 2014). These black ash wetlands currently exist in a saturated state for a majority of the year, and following EAB infestation and black ash mortality, the saturation levels should increase (Shannon et al. 2022; M. Van Grinsven et al. 2017). This increase could cause the nitrogen cycle to tip further towards NH_4^+-N as the dominant inorganic form.

Furthermore, I hypothesize that water levels will be the predominant driver of nitrogen species type and availability across seasons and as EAB induced mortality progresses. Since these wetland systems typically display increased surface water levels following canopy removal, a more anoxic environment will occur in these wetlands. As a result, NH_4^+-N will not be converted to $NO_3^-.NO_2^-$-N via microbial processes that require the presence of oxygen, thus $NO_3^-.NO_2^-$-N will not be common in the black ash wetland ecosystem.

2.4 Methods

Nitrogen availability was sampled using UNIBEST® Ion-Exchange Resin Capsules (IERCs). IERCs mimic roots by releasing resin ions (H^+ & OH^-) in exchange for nitrogen (NH_4^+-N, $NO_3^-.NO_2^-$-N, organic N) and other elements (Ca^{2+}, Mg^{2+}, Na^+, or PO_4^{3-}) in the soil (UNIBEST International 2023). When IERCs were replaced, the capsules were extracted by USFS Northern Research Station, Forestry Sciences Laboratory, Houghton, MI, and nutrient contents were analyzed by USFS Northern Research Station, Forestry Sciences Laboratory, Grand Rapids, MN. Three seasonal retrievals were timed with phenological events (i.e. leaf on, onset of senescence, and leaf off) to capture changes over the growing season and from year to year as EAB infestation progressed through the black ash wetland (Davis et al. 2019). Exact dates of IERC collections are listed in Table 2.1.

2.4.1 Research Sites

Research sites center in a gradient of black ash wetlands that were infected with EAB starting in 2020 in the Ottawa National Forest in the western Upper Peninsula of Michigan, USA (Figure 2.1). Sites are characterized by small, riparian forested wetlands ranging from 6 to 40 meters wide and 1.2 to 2.4 kilometers long, with black ash as the dominant canopy species. These wetlands serve as headwaters for the Silver River, which originates in Houghton County, MI, and drains to the Sturgeon River which empties into Keweenaw Bay of Lake Superior (note that this Silver River is not related to Silver River near L'Anse, MI). Situated in a slight landscape depression left by glacial retreat, these sites are among several parallel drains situated in the larger watershed area.

Water levels increase following precipitation, peak in spring with snowmelt, and rise again in the fall with late season rains (Davis et al. 2019). It is common for the drains to dry completely in summer, particularly during periods of drought (Van Grinsven 2015). Watershed streamflow and transpiration predominantly drive the changes in hydrology. During periods of snowmelt or high volumes of precipitation, the wetland drains will collect water and become more saturated (Shannon et al. 2018). Pools of water and surface flow are common in spring following snowmelt and in fall following periods of high precipitation and low evapotranspiration.

Average annual precipitation was 82.0 cm and average temperature was 5.0 ^{o}C from 1991-2020. The January average precipitation from 1991-2020 was 4.3 cm and January average temperature was -9.6 ^{o}C. For a comparison, July average for precipitation from 1991-2020 was 9.3 cm and July average temperature was 18.9 ^{o}C. Water table, temperature, and precipitation trends throughout an average year in a black ash wetland are displayed in Figure 2.2 (see also Appendix V). The degree of seasonal drying and wetting is directly related to precipitation (including snowmelt) and transpiration. Intensity of seasonal changes varies with temperature, precipitation, and seasonal weather patterns for a given year.

There is a distinct transition from the wetland to the upland forest ecosystem. Adjacent to the forested black ash wetland, the ground slopes upwards at 2-5 percent. Wetland soils consist of Gay-Leafriver complex, 0 to 2 percent slopes (NRCS Soil Map classification 8125) while adjacent upland ecosystems consist of Nunica silt loam, 1 to 6 percent slopes (NRCS Soil Map classification 8126B). The distribution of these soil types is consistent across all treatment sites.

The Leafriver soil series is characterized by a shallow organic layer atop a poorly draining mineral layer. The mineral layer is comprised of loamy sand or sandy loam, left in depressions created by glacial activity. Depth to water level remains between 0 and 0.5 m below the surface during dry periods. Surface water is regularly present throughout the year reaching a depth of up to 15 cm during peak snowmelt, except for very dry years when the level of ponding is lower. This soil series regularly supports a wetland plant community of sedges, reeds, and willow. Tree species are less common, but black ash, quaking aspen (*Populus tremuloides* Michaux), balm of gilead (*Populus balsamifera* L.), tamarack (*Larix laricina* (Du Roi) K.Koch), and/or black spruce (*Picea mariana* (Miller) Britton, Sterns & Poggenburg) regularly grow in these soils.

The Nunica soil series is characterized by hardwood litter atop silty loam or silt loam mineral soil. Following glacial melt, these soils formed on lacustrine deposits. This soil is well drained, with moderate to rapid surface runoff and does not routinely support ponding surface water. When water does flow across the surface, it regularly collects in adjacent wetlands composed of Leafriver soils. Vegetation communities are composed of northern hardwood species including sugar maple (*Acer saccharum* Marshall), basswood (*Tilia americana* L.), eastern hemlock (*Tsuga canadensis* L.), and yellow birch (*Betula alleghaniensis* Britton).

2.4.2 Site Treatments

The treatment sites were selected along a gradient of EAB infestation severity. The EAB severity ranges from: high infestation severity (trees showing advanced signs of EAB infection and complete tree mortality), moderate (canopy dieback is beginning), low (signs of declining tree health), and no EAB present (healthy ash trees and intact wetland ecosystem function) (Figure 2.3). All vegetation and environmental monitoring data was collected at all gradient treatment sites unless otherwise noted. In total, twelve plots were established, with three in each EAB infestation gradient site.

2.4.3 Tube Construction

IERC deployment tubes were made of two different diameter PVC tubes. The smaller (inner, ¾ inch) tubes were inserted into larger (outer, 1¼ inch) tubes that remained fixed in the soil over the study period. The IERCs were attached to the inner tubes, which slid in and out for removal and replacement. The tubes were capped to prevent contamination from above soils and litters. A spring was located on top of the IERC and below the cap, to ensure the IERC was in contact with the soil below when the inner tube and cap were in place. The inner tube was longer than the outer tube to not cover up the IERC when deployed (C. Iversen, personal communication, Feb 22, 2021). Tubes varied in length (30 cm and 47 cm) to allow sampling at two depths in the soil. Tubes were deployed in groups of two, reaching depths of 10 cm and 25 cm below the surface with replicates of three (one set per plot) in each of the four treatment sites (Figure 2.3). A total of 24 tubes were placed throughout all treatment sites.

2.4.4 Resin Preparation

IERC preparation for analysis followed the procedure used on the Ottawa National Forest treatment sites, a modification from Giblin et al. (1994). Upon sample retrieval, IERCs were cleaned with deionized water to remove soil debris and organic matter and chilled on ice in polyethylene bags while in the field. In the lab, they were prepared via 2M KCl extraction following the procedure outlined by Jimenez (2007) and modified by Iversen

(2010), of the Protocol for Ecosystem Laboratory, Oak Ridge National Laboratory (Appendix IV). After sample preparation was completed, extracted samples were stored at -20°C before being shipped for sample analysis.

2.4.5 Resin Sample Analysis

Mass of nitrogen in NH_4^+-N, NO_3^-.NO_2^--N, and Total Nitrogen (TN) eluted from IERCs was determined using a Lachat QuikChem 8500 Series 2 Flow Injection Analysis System and then the concentration (mg/L) of nitrogen species was calculated for each sample.

Lab analysis was performed at the USFS Northern Research Station, Forestry Sciences Laboratory in Grand Rapids, MN.

Total organic nitrogen (TON) was not measured but calculated based on the mass balance of Lachat analysis.

$$TN - NH_4^+\text{-N} - NO_3^-.NO_2^-\text{-N} = TON$$

The difference between TN and the sum of NH_4^+-N and $NO3^-$.$NO2^-$-N represents total organic nitrogen (Jones and Willett 2006). It is generally assumed that this difference in primarily aquatic or flooded systems is stored in forms of organic nitrogen (Hansell 1993; Cornell et al. 2003; Lee and Westerhoff 2005; Saunders et al. 2017). Hereinafter, the difference will refer to TON.

Elemental mass of Ca^{2+}, Mg^{2+}, Na^+, and Total P eluted from IERCs was determined using a Thermo Scientific iCAP 7000 Series ICP-OES and then the density (mg/L) of each element was calculated for each sample. Henceforth, analytical results will be expressed without valences as Ca, Mg, Na, and P. Lab analysis was performed at the USFS Northern Research Station, Forestry Sciences Laboratory in Grand Rapids, MN.

2.4.6 Statistical Analysis

Data was grouped by treatment site for analysis purposes. Statistical analysis was performed in Microsoft Excel. Significant values were tested using analysis of variance tests (ANOVA) for comparison of means across collections of parameters (i.e., EAB treatment, season, depth, nutrient). When comparing means of two groupings (i.e., between seasons for a given EAB treatment), t test: paired two sample for means was used. Significance was recorded as $p < 0.10$, with notations when significance was recorded as $p < 0.05$, $p < 0.01$, $p < 0.001$.

2.5 Results

Variable forms of nitrogen existed in near-surface soils. Soil NH_4^+-N was the dominant available form of nitrogen throughout all seasons and treatment sites, accounting for up to

94% of TN at some sites. $NO_3^-.NO_2^-$-N and TON were much lower, but still contributed to TN. Across all sites and all treatments, percent of TN ± standard deviation for NH_4^+-N accounted for 62 ± 2.77% of TN, $NO_3^-.NO_2^-$-N accounted for 5 ± 0.65% of TN, and TON accounted for 33 ± 0.49% of TN. Nitrogen variation among seasons across all sites was statistically significant for NH_4^+-N ($p < 0.01$), TON ($p < 0.001$), and TN ($p < 0.01$). More nitrogen was available at sites in the summer and spring than in the fall (Figure 2.4). It is important to note average measures of TON in 2022 were greater than those from 2021.

2.5.1 EAB Treatment Site Effects on Near-surface Soil Nitrogen

Treatment site variation was observed within individual seasons and when compared to other seasons (Figure 2.5). A greater mean amount of NH_4^+-N was available in HighEAB treatment sites than other treatment sites across all seasons. Less TON was present in sites impacted by EAB, displaying less organic production when ash is unhealthy or removed from the environment. Nitrogen species variation between treatment sites across all seasons was statistically significant for NH_4^+-N ($p < 0.001$), $NO_3^-.NO_2^-$-N ($p < 0.01$), TON ($p < 0.001$), and TN ($p < 0.001$). Significant values are in accordance with treatment site infestation gradient and environmental impacts from EAB and their expected influence on biogeochemical cycling of nitrogen.

2.5.2 Depth Effects on Near-surface Soil Nitrogen

The depth of samples also provided insight into nitrogen availability in these black ash wetlands. Nitrogen mean and range values at all treatment sites across all seasons were greater at 10 cm from the soil surface for $NO_3^-.NO_2^-$-N and TON (Figure 2.6). The differences in values between the 10 cm depth and 25 cm depth remained statistically significant for NH_4^+-N ($p < 0.05$), $NO_3^-.NO_2^-$-N ($p < 0.05$), TON ($p < 0.1$), and TN ($p < 0.1$) at all treatment sites for all seasons. At 10 cm depth, mean (± standard error) was 0.44 ± 0.11 ppm for $NO_3^-.NO_2^-$-N and 2.07 ± 0.06 ppm for TON compared to the 25 cm depth where mean values were 0.16 ± 0.02 ppm for $NO_3^-.NO_2$-N and 1.92 ± 0.07 ppm for TON. The mean value for NH_4^+-N was greatest at 25 cm from the surface (4.44 ± 0.32 ppm) compared to 3.15 ± 0.37 ppm at 10 cm from the surface. The greater amount of NH_4^+-N drove TN values, which were likewise greater at 25 cm than 10 cm, where mean values were 6.52 ± 0.30 and 5.66 ± 0.35 ppm, respectively.

Depending on the treatment, nitrogen was frequently more available at 25 cm below the surface than at 10 cm below the surface. (Figure 2.7). The 10 cm depth and 25 cm depth displayed statistically significant differences in mean nitrogen availability between depths. The values when comparing the means of the 10 cm depth and 25 cm depth were for NH_4^+-N ($p < 0.001$), $NO_3^-.NO_2^-$-N ($p < 0.01$), TON ($p < 0.001$), and TN ($p < 0.01$) at all treatment sites for all seasons. Water table levels, seasonal variation, and EAB treatment site all influenced nitrogen species availability. The HighEAB treatment had more TN at both 10 cm and 25 cm below the surface (mean levels of 7.94 ppm and 8.66 ppm, respectively) than any other treatment site or depth (Table 2.2). The HighEAB treatments at 10 cm and 25 cm also had more NH_4^+-N than any other treatment site or depth (mean levels of 5.93 ppm and 6.85 ppm, respectively). The amount of $NO_3^-.NO_2^-$-N and TON fluctuated between treatment sites and depths. Since these nitrogen species were a much smaller component of the nitrogen budget at the treatment sites, the differences observed may be a product of the specific sites and not treatment effects.

Also, comparing only HighEAB sites to NoEAB sites (Figure 2.10) creates a direct comparison of extremes of EAB impact. HighEAB represents advanced ecosystem changes and NoEAB serves as the control without impacts from EAB. Nitrogen variation between depths for specific seasons was not significant across all treatment sites (Table 2.4). Individual sites showed significance in some seasons.

2.5.3 Seasonal Effects on Near-surface Soil Nitrogen

Nitrogen species variation between treatment sites for a given season were statistically significant for some seasons and some species of nitrogen (Table 2.3). A greater number of significant differences were observed in 2021 (87.5% of combined all measures) than in 2022 (50% of combined all measures). Significant differences for NH_4^+-N were observed in Summer21, Fall21, Spring22, and Summer22. Significant differences for $NO_3^-.NO_2^-$-N were observed in Summer21, Summer22, and Fall22. Significant differences for TON were observed in Summer21, Fall21, and Fall22. Significant differences for TN were observed in Summew21, Fall21, and Spring22. The top row of figures in Figure 2.8 labeled All Depths corresponds to these tests. A complete display of nitrogen species, treatment sites, and season demonstrates the variation between seasons, sites, and nitrogen species in a single visible display.

Nitrogen variation between depth for specific seasons was not significant across all treatment sites. Individual sites showed significance in some seasons. A greater number of significant differences were observed for the 10 cm depth from the soil surface (45% of sites and seasons) compared to the 25 cm depth from the soil surface (40%). In Summer21, significance was observed for both depths for NH_4^+-N and TN, only for the 10 cm depth for $NO_3^-.NO_2^-$-N, and not at all for TON. In Fall21, significance was observed for both depths for NH_4^+-N, only the 10 cm depth for TON, only the 25 cm depth for TN, and not at all for $NO_3^-.NO_2^-$-N. Spring22 represents the first year post drought conditions experienced during 2021, and produced significant differences for only the 10 cm depth of TON, the 25 cm depth of NH_4^+-N, and the 25 cm depth of TN. Summer22 only yielded two significant differences for the 10 cm depth of $NO_3^-.NO_2^-$-N and the 25 cm depth of NH_4^+-N, and therefore was the least significant season when comparing depth from surface. Fall22 produced significant differences for both depths of TON and the 10 cm depth of $NO_3^-.NO_2^-$-N. The middle row of Table 2.3 represents 10 cm depth and the bottom row of Table 2.3 represents 25 cm depth, respectively. Due to small sample sizes of n = 6 for top table (Table 2.3), n = 3 for middle table (Table 2.3), and n = 3 for bottom table (Table 2.3), a range of statistical significance was observed.

2.5.4 Other Nitrogen Analysis

Additional data analysis was conducted. Specifically, adjusting nitrogen species value by the mean of NoEAB (control) sites to standardize values based on the difference from normal (Figure 2.9). This created a normalized value for HighEAB, IntEAB, and LowEAB Treatment sites for comparison against the control NoEAB. Nitrogen variation between depth for specific seasons was not significant across all treatment sites (Table 2.4). Individual sites showed significance in some seasons.

2.5.5 Other Elements

Elemental variation between seasons across all sites was statistically significant for Ca ($p < 0.001$), Mg ($p < 0.001$), and P ($p < 0.001$). Changes in Na observed between seasons for 2022 was not significant, likely due to a smaller sample size (n = 3, compared to n = 5 for other elements). More Ca, Mg, P, and Na was available at sites in the summer than in the spring or fall (Figure 2.11). Spring regularly had the lowest amount of available elements, based on mean amount available (Table 2.5).

Treatment site variation was also observed within individual seasons and when compared to other seasons (Figure 2.12). A greater mean amount of Ca and Mg was available in HighEAB treatment sites than other treatment sites across all seasons. Elemental variation between treatment sites across all seasons was statistically significant for Ca ($p < 0.001$), Mg ($p < 0.001$), and Na ($p < 0.001$). P produced inconsistent results, likely due to sample measurements being near or below detection limit for ICP analysis.

2.6 Discussion

2.6.1 EAB Treatment Site Effects

As EAB moved into black ash wetlands, more TN availability was observed at sites with greater levels of disturbance (Figure 2.5). HighEAB treatment sites had the most TN available, followed by IntEAB/LowEAB, and finally NoEAB (Figure 2.7). The NoEAB control sites served as a baseline for comparison, since they represent a non-infested black ash wetland similar to the infested treatment site wetlands prior to EAB invasion.

Numerous studies indicated an increase of nitrogen availability following tree mortality caused by insect pests (Orwig et al. 2008; Griffin, Turner, and Simard 2011; Toczydlowski, Slesak, Kolka, and Venterea 2020). Notably, coarse woody debris inputs following black ash mortality in simulated EAB treatment sites (Davis et al. 2017) and green ash mortality in riparian forests have shown greater inputs of organic nitrogen than comparable plots of healthy forests lacking ash (Engelken, Benbow, and McCullough 2020). With additional nitrogen available following EAB disturbance, the net changes in the availability between sites was impacted by alternative species taking advantage of the increased nitrogen levels in the environment (Westbrook and Devito 2004; Nakagawa et al. 2012).

NH_4^+-N was the primary component of TN across seasons and treatment sites (Figure 2.6). Greater soil NH_4^+-N availability was observed at more disturbed sites in simulated EAB infestations in black ash wetlands (Toczydlowski et al. 2020; Davis et al. 2019). An increase in available NH_4^+-N is common following other tree mortality events caused by insect pests such as hemlock woolly adelgid (Cobb, Orwig, and Currie 2006; Jenkins, Aber, and Canham 1999; Orwig et al. 2008). With a larger input of organic nitrogen from black ash mortality, microbes readily mineralize organic nitrogen from black ash into forms of inorganic nitrogen like NH_4^+-N.

Water table changes following ash canopy removal from EAB mortality also explained the changes in soil nitrogen availability (Davis et al. 2019; Toczydlowski et al. 2020). All treatment sites had a water table closer to the soil surface for summer 2022 than for summer 2021. As EAB caused tree mortality, and tree mortality led to less evapotranspiration at sites, the water table rose and created an anoxic environment for a greater portion of the year. Since there was not oxygen available for further denitrification to occur in the anoxic environment created by rising water levels, microbes were unable to convert NH_4^+-N via oxidation (Zak et al. 1990). The lack of oxygen resulted in an environmental accumulation of NH_4^+-N, as observed by the greater amount of NH_4^+-N than other nitrogen species at all sites. $NO_3^-.NO_2^-$-N was the least abundant component of TN. Since these wetlands existed in saturated conditions for most of the year, nitrate was not produced regularly (Spoelstra et al. 2010).

When nitrogen is readily available, black ash trees capitalize on the resource and grow rapidly. Less TN was measured at the NoEAB site regularly throughout this study due to the hyper-consumption of nitrogen by the intact vegetation community. In contrast, there was more exchangeable nitrogen and other elements present in the HighEAB sites. The HighEAB site represents the forest with the greatest level of disturbance, since EAB has been present at the site for a longer period. Similarly, hemlock wooly adelgid infestations caused more inorganic nitrogen to become available as foliage was desiccated and allowed nitrogen to leach into the environment (Orwig et al. 2008; Jenkins, Aber, and Canham 1999). Mountain pine beetle infestation likewise returned nitrogen to the environment as snags fell and began to decompose (Griffin, Turner, and Simard 2011; Keville, Reed, and Cleveland 2013). Since the black ash were dying due to EAB infestation, the nitrogen and other elements incorporated into black ash organic matter were decomposed and returned to the environment.

2.6.2 Depths Effects

Often, more nitrogen was present at the 25 cm depth than the 10 cm depth (Figure 2.7). The microbial community, plant demands, and ecosystem return via litter and throughfall influence the upper soil layers more than lower soil layers and lead to a reduction in available nitrogen (Durán et al. 2017). Since black ash wetlands regularly exist in a saturated condition, when the soil does have a chance to dry during the summer growing season, oxygen can enter the upper soil layers (Grigal and Homann 1994). Frequently, the

10 cm depth was above the water table during this two-year study period which may have led to increased demand for nitrogen in soils closest to the surface.

Depending on the season, a difference in water table level further influenced nitrogen availability. If the water table resided between the 10 cm and 25 cm sampling depths, the presence or absence of oxygen influenced nitrogen species distribution (Murphy et al. 2009). Specifically, during the summer collection when sites were at their driest, the 25 cm depth was regularly below the water table, creating an anoxic environment while there was the presence of oxygen at the 10 cm depth (Figure 2.8).

Apart from nitrogen species, depth did not have a significant influence on other elements measured. The measurements of other elements at two depths helped provide a reference for the site impacts on the nitrogen species. Demand was greater in the top 10 cm, which likely decreased the availability for other elements such as major cations as they were readily incorporated by vegetation.

2.6.3 Seasonal Effects

More TN was available at sites in summer and spring than in fall (Figure 2.4). Seasonal variations were expected since plant demand on nitrogen changes throughout the year (Westbrook and Devito 2004). Belowground storage in the fall sequesters available nutrients for use in the following growing season (Zak et al. 1990). All treatment sites followed similar trends across seasons, with a greater level of TN present in the environment in spring and summer than fall. In fall, vegetation is storing accumulated nitrogen or has already released organic nitrogen back into the environment, resulting in the lower levels of TN measured in fall.

Significant TON changes may not be measurable between treatments since plants and microbes in the saturated environment were more readily able to absorb available sources of nitrogen following disturbance but may be significant over seasons. TON primarily represented organic nitrogen in the environment (Jones and Willett 2006), and the calculated values of TON from measurements in this experiment supported these arguments. There was more TON throughout 2022, likely due to higher levels of precipitation allowing plants to produce more leaf mass per area and increased overall plant growth. Since 2021 was drastically dryer than average and also dryer than 2022, a lack of water and other available resources likely led to decreased vegetation production and subsequent return of TON to the environment (Landesman and Dighton 2010). When leaves were annually returned to the wetlands in fall, greater levels of TON were present in the environment.

When black ash wetlands dried in fall, higher $NO_3^-.NO_2^-$—N concentration was measured (Figure 2.5). Since the water table dropped throughout the growing season, this observation aligned with expected outcomes of nitrogen cycling (Davis et al. 2019; Toczydlowski, Slesak, Kolka, Venterea, et al. 2020). When $NO_3^-.NO_2^-$-N was available in the environment, it was readily incorporated into plant tissues (Spoelstra et al. 2010).

This steady uptake further explained the lower levels of $NO_3^-.NO_2^-$-N during the growing seasons (spring and summer). Most nitrite remained in the soil environment and was not denitrified into gases in these wetlands due to the absence of oxygen. The aquatic nature of these forested wetlands encourages a microbial community to develop that readily consumes $NO_3^-.NO_2^-$-N, since these microbes are able to derive oxygen from $NO_3^-.NO_2^-$-N in the otherwise anoxic environment (Peralta, Matthews, and Kent 2010). As a result, levels of $NO_3^-.NO_2^-$-N were lower than other available forms of nitrogen in the environment.

Seasonal variation of other elements was correlated to changes in nitrogen availability. In more impacted treatment sites, a greater amount of NH_4^+-N, TN, Ca, Mg, and Na were measured (Figures 2.8 and 2.12). During the winter, trees store resources below ground to enable leaf out during the subsequential growing season. Since nutrients and other resources are stored, there is less available in the soil immediately in spring following the period of winter dormancy. During the summer growing season, resources are incorporated into organic matter and used to facilitate further plant growth and development. The summer growing season is especially truncated in black ash wetlands, due to late leaf out in an attempt to allow spring snowmelt to dissipate and dry the environment.

2.6.4 Most Impactful Variable on Nitrogen

Nitrogen availability across EAB treatment sites, seasons, and depths varied. Nitrogen fluctuations are common throughout an ecosystem. Disturbance, seasonality, and depth below the surface all influence how nitrogen is distributed throughout the environment (Lovett et al. 2006). Changes are common, especially when wetland community composition changes following ash mortality and also shift demands for nitrogen. How nitrogen is distributed and the forms in which it is available influences how the vegetation communities develop, grow, and change (Jicha et al. 2014). In black ash wetlands, influences that alter the water level and soil saturation in the sites have the greatest impact on nitrogen species, other elements, and vegetation composition changes.

EAB disturbance has demonstrated an increase in water table levels as infestation progresses. With less ash present following mortality, there is less net transpiration and water table draw down does not occur to the previous magnitude observed prior to EAB infestation. Site saturation plays a large role in $NO_3^-.NO_2^-$-N availability in the environment, but it is noteworthy that $NO_3^-.NO_2^-$-N also leaches readily below the rooting zone. Certain studies have noted export via hydrologic pathways following insect caused tree mortality as an explanation for the absence of $NO_3^-.NO_2^-$-N in disturbed environments (Cessna and Nielsen 2012). Also, alder has been observed as a newly occurring species in EAB infested sites. Alder is a nitrogen fixer and may serve as an important new source for nitrogen in the newly emerging ecosystem (Kiernan, Hurd, and Raynal 2003). As the nitrogen budget shifts following the loss of black ash, new members of the vegetation community may help supplement the entire ecosystem with new sources of nitrogen.

As surface water flows from sites, an increased nitrogen export via hydrologic systems draining forested wetlands has been measured in certain riparian forest communities (Bayley et al. 1992; Nieminen 2004). The degree of water level rise was greatest at more disturbed sites. Studies of simulated EAB in black ash wetlands showed greatest water level increases in more disturbed black ash wetlands (Shannon et al. 2018; Slesak et al. 2014). Hydrologic observations from impacts of EAB in other ash ecosystems likewise showed an increase in water levels following EAB disturbance. (Engelken, Benbow, and McCullough 2020; Robertson, Robinett, and McCullough 2018).

2.7 Conclusions

Nitrogen cycle responses following emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) infestation and subsequent black ash (*Fraxinus nigra* Marshall) mortality in forested wetlands will have novel impacts on ecosystem composition and function, in part due to the ecological niche these forests occupy and their impact on hydrologic cycling for northern hardwood forests. More TN was available at more impacted sites, resulting in the greatest amount of available nitrogen at the HighEAB site. Due to the aquatic nature of these forested wetlands, NH_4^+-N was the primary component of TN at all sites. NO_3^-.NO_2^--N was far less abundant since the anoxic environment quickly facilitated its use by the microbial community. As black ash died and fell to the wetland, more TON was returned to the environment and promptly incorporated into the growing shrub and sapling layers. Vegetation community changes could be intertwined with nitrogen cycle disturbances following EAB infestation. If nitrogen availability in shallow soils increases and other plant species do not colonize the environment and uptake it, nitrogen is likely to leave the system as runoff in streams.

The season and EAB infestation severity played a greater role in determining the availability of nitrogen and other elements than depth. Gaining an understanding of how the nitrogen budget of these forests will shift will have important implications for future forest management activities, plant community changes, and the future restoration of these forested wetland ecosystems. With more nitrogen species available at sites infested with EAB, long term ecosystem integrity will require future species to have access to these nutrient resources. If long term changes and nitrogen loss decrease site fertility, future plant communities in these sites may be in jeopardy. Likewise, these forested sites may no longer serve as the biogeochemical stores or landscape habitat they presently create.

2.8 Future Research

At present, a baseline of near surface soil nitrogen availability has been established. With changes in nitrogen availability in response to EAB infestation documented, this data may serve as a baseline for the sites moving forward. Continued monitoring of vegetation changes and nitrogen species composition down the road will enable comparison and a greater understanding of long term EAB impacts on these forested wetland ecosystems.

In the future, pairing these studies with microbial analysis, throughfall, or leaflitter analysis will provide a more complete description of the nitrogen cycle in these altered forested wetland habitats.

Future research to expand and further validate EAB impacts in black ash wetlands should include expansion the number of impacted black ash wetlands being monitored. This expansion would create additional sites within the HighEAB to NoEAB gradient to further display the impacts of EAB. A greater number of replicates may lead to more statistical significance and further validate these findings. Also, monitoring the entire watershed that these parallel wetland drains are within may lead to an entire ecosystem assessment, since these sites are aquatic in nature and all drain to a common point of the Silver River.

2.9 Tables

Table 2.1. Collection dates for specific seasons. Seasons were timed with phenological events (i.e., leaf on, onset of senescence, and leaf off), to capture changes over the growing season and year to year.

Season	**Collection Date**
Summer21	09/02/2021
Fall21	10/29/2021
Spring22	06/13/2022
Summer22	09/04/2022
Fall22	10/29/2022

Table 2.2. Mean values for specific nitrogen species (in ppm) and EAB treatment site. Values combine all measures across all seasons for a given treatment intensity (n = 15). Percent of TN is shown in parenthesis following mean values.

10 cm	HighEAB	IntEAB	LowEAB	NoEAB
NH_4^+-N	5.93 (74.69%)	2.48 (53.91%)	3.01 (51.63%)	1.16 (27.17%)
NO_3^-.NO_2^--N	0.09 (1.13%)	0.10 (2.17%)	0.86 (14.75%)	0.72 (16.86%)
TON	1.92 (24.18%)	2.01 (43.70%)	1.96 (33.62%)	2.39 (55.97%)
TN	7.94 (100%)	4.60 (100%)	5.83 (100%)	4.27 (100%)

25 cm	HighEAB	IntEAB	LowEAB	NoEAB
NH_4^+-N	6.85 (79.10%)	4.44 (68.62%)	4.09 (65.97%)	2.37 (49.89%)
NO_3^-.NO_2^--N	0.09 (1.04%)	0.10 (1.55%)	0.26 (4.19%)	0.20 (4.21%)
TON	1.71 (19.75%)	1.93 (29.83%)	1.85 (29.84%)	2.18 (45.89%)
TN	8.66 (100%)	6.47 (100%)	6.20 (100%)	4.75 (100%)

Table 2.3. ANOVA's *p* values for each display in Figure 2.8, organized by row. The first, second, and third section corresponds to values at all depth, at 10 cm depth, at 25 cm depth. Green highlights indicate significance at $p < 0.05$ and yellow ones indicate significance at $p < 0.10$.

All	Summer21	Fall21	Spring22	Summer22	Fall22
NH_4^+-N	9.03E-06	6.45E-05	0.0085	0.0843	0.109
$NO_3^-.NO_2^-$-N	0.0355	0.167	0.117	0.015	0.0998
TON	0.0645	0.00572	0.124	0.337	0.00017
TN	1.33E-05	0.0016	0.1	0.12	0.236

10 cm	Summer21	Fall21	Spring22	Summer22	Fall22
NH_4^+-N	0.0016	0.0177	0.2360	0.1550	0.2670
$NO_3^-.NO_2^-$-N	0.0350	0.4000	0.2090	0.0013	0.0563
TON	0.1800	0.0234	0.0066	0.3580	0.0582
TN	0.0070	0.2170	0.5310	0.2080	0.3620

25 cm	Summer21	Fall21	Spring22	Summer22	Fall22
NH_4^+-N	0.0011	0.0006	0.0357	0.0771	0.3130
$NO_3^-.NO_2^-$-N	0.4870	0.5160	0.5810	0.4880	0.9570
TON	0.1950	0.2440	0.6530	0.6620	0.0077
TN	0.0015	0.0057	0.0986	0.1020	0.7530

Table 2.4. ANOVA *p* values for figures displayed in 3x5 figure displays in Figure 2.8, Figure 2.5, and Figure 2.6. The Values row corresponds to Figure 2.8. The Differences row corresponds to Figure 2.5. The HighEAB v NoEAB row corresponds to Figure 2.6. The All column for each season combines the depth measurements for the specific season. Green cells indicate significance at $p < 0.05$ and yellow cells indicate significance at $p < 0.10$.

			Summer 21				Fall 21				Spring 22				Summer 22				Fall 22	
NH_4^+-N		All	10 cm	25 cm		All	10 cm	25 cm		All	10 cm	25 cm		All	10 cm	25 cm		All	10 cm	25 cm
Values		0.0000	0.0016	0.0011		0.0001	0.0177	0.0006		0.0085	0.2360	0.0357		0.0843	0.1550	0.0771		0.1090	0.2670	0.3130
Differences		0.0000	0.0016	0.0011		0.0000	0.0177	0.0006		0.0057	0.2358	0.0357		0.0735	0.1546	0.0771		0.1346	0.2667	0.3125
HighEAB v NoEAB		0.0002	0.0116	0.0045		0.0002	0.0384	0.0017		0.0103	0.1828	0.0494		0.0301	0.2030	0.0765		0.1062	0.2297	0.1896
			Summer 21				Fall 21				Spring 22				Summer 22				Fall 22	
$NO_3^-.NO_2^-$-N		All	10 cm	25 cm		All	10 cm	25 cm		All	10 cm	25 cm		All	10 cm	25 cm		All	10 cm	25 cm
Values		0.0355	0.0350	0.4870		0.1670	0.4000	0.5160		0.1170	0.2090	0.5810		0.0150	0.0013	0.4880		0.0998	0.0563	0.9570
Differences		0.0372	0.0350	0.4869		0.1528	0.3997	0.5163		0.2474	0.2091	0.5814		0.1351	0.0013	0.4877		0.0785	0.0563	0.9573
HighEAB v NoEAB		0.0246	0.3757	0.0518		0.0662	0.2007	0.0023		0.1548	0.2325	0.4194		0.0226	0.0151	0.4155		0.1305	0.1117	0.7680
			Summer 21				Fall 21				Spring 22				Summer 22				Fall 22	
TON		All	10 cm	25 cm		All	10 cm	25 cm		All	10 cm	25 cm		All	10 cm	25 cm		All	10 cm	25 cm
Values		0.0645	0.1800	0.1950		0.0057	0.0234	0.2440		0.1240	0.0066	0.6530		0.3370	0.3580	0.6620		0.0002	0.0582	0.0077
Differences		0.0378	0.1801	0.1949		0.0038	0.0234	0.2442		0.0819	0.0066	0.6527		0.3989	0.3584	0.6615		0.0001	0.0582	0.0077
HighEAB v NoEAB		0.0364	0.1881	0.1892		0.0048	0.0100	0.1184		0.1083	0.0211	0.3848		0.2035	0.4028	0.3528		0.0008	0.0008	0.0158
		e	Summer 21				Fall 21				Spring 22				Summer 22				Fall 22	
TN		All	10 cm	25 cm		All	10 cm	25 cm		All	10 cm	25 cm		All	10 cm	25 cm		All	10 cm	25 cm
Values		0.0000	0.0070	0.0015		0.0016	0.2170	0.0057		0.1000	0.5310	0.0986		0.1200	0.2080	0.1020		0.2360	0.3620	0.7530
Differences		0.0000	0.0070	0.0015		0.0029	0.2173	0.0057		0.1445	0.5306	0.0986		0.0948	0.2084	0.1017		0.2448	0.3617	0.7529
HighEAB v NoEAB		0.0002	0.0088	0.0048		0.0007	0.0905	0.0033		0.0696	0.4563	0.0989		0.0445	0.2726	0.0863		0.2302	0.3439	0.4482

Table 2.5. Summary statistics for other elements analyzed, broken down by season and year. Values listed include mean, variance, standard deviation, and standard error.

Ca	Mean	Variance	Standard Deviation	Standard Error
Summer21	61.65	1259.78	35.49	7.25
Fall21	27.90	330.62	18.18	3.71
Spring22	17.23	513.26	22.66	4.62
Summer22	39.72	1072.60	32.75	6.69
Fall22	20.76	364.43	19.09	3.98
Mg	Mean	Variance	Standard Deviation	Standard Error
Summer21	14.19	57.69	7.60	1.55
Fall21	6.67	14.56	3.82	0.78
Spring22	4.68	33.40	5.78	1.18
Summer22	10.25	72.86	8.54	1.74
Fall22	5.56	27.80	5.27	1.10
Na	Mean	Variance	Standard Deviation	Standard Error
Summer21	x	x	x	x
Fall21	x	x	x	x
Spring22	5.08	17.31	4.16	0.85
Summer22	7.68	32.35	5.69	1.16
Fall22	5.40	16.48	4.06	0.85
P	Mean	Variance	Standard Deviation	Standard Error
Summer21	0.23	0.02	0.14	0.03
Fall21	0.05	0.00	0.06	0.01
Spring22	0.04	0.00	0.07	0.01
Summer22	0.05	0.00	0.04	0.01
Fall22	0.04	0.00	0.07	0.01

2.10 Figures

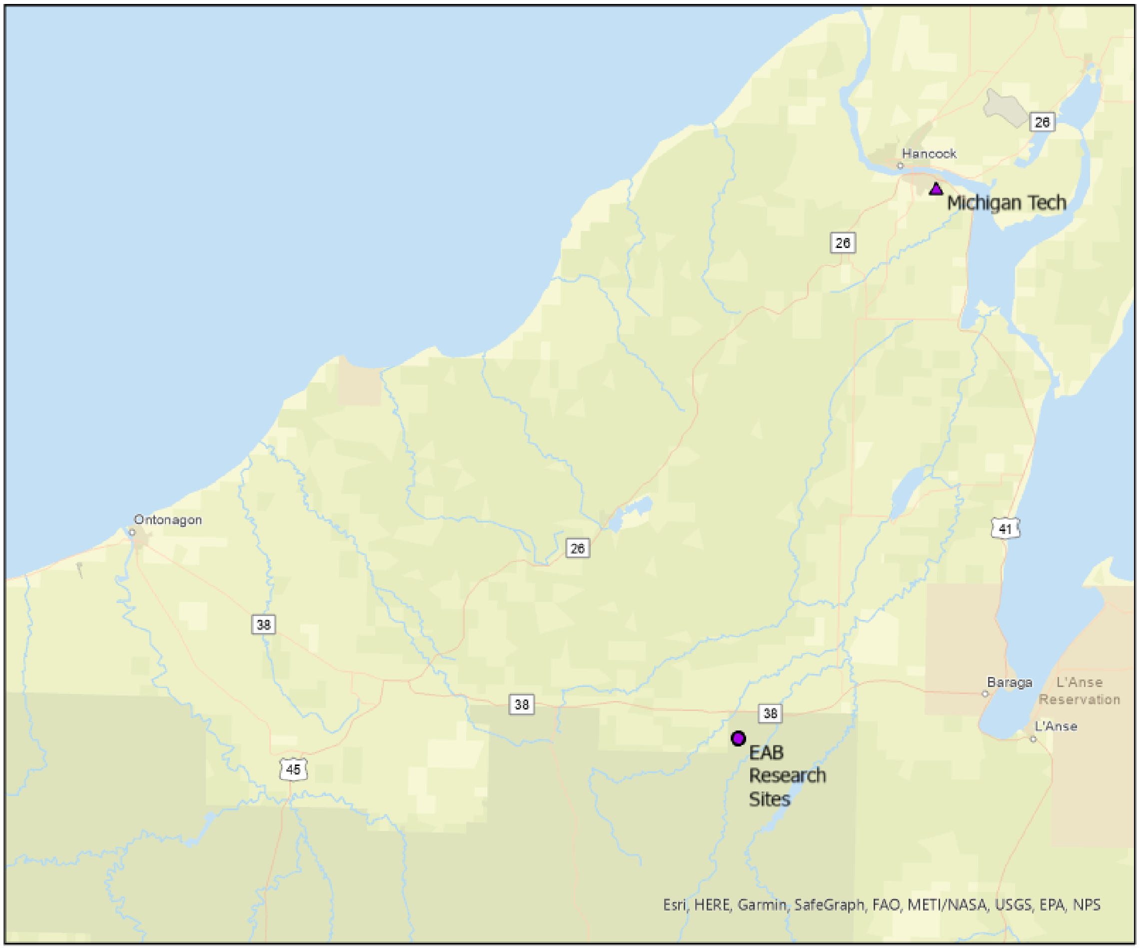

Figure 2.1. Overview map showing location of EAB research sites in western Upper Peninsula, Michigan, USA. Sites are approximately 27 miles south of Michigan Tech (Houghton, Michigan, USA), 12 miles west of Baraga (Michigan, USA), and 28 miles east of Ontonagon (Michigan, USA).

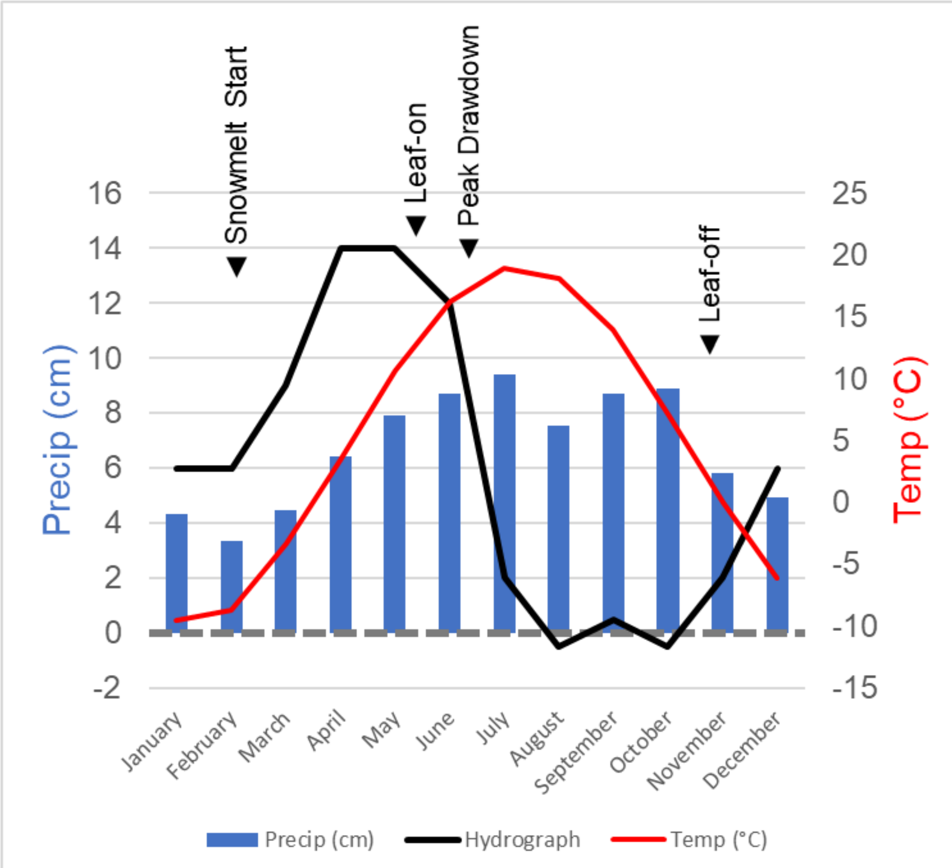

Figure 2.2. Seasonal hydroperiod of a black ash wetland (black line) relative to ground surface symbolized at Precip = 0 cm with the black dashed horizontal line. Precipitation (cm) is shown with blue bars based on 30-year normal from 1991-2020. Temperature (°C) is shown with red line based on 30-year normals.

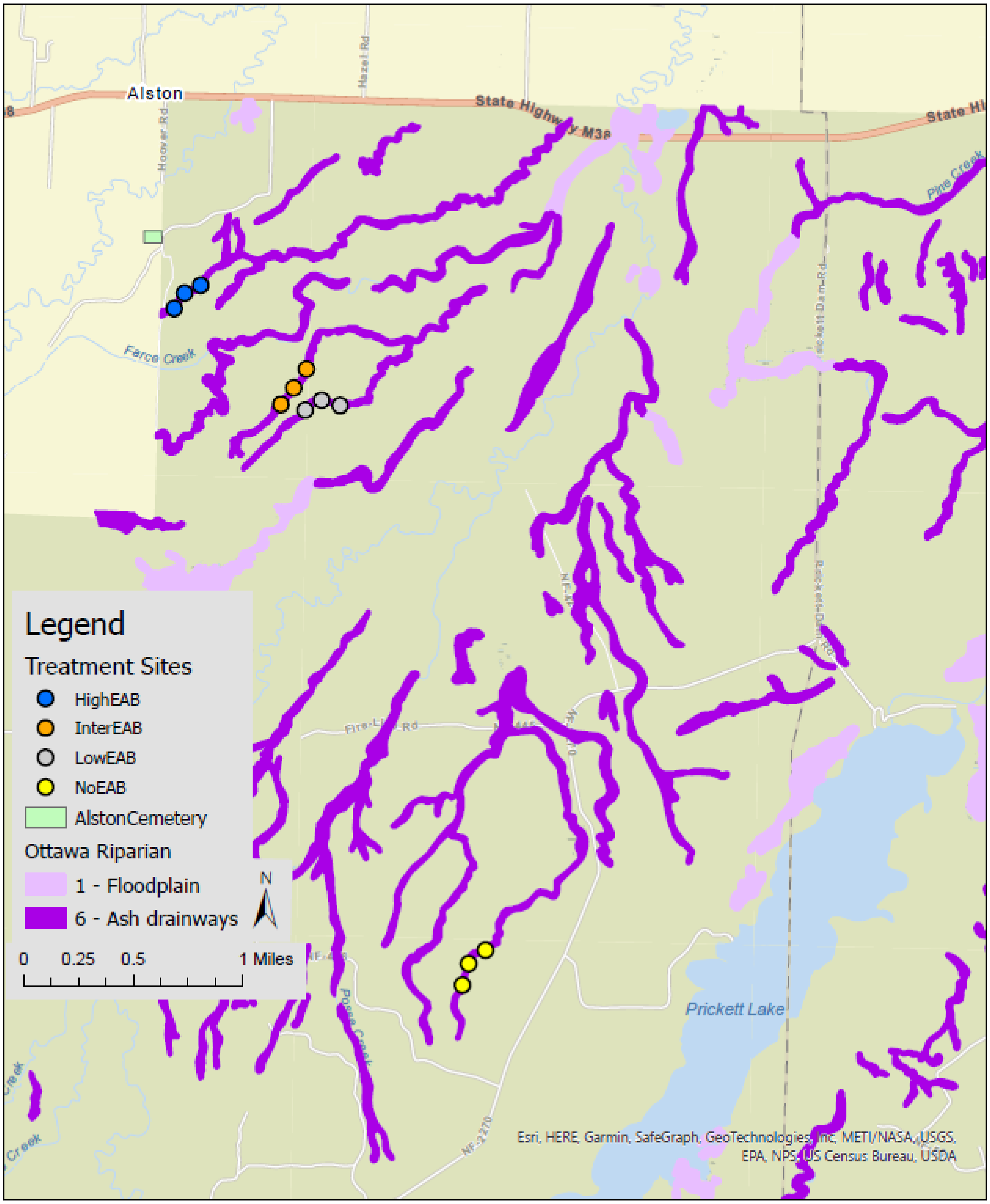

Figure 2.3. Research treatment sites map near Alston, Michigan, USA. Sites are located in the SWSW, Sec. 6; NE, NWNW, Sec. 7; SWSE, Sec. 20; NWNE, Sec. 29, T. 50 N., R. 35 W., Houghton County, MICHIGAN MERIDIAN. EAB gradient moves from high EAB impact (HighEAB) in the northwest to intermediate EAB impact (IntEAB), low EAB impact (Low EAB), and finally no EAB impact (NoEAB) in the southeast.

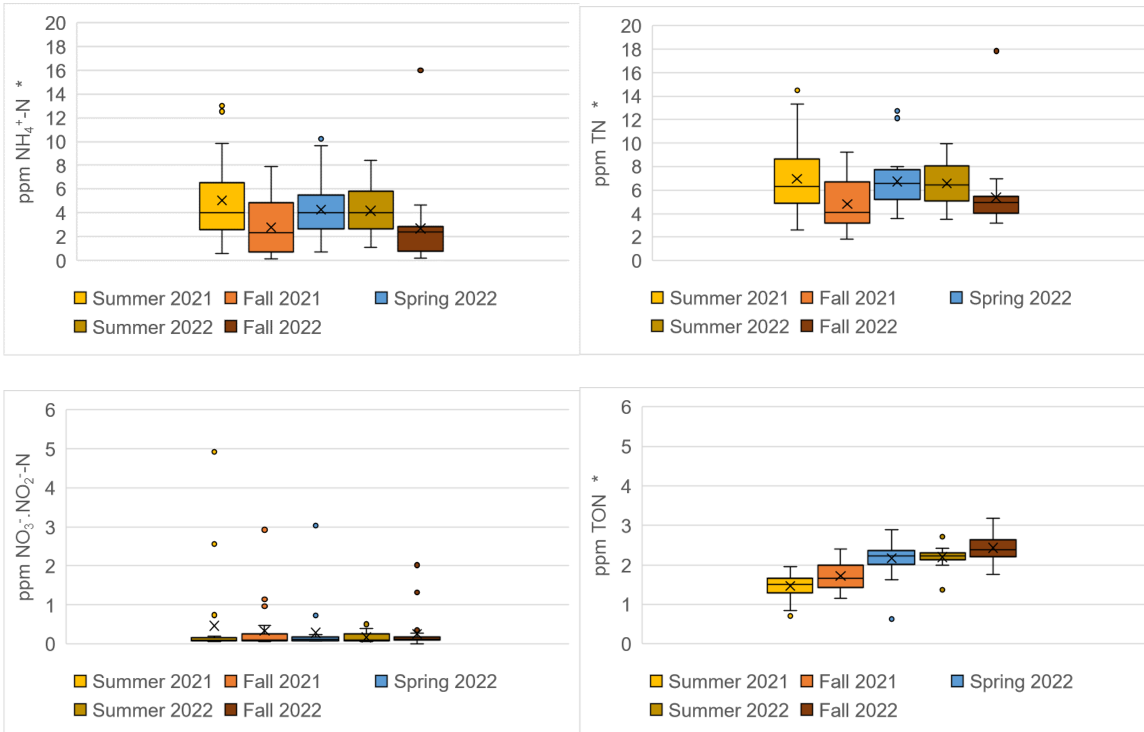

Figure 2.4. Total values of individual nitrogen species by season. Depth was not separated when producing this figure. A combination of EAB infestation and hydrology (seasonal variation and drought stress) explained much of the variation between seasons and years. The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value. Nitrogen species labeled with an asterisk are statistically different across seasons.

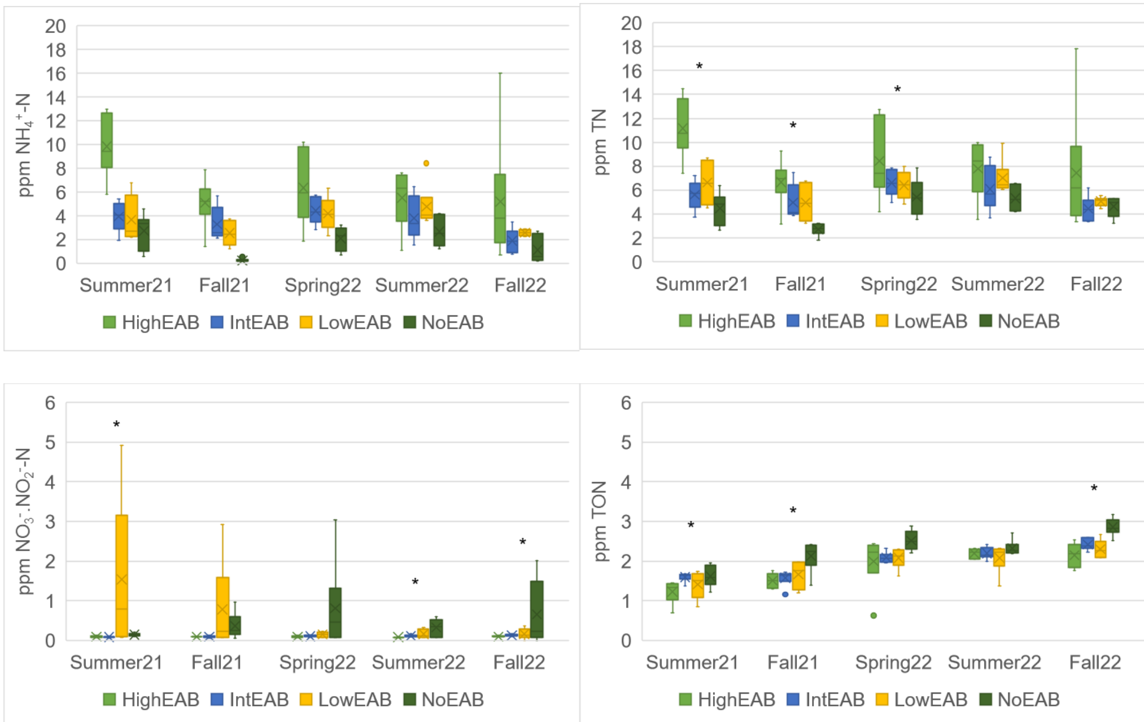

Figure 2.5. Display of values of nitrogen species as they vary across treatment sites by season. Depth was not separated when producing this figure. The sum of values in an individual season across all treatment sites (i.e., NH_4^+-N measurements in Summer 21, HighEAB, IntEAB, LowEAB, and NoEAB) is equal to the corresponding individual season in Figure 2.4 (i.e., NH_4^+-N measurements for all of Summer 2021). The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value. Seasons marked with an asterisk are statistically significant when comparing EAB site treatment.

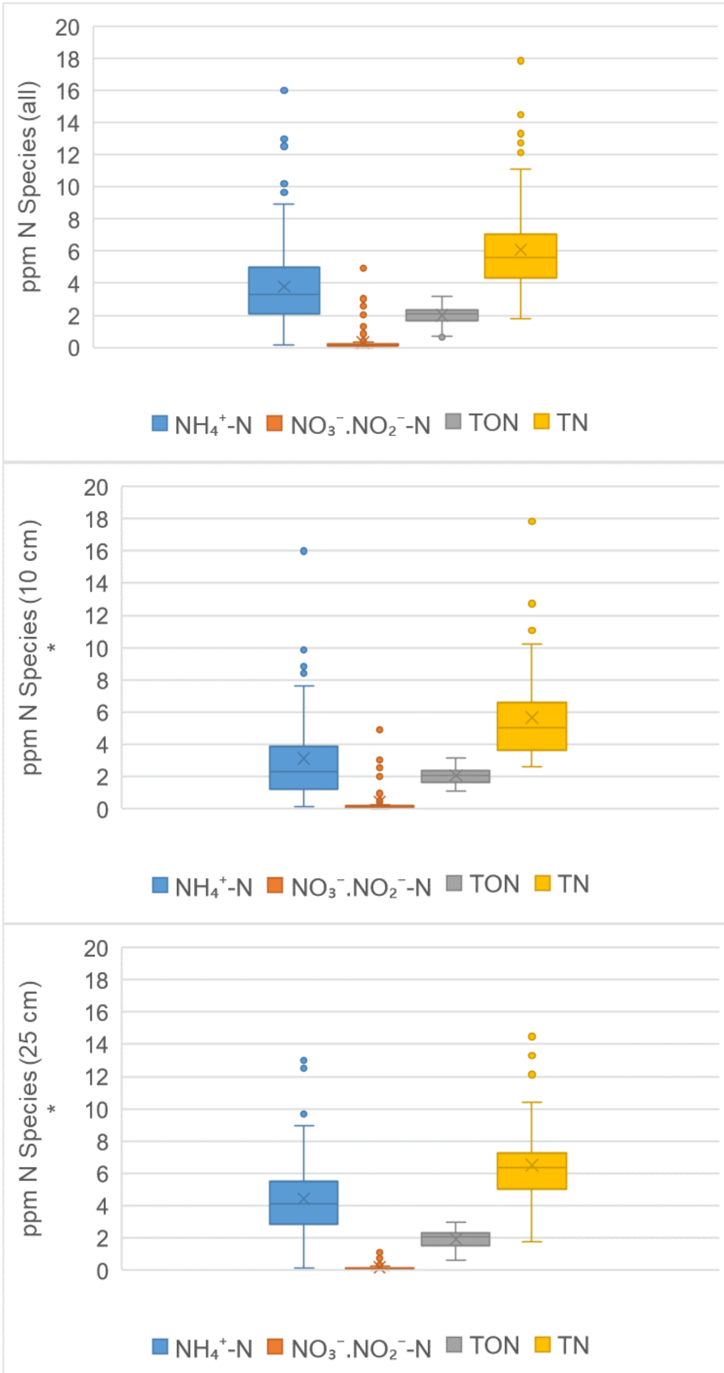

Figure 2.6. Variation of nitrogen species by depth of all seasons (summer21, fall21, spring22, summer22, fall22) and treatments (HighEAB, IntEAB, LowEAB, NoEAB) combined. Adding values from 10 cm and 25 cm will produce the results displayed in All Depth, All Season. The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value. Depth charts marked with an asterisk are significantly different from one another.

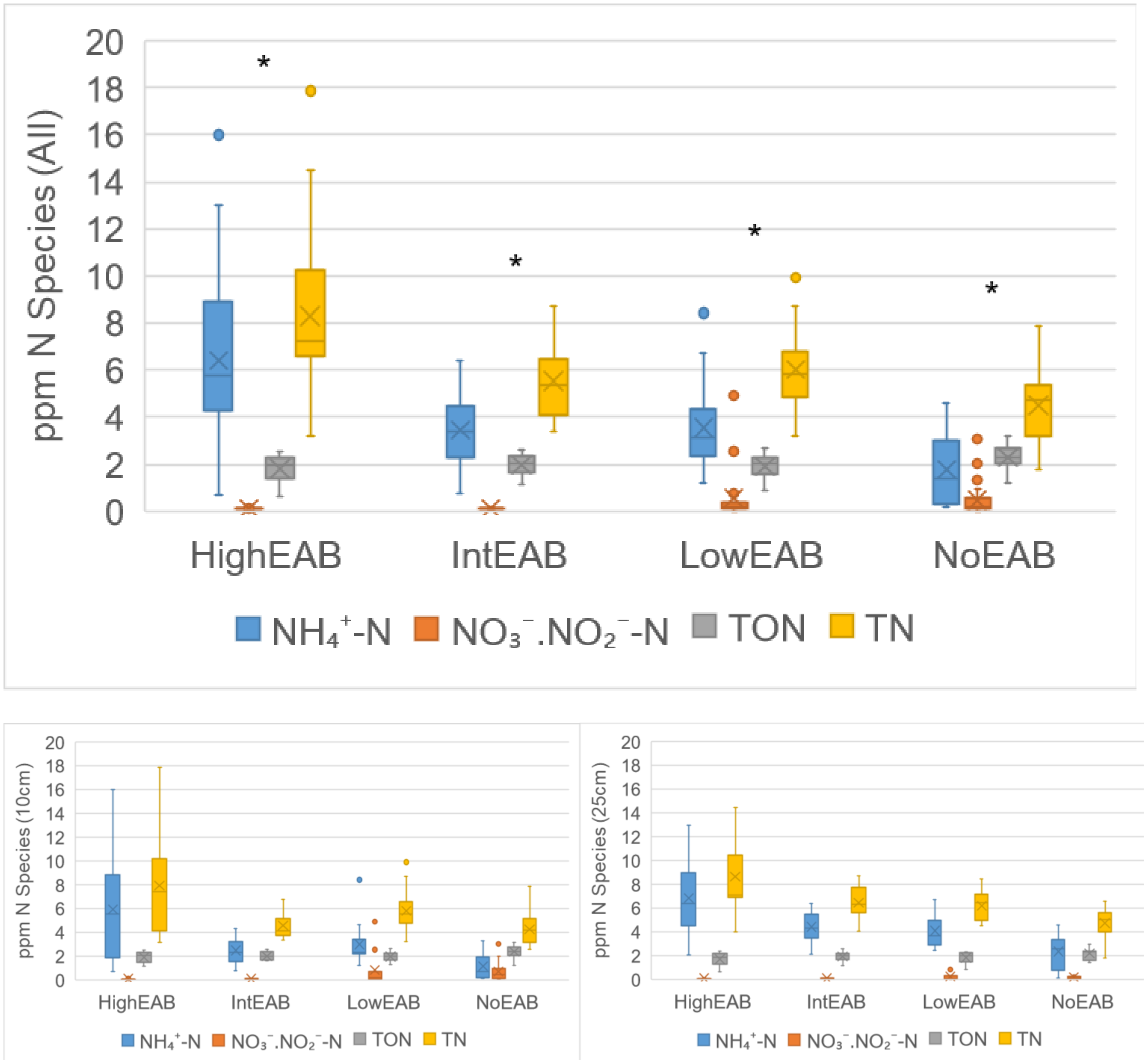

Figure 2.7. Variation of nitrogen species by depth of all seasons (summer21, fall21, spring22, summer22, fall22) and individual treatments (HighEAB, IntEAB, LowEAB, NoEAB). The sum of values in an individual depth across all treatment sites is equal to the corresponding individual season in Figure 2.6. The displays for 10 cm and 25 cm display their respective depths. The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value. EAB treatments marked with an asterisk are statistically significant when comparing nitrogen species means between depths.

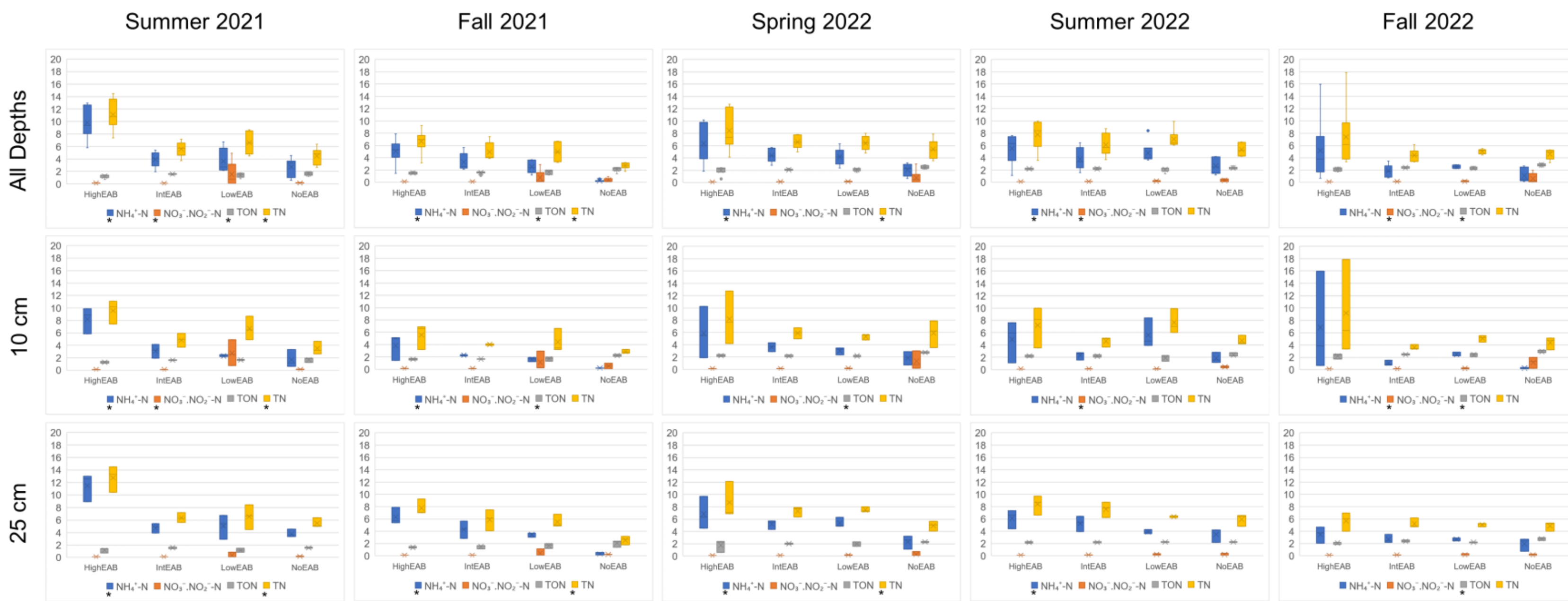

Figure 2.8. Variation in nitrogen species by treatment site, season, and depth across the duration of the study. A complete visual display. Significance values as reported in Table 2.3 are summarized in Table 2.4 with additional ANOVA *p* values. The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value. Legend labels marked with an asterisk are statistically significant when comparing values of nitrogen species across a specific depth for a specific season.

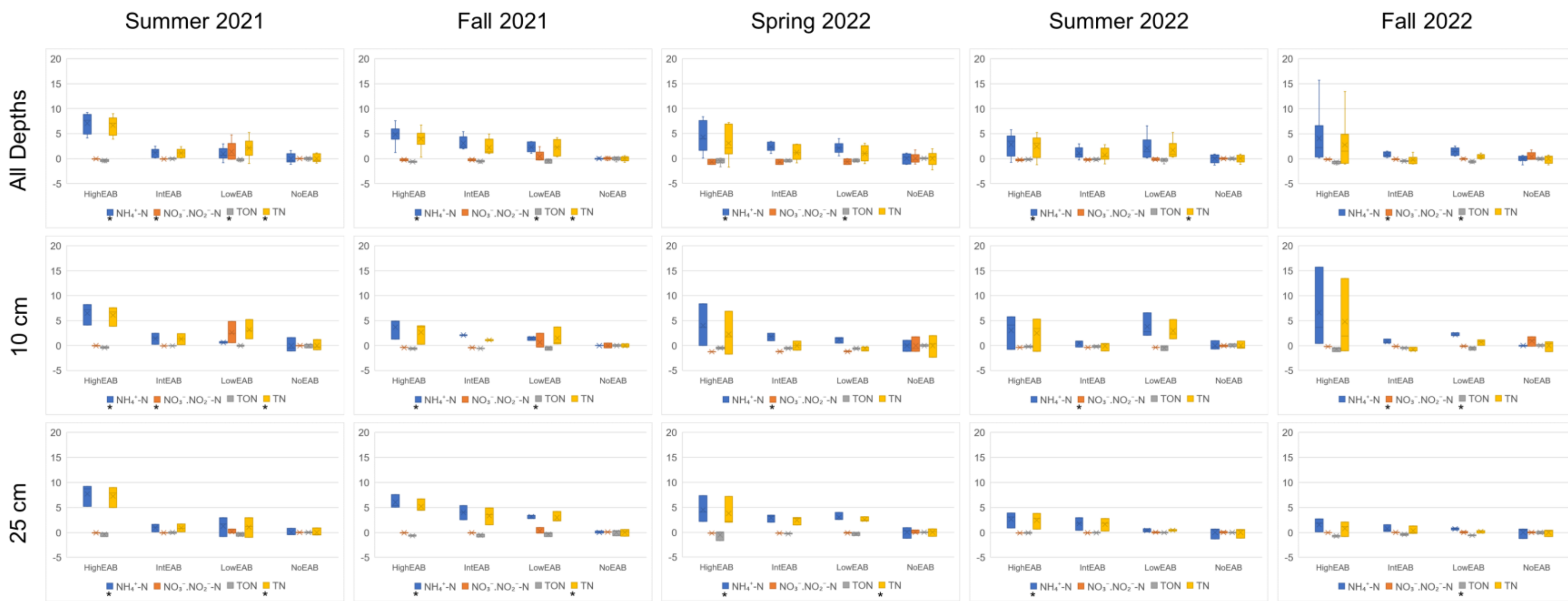

Figure 2.9. Variation in normalized nitrogen species values by treatment site, season, and depth across the duration of the study. Significance values are reported in Table 2.4, in the row titled Differences. The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value. Legend labels marked with an asterisk are statistically significant when comparing values of nitrogen species across a specific depth for a specific season.

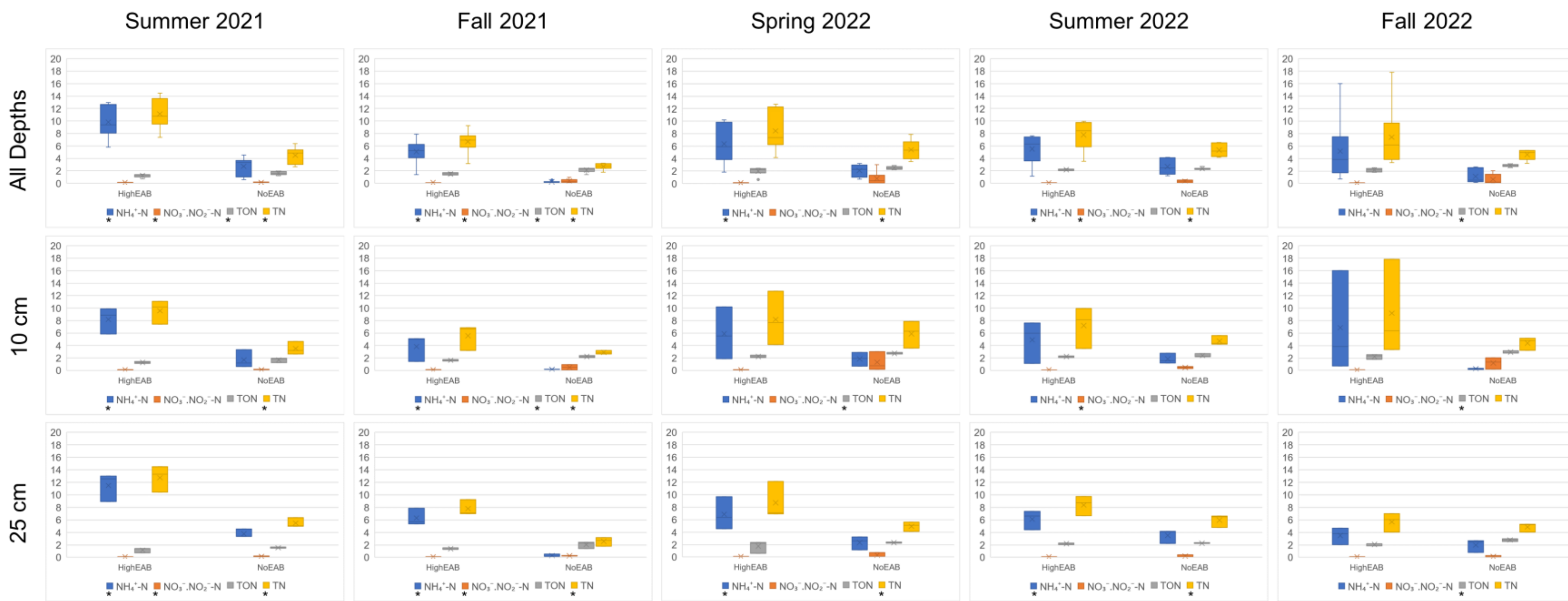

Figure 2.10. Variation in HighEAB and NoEAB nitrogen species values by treatment site, season, and depth across the duration of the study. Significance values are reported in Table 2.4, in the row entitled HighEAB v NoEAB. The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value. Legend labels marked with an asterisk are statistically significant when comparing values of nitrogen species across a specific depth for a specific season.

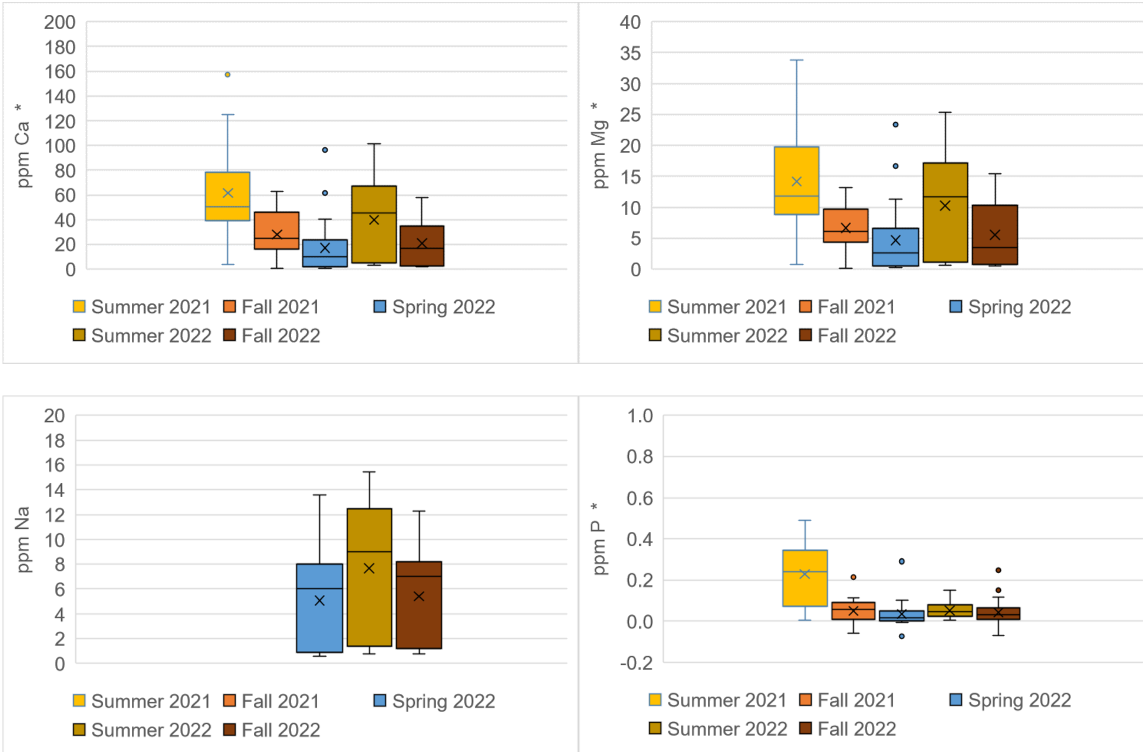

Figure 2.11. Total values of Ca, Mg, Na, and P across all depths by season. A combination of EAB infestation and hydrology (seasonal variation and drought stress) explained much of the variation between seasons and years. Na was only measured in 2022. The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value. Elements labeled with an asterisk are statistically different across seasons.

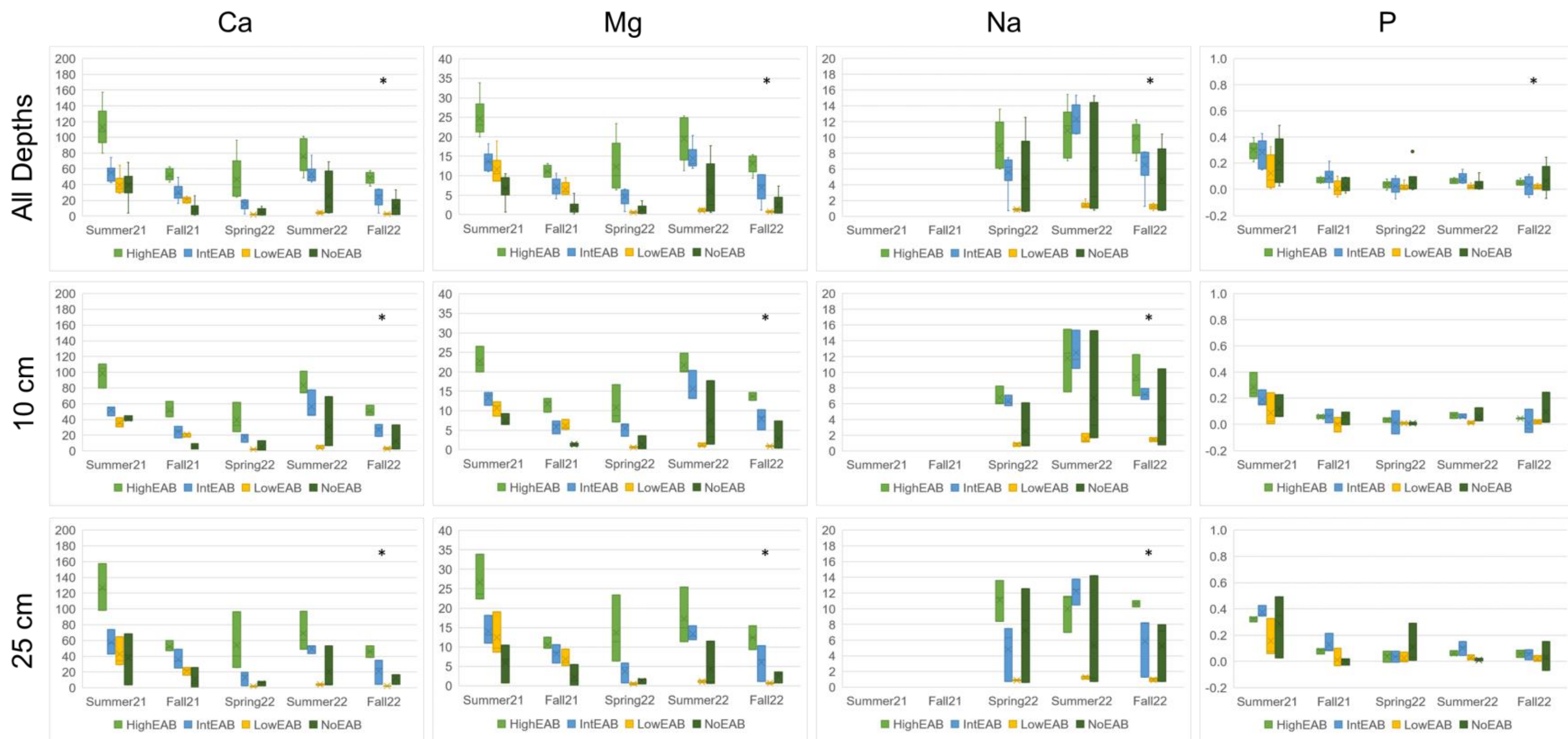

Figure 2.12. Display of Ca, Mg, Na, and P as they vary across treatment site(HighEAB, IntEAB, LowEAB, NoEAB) and season (summer21, fall21, spring22, summer22, fall22). The All Depths row combines both depth measurements (10cm and 25cm) while 10cm and 25cm represent their respective depth below the surface. The X related to a box and whisker plot represents the mean value and the horizontal bar in the interquartile range represents the median value. Displays marked with an asterisk are statistically significant within a treatment and comparing across seasons.

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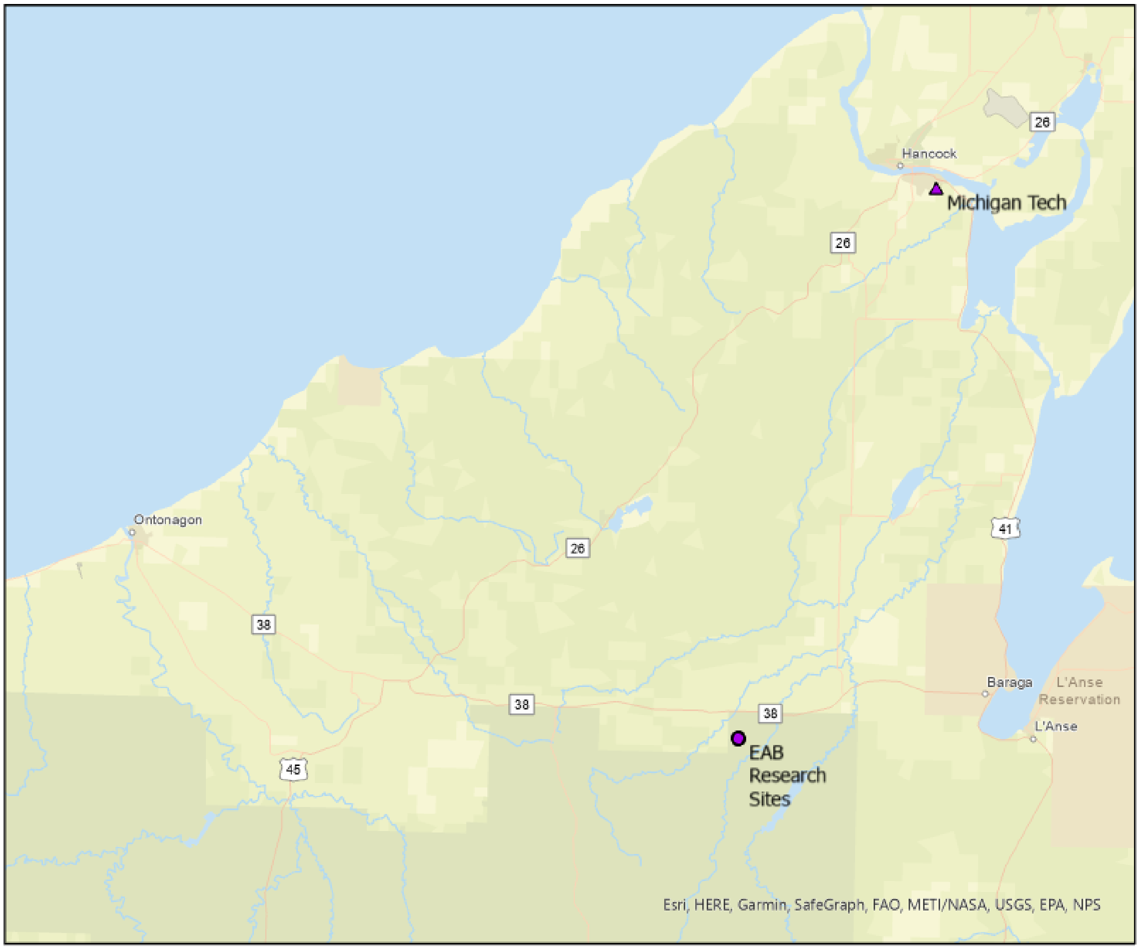
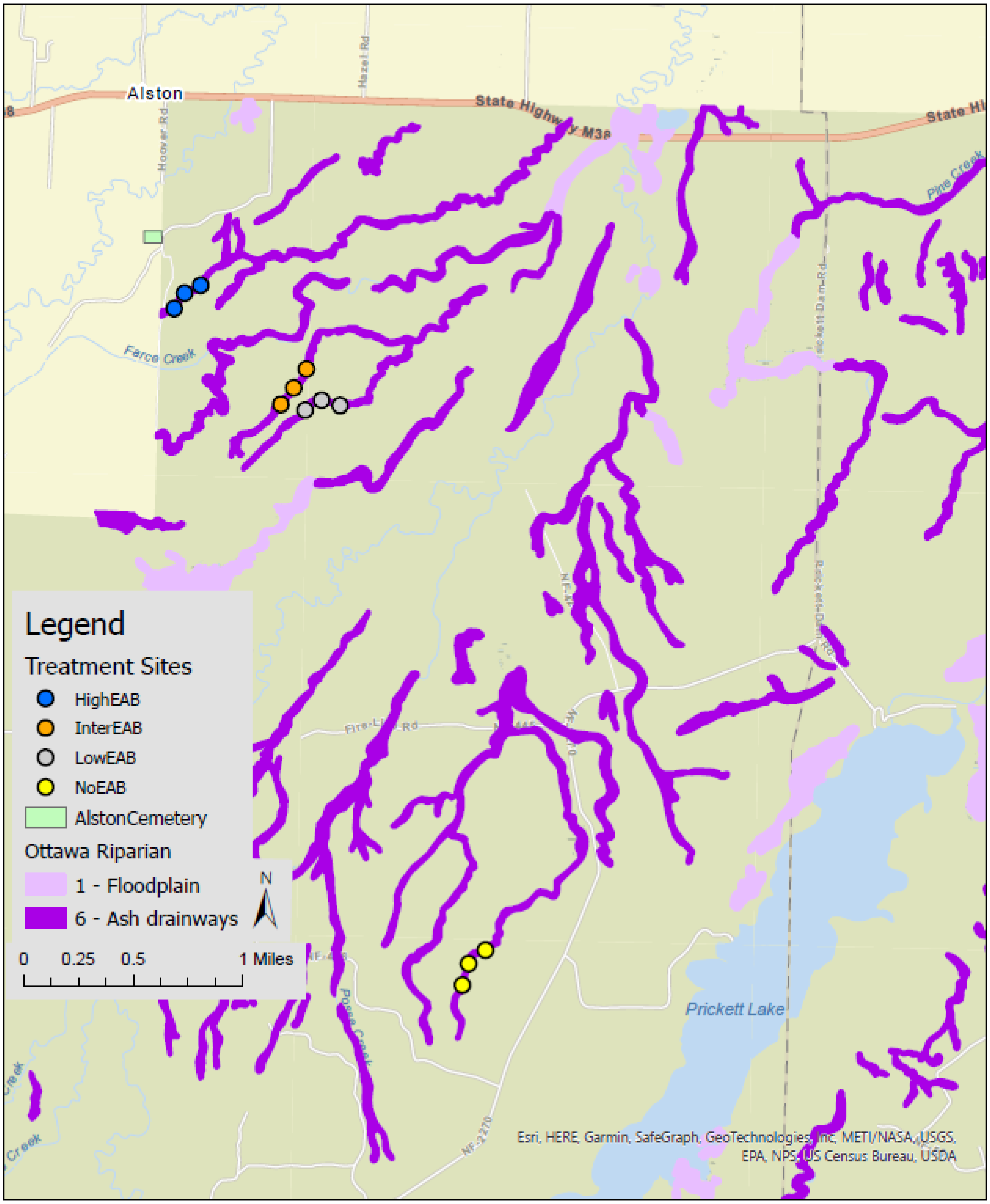
A Appendix

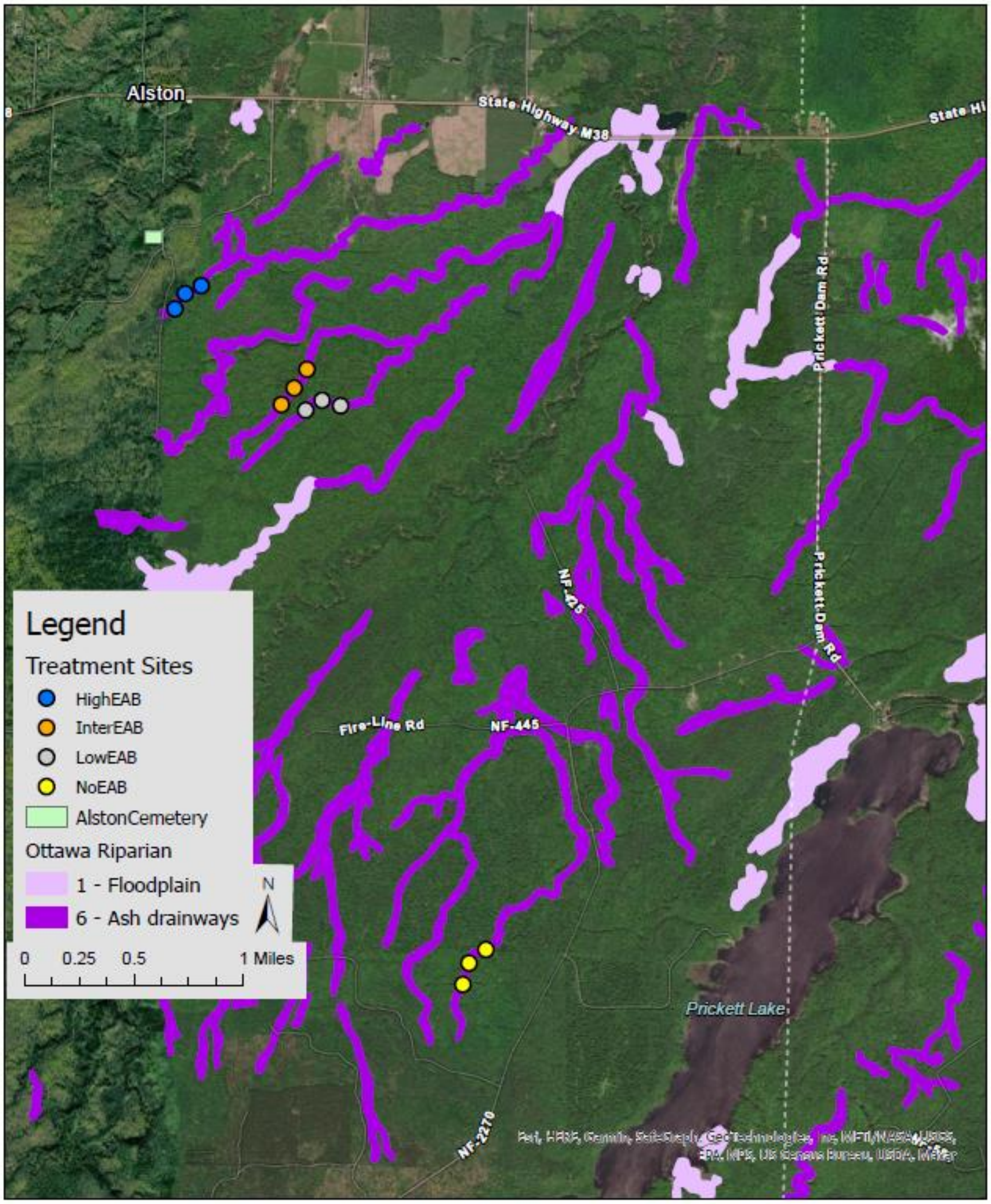
A.1 Maps



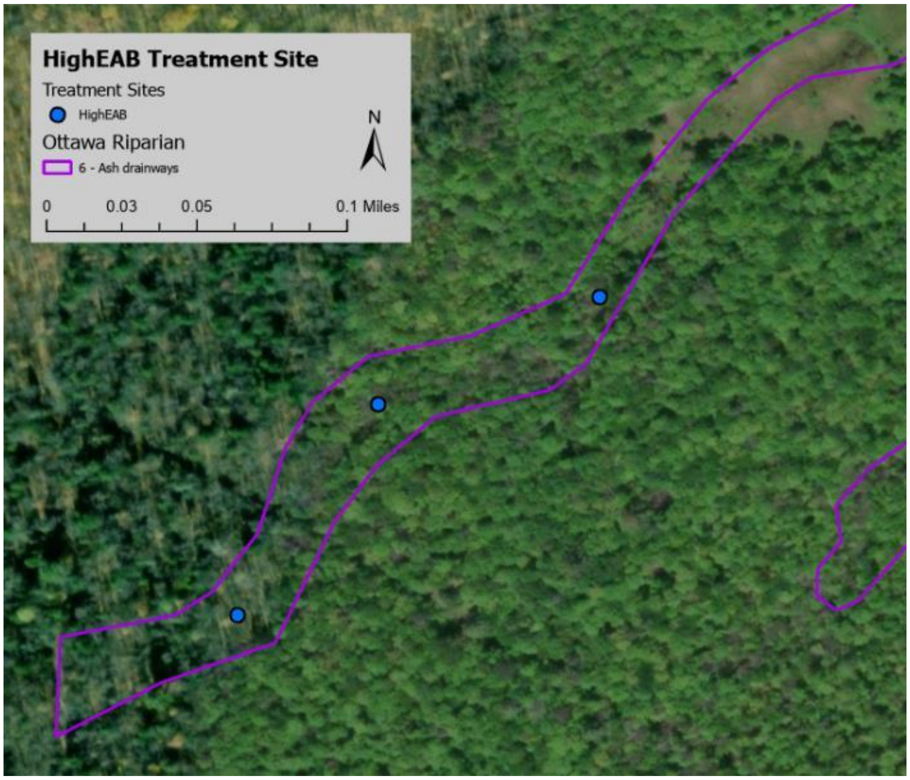
Map 1. Overview map showing location of EAB research sites in western Upper Peninsula, Michigan, USA. Sites are approximately 27 miles south of Michigan Tech (Houghton, Michigan, USA), 12 miles west of Baraga (Michigan, USA), and 28 miles east of Ontonagon (Michigan, USA).



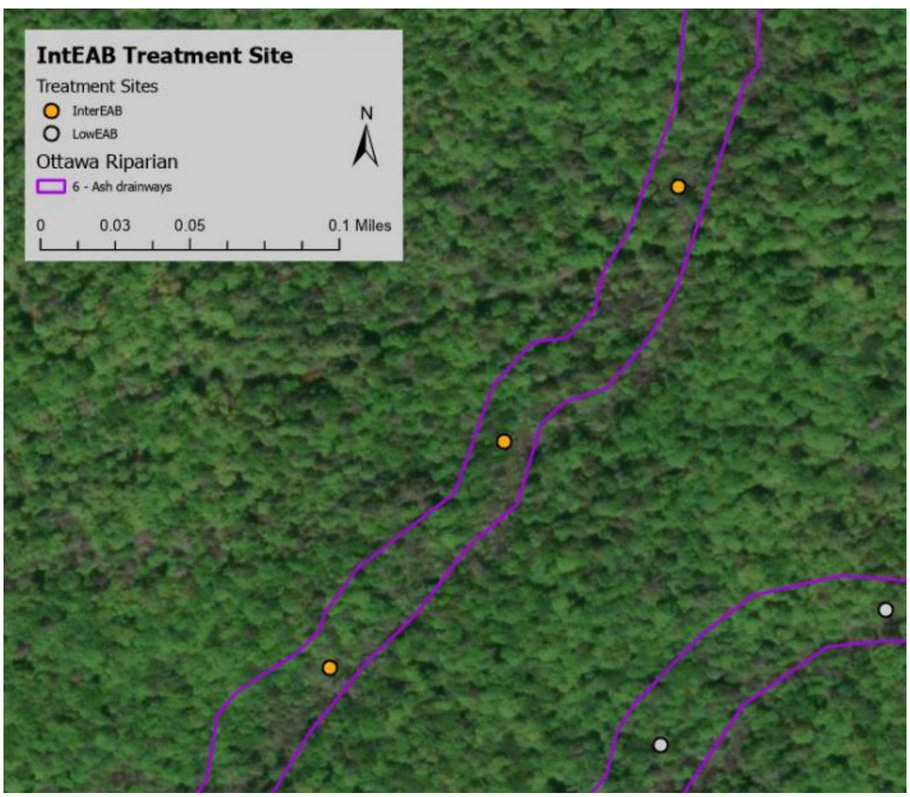
Map 2.1. Research treatment sites map near Alston, Michigan, USA. Sites are located in the SWSW, Sec. 6; NE, NWNW, Sec. 7; SWSE, Sec. 20; NWNE, Sec. 29, T. 50 N., R. 35 W., Houghton County, MICHIGAN MERIDIAN. EAB gradient moves from high EAB impact (HighEAB) in the northwest to intermediate EAB impact (IntEAB), low EAB impact (Low EAB), and finally no EAB impact (NoEAB) in the southeast.



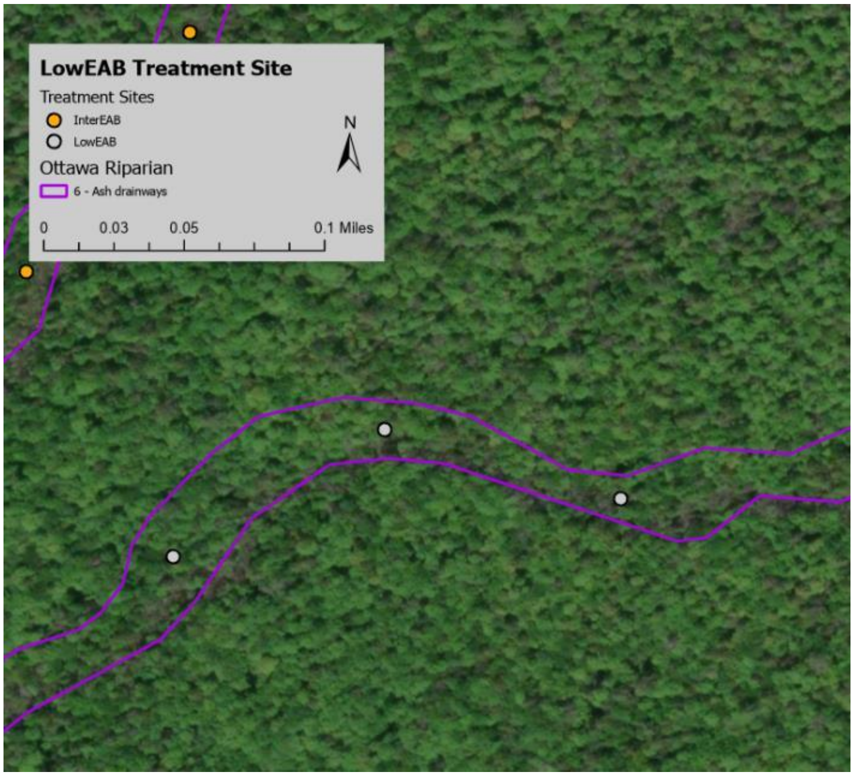
Map 2.2. Map 2. Research treatment sites satellite image map near Alston, Michigan, USA. Sites are located in the SWSW, Sec. 6; NE, NWNW, Sec. 7; SWSE, Sec. 20; NWNE, Sec. 29, T. 50 N., R. 35 W., Houghton County, MICHIGAN MERIDIAN. EAB gradient moves from high EAB impact (HighEAB) in the northwest to intermediate EAB impact (IntEAB), low EAB impact (Low EAB), and finally no EAB impact (NoEAB) in the southeast.



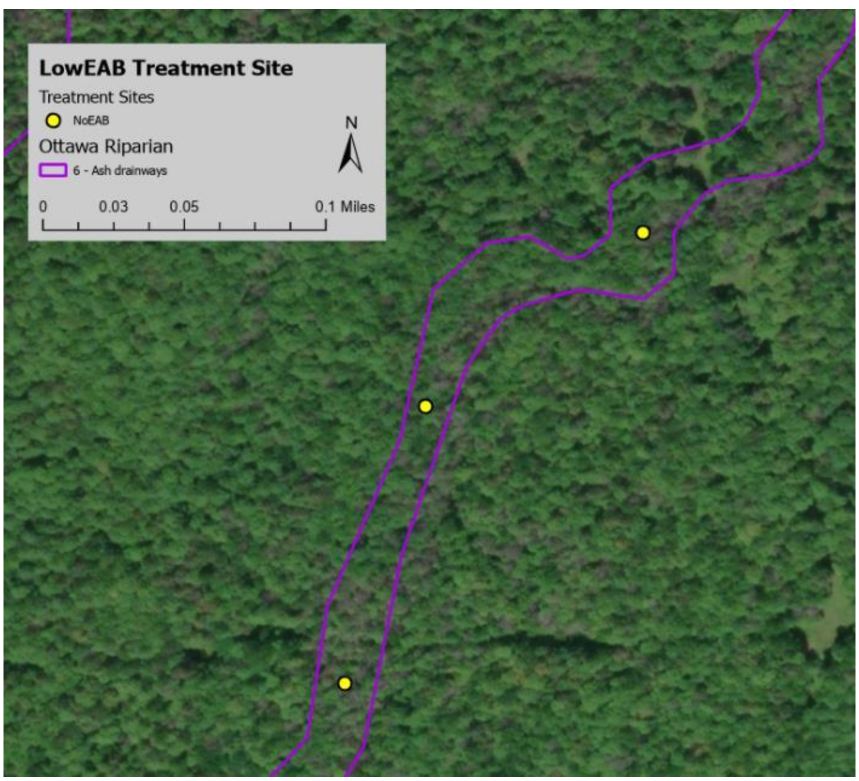
Map 3.1. HighEAB treatment sites.



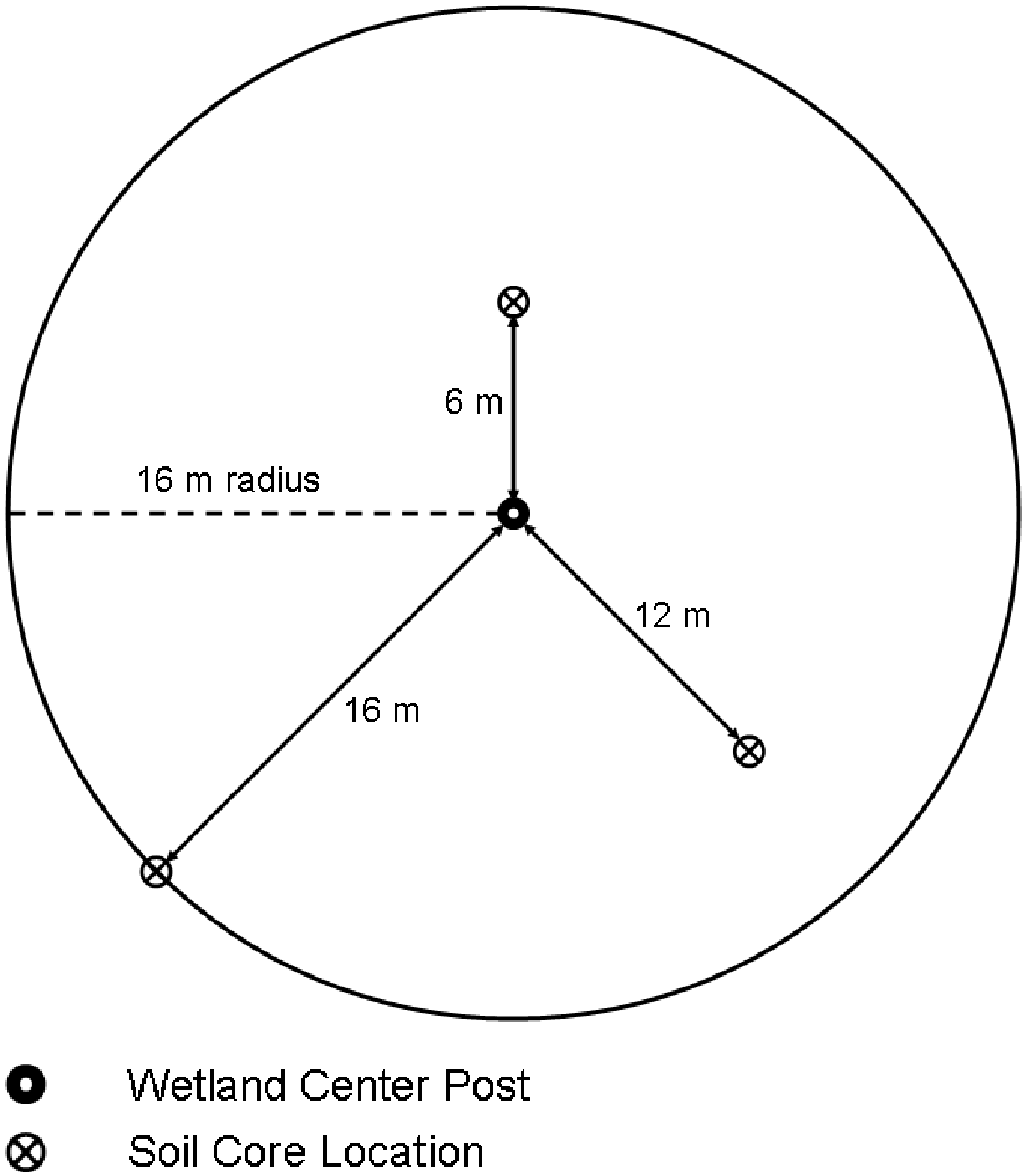
Map 3.2. IntEAB treatment sites.



Map 3.3. LowEAB treatment sites.



Map 3.4. NoEAB treatment sites. NoEAB treatment serves as the control.



Map 4. Treatment site sampling layout map. All overstory trees (>10 cm DBH) and woody shrubs(<10 cm DBH) within the 16 m radius plot were measured and counted. Soil cores were taken at 0°, 120°, and 240° at 6 m, 12 m, and 16 m distance from wetland center post, respectively, to 30 cm depth. Soil cores were separated into three depth ranges (0-10 cm, 10-20 cm, 20-30 cm).

A.2 Ash and EAB Symptom Field Identification


Photo II.1. Epicormic branching high in summer.


Photo II.2. Epicormic branching low in winter.


Photo II.3. Epicormic branching low in fall.


Photo II.4. Epicormic branching along tree trunk.

Photo II.5. Bark blonding high.

Photo II.6. Bark blonding low.


Photo II.7. Canopy dieback following EAB induced ash mortality.


Photo II.8. EAB exit holes in black ash.

Photo II.9. EAB feeding gallery.

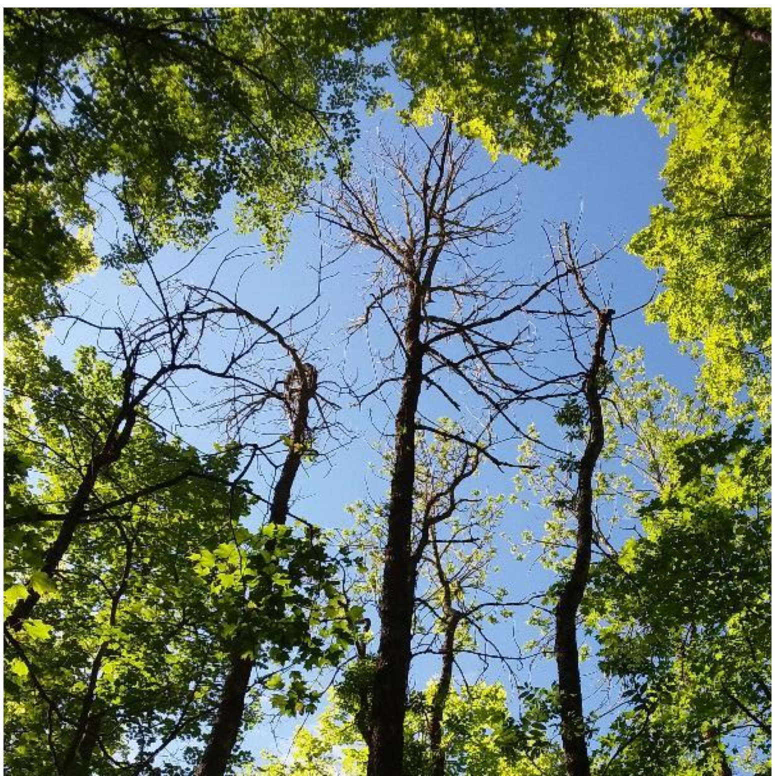


Photo II.10. Ash mortality following EAB infestation.

A.3 FS Permit

Authorization ID: ONT118
Contact Name: DAN BEYER
Expiration Date: **12/31/2025**
Use Code: 422

FS-2700-4 (VER. 03/17)
OMB 0596-0082

U.S. DEPARTMENT OF AGRICULTURE
FOREST SERVICE

SPECIAL USE PERMIT

Authority: ORGANIC ADMINISTRATION ACT June 4, 1897

DAN BEYER, of 219A MICHIGAN STREET, ONTONAGON, MI, 49953 (hereinafter "the holder") is authorized to use or occupy National Forest System lands in the OTTAWA NATIONAL FOREST subject to the terms and conditions of this special use permit (the permit).

This permit covers approx. 0.01 acres in:

SWSW, Sec. 6; NE, NWNW, Sec. 7; SWSE, Sec. 20; NWNE, Sec. 29, T. 50 N., R. 35 W., Houghton County, MICHIGAN MERIDIAN, ("the permit area"), as shown on the map attached as **Exhibit A**. This and any other appendices to this permit are hereby incorporated into this permit.

This permit issued for the purpose of:

Conducting research to determine the spread and progression of Emerald Ash Borer (EAB) from first detected sites on the Ottawa National Forest, by documenting vegetation changes and changes in nutrient availability using ion-exchange resin capsules.

Methodology
Vegetation plots with a 36-foot radius will be established at 12 sites on the Ontonagon District. Non-invasive sampling will be conducted within each plot to determine any changes and note the signs and symptoms of EAB as it spreads. Tree tags and rebar will be used to monument the plot centers.

At each plot, within a 2-foot diameter area, two 1.25" PVC tubes will be installed at depths of 10 and 25 cm. Ion-exchange resin capsules will be placed in the tubes, to assess nutrient availability in EAB infested black ash wetlands and will be replaced seasonally.

Sites will be accessed by foot. For the northern sites, access will be via Alston Cemetery Road, and for the southern sites, access will be from Prickett Dam Road.

TERMS AND CONDITIONS

I. GENERAL TERMS

A. AUTHORITY. This permit is issued pursuant to the Organic Administration Act of June 4, 1897and 36 CFR Part 251, Subpart B, as amended, and is subject to their provisions.

B. AUTHORIZED OFFICER. The authorized officer is the Forest or Grassland Supervisor or a subordinate officer with delegated authority.

C. **TERM.** This permit shall expire at midnight on **12/31/2025**.

D. CONTINUATION OF USE AND OCCUPANCY. This permit is not renewable. Prior to expiration of this permit, the holder may apply for a new permit for the use and occupancy authorized by this permit. Applications for a new permit must be submitted at least 6 months prior to expiration of this permit. Issuance of a new permit is at the sole discretion of the authorized officer. At a minimum, before issuing a new permit, the authorized officer shall ensure that (1) the use and occupancy to be authorized by the new permit is consistent with the standards and guidelines in the applicable land management plan; (2) the type of use and occupancy to be authorized by the new permit is the same as the type of use and occupancy authorized by this permit; and (3) the holder is in compliance with all the terms of this permit. The authorized officer may prescribe new terms and conditions when a new permit is issued.

E. AMENDMENT. This permit may be amended in whole or in part by the Forest Service when, at the discretion of the authorized officer, such action is deemed necessary or desirable to incorporate new terms that may be required by law, regulation, directive, the applicable forest land and resource management plan, or projects and activities implementing a land management plan pursuant to 36 CFR Part 215.

F. COMPLIANCE WITH LAWS, REGULATIONS, AND OTHER LEGAL REQUIREMENTS. In exercising the rights and privileges granted by this permit, the holder shall comply with all present and future federal laws and regulations and all present and future state, county, and municipal laws, regulations, and other legal requirements that apply to the permit area, to the extent they do not conflict with federal law, regulation, or policy. The Forest Service assumes no responsibility for enforcing laws, regulations, and other legal requirements that fall under the jurisdiction of other governmental entities.

G. NON-EXCLUSIVE USE. The use or occupancy authorized by this permit is not exclusive. The Forest Service reserves the right of access to the permit area, including a continuing right of physical entry to the permit area for inspection, monitoring, or any other purpose consistent with any right or obligation of the United States under any law or regulation. The Forest Service reserves the right to allow others to use the permit area in any way that is not inconsistent with the holder's rights and privileges under this permit, after consultation with all parties involved. Except for any restrictions that the holder and the authorized officer agree are necessary to protect the installation and operation of authorized temporary improvements, the lands and waters covered by this permit shall remain open to the public for all lawful purposes.

H. ASSIGNABILITY. This permit is not assignable or transferable.

II.IMPROVEMENTS

A. LIMITATIONS ON USE. Nothing in this permit gives or implies permission to build or maintain any structure or facility or to conduct any activity, unless specifically authorized by this permit. Any use not specifically authorized by this permit must be proposed in accordance with 36 CFR 251.54. Approval of such a proposal through issuance of a new permit or permit amendment is at the sole discretion of the authorized officer.

B. PLANS. All plans for development, layout, construction, reconstruction, or alteration of improvements in the permit area, as well as revisions to those plans must be prepared by a professional engineer, architect, landscape architect, or other qualified professional based on federal employment standards acceptable to the authorized officer. These plans and plan revisions must have written approval from the authorized officer before they are implemented. The authorized officer may require the holder to furnish as-built plans, maps, or surveys upon completion of the work.

C. CONSTRUCTION. Any construction authorized by this permit shall commence by N/A and shall be completed by N/A.

III. OPERATIONS.

A. PERIOD OF USE. Use or occupancy of the permit area shall be exercised at least 1 day each year.

B. CONDITION OF OPERATIONS. The holder shall maintain the authorized improvements and permit area to standards of repair, orderliness, neatness, sanitation, and safety acceptable to the authorized officer and consistent with other provisions of this permit. Standards are subject to periodic change by the authorized officer when deemed necessary to meet statutory, regulatory, or policy requirements or to protect national forest resources. The holder shall comply with inspection requirements deemed appropriate by the authorized officer.

C. MONITORING BY THE FOREST SERVICE. The Forest Service shall monitor the holder's operations and reserves the right to inspect the permit area and transmission facilities at any time for compliance with the terms of this permit. The holder shall comply with inspection requirements deemed appropriate by the authorized officer. The holder's obligations under this permit are not contingent upon any duty of the Forest Service to inspect the permit area or transmission facilities. A failure by the Forest Service or other governmental officials to inspect is not a justification for noncompliance with any of the terms and conditions of this permit.

IV. RIGHTS AND LIABILITIES

A. LEGAL EFFECT OF THE PERMIT. This permit, which is revocable and terminable, is not a contract or a lease, but rather a federal license. The benefits and requirements conferred by this authorization are reviewable solely under the procedures set forth in 36 CFR 214 and 5 U.S.C. 704. This permit does not constitute a contract for purposes of the Contract Disputes Act, 41 U.S.C. 601. The permit is not real property, does not convey any interest in real property, and may not be used as collateral for a loan.

B. VALID EXISTING RIGHTS. This permit is subject to all valid existing rights. Valid existing rights include those derived under mining and mineral leasing laws of the United States. The United States is not liable to the holder for the exercise of any such right.

C. ABSENCE OF THIRD-PARTY BENEFICIARY RIGHTS. The parties to this permit do not intend to confer any rights on any third party as a beneficiary under this permit.

D. SERVICES NOT PROVIDED. This permit does not provide for the furnishing of road or trail maintenance, water, fire protection, search and rescue, or any other such service by a government agency, utility, association, or individual.

E. RISK OF LOSS. The holder assumes all risk of loss associated with use or occupancy of the permit area, including but not limited to theft, vandalism, fire and any fire-fighting activities (including prescribed burns), avalanches, rising waters, winds, falling limbs or trees, and other forces of nature. If authorized temporary improvements in the permit area are destroyed or substantially damaged, the authorized officer shall conduct an analysis to determine whether the improvements can be safely occupied in the future and whether rebuilding should be allowed. If rebuilding is not allowed, the permit shall terminate.

F. DAMAGE TO UNITED STATES PROPERTY. The holder has an affirmative duty to protect from damage the land, property, and other interests of the United States. Damage includes but is not limited to fire suppression costs and damage to government-owned improvements covered by this permit.

1. The holder shall be liable for all injury, loss, or damage, including fire suppression, prevention and control of the spread of invasive species, or other costs in connection with rehabilitation or restoration of natural resources resulting from the use or occupancy authorized by this permit. Compensation shall include but not be limited to the value of resources damaged or destroyed, the costs of restoration, cleanup, or other mitigation, fire suppression or other types of abatement costs, and all administrative, legal (including attorney's fees), and other costs. Such costs may be deducted from a performance bond required under clause IV.J.

2. The holder shall be liable for damage caused by use of the holder or the holder's heirs, assigns, agents,

employees, contractors, or lessees to all roads and trails of the United States to the same extent as provided under clause IV.F.1, except that liability shall not include reasonable and ordinary wear and tear.

G. <u>HEALTH AND SAFETY</u>. The holder shall take all measures necessary to protect the health and safety of all persons affected by the use and occupancy authorized by this permit. The holder shall promptly abate as completely as possible and in compliance with all applicable laws and regulations any physical or mechanical procedure, activity, event, or condition existing or occurring in connection with the authorized use and occupancy during the term of this permit that causes or threatens to cause a hazard to the health or safety of the public or the holder's employees or agents. The holder shall as soon as practicable notify the authorized officer of all serious accidents that occur in connection with these procedures, activities, events, or conditions. The Forest Service has no duty under the terms of this permit to inspect the permit area or operations of the holder for hazardous conditions or compliance with health and safety standards.

H. <u>ENVIRONMENTAL PROTECTION</u>.

1. For purposes of clause IV.H and section V, "hazardous material" shall mean (a) any hazardous substance under section 101(14) of the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), 42 U.S.C. 9601(14); (b) any pollutant or contaminant under section 101(33) of CERCLA, 42 U.S.C. 9601(33); (c) any petroleum product or its derivative, including fuel oil, and waste oils; and (d) any hazardous substance, extremely hazardous substance, toxic substance, hazardous waste, ignitable, reactive or corrosive materials, pollutant, contaminant, element, compound, mixture, solution or substance that may pose a present or potential hazard to human health or the environment under any applicable environmental laws.

2. The holder shall avoid damaging or contaminating the environment, including but not limited to the soil, vegetation (such as trees, shrubs, and grass), surface water, and groundwater, during the holder's use and occupancy of the permit area. Environmental damage includes but is not limited to all costs and damages associated with or resulting from the release or threatened release of a hazardous material occurring during or as a result of activities of the holder or the holder's heirs, assigns, agents, employees, contractors, or lessees on, or related to, the lands, property, and other interests covered by this permit. If the environment or any government property covered by this permit becomes damaged in connection with the holder's use and occupancy, the holder shall as soon as practicable repair the damage or replace the damaged items to the satisfaction of the authorized officer and at no expense to the United States.

3. The holder shall as soon as practicable, as completely as possible, and in compliance with all applicable laws and regulations abate any physical or mechanical procedure, activity, event, or condition existing or occurring in connection with the authorized use and occupancy during or after the term of this permit that causes or threatens to cause harm to the environment, including areas of vegetation or timber, fish or other wildlife populations, their habitats, or any other natural resources.

I. <u>INDEMNIFICATION OF THE UNITED STATES</u>. The holder shall indemnify, defend, and hold harmless the United States for any costs, damages, claims, liabilities, and judgments arising from past, present, and future acts or omissions of the holder in connection with the use or occupancy authorized by this permit. This indemnification provision includes but is not limited to acts and omissions of the holder or the holder's heirs, assigns, agents, employees, contractors, or lessees in connection with the use or occupancy authorized by this permit which result in (1) violations of any laws and regulations which are now or which may in the future become applicable; (2) judgments, claims, demands, penalties, or fees assessed against the United States; (3) costs, expenses, and damages incurred by the United States; or (4) the release or threatened release of any solid waste, hazardous waste, hazardous materials, pollutant, contaminant, oil in any form, or petroleum product into the environment. The authorized officer may prescribe terms that allow the holder to replace, repair, restore, or otherwise undertake necessary curative actions to mitigate damages in addition to or as an alternative to monetary indemnification.

J. <u>BONDING</u>. The authorized officer may require the holder to furnish a surety bond or other security for any of the obligations imposed by the terms and conditions of this permit or any applicable law, regulation, or order.

V. RESOURCE PROTECTION

A. COMPLIANCE WITH ENVIRONMENTAL LAWS. The holder shall in connection with the use or occupancy authorized by this permit comply with all applicable federal, state, and local environmental laws and regulations, including but not limited to those established pursuant to the Resource Conservation and Recovery Act, as amended, 42 U.S.C. 6901 et seq., the Federal Water Pollution Control Act, as amended, 33 U.S.C. 1251 et seq., the Oil Pollution Act, as amended, 33 U.S.C. 2701 et seq., the Clean Air Act, as amended, 42 U.S.C. 7401 et seq., CERCLA, as amended, 42 U.S.C. 9601 et seq., the Toxic Substances Control Act, as amended, 15 U.S.C. 2601 et seq., the Federal Insecticide, Fungicide, and Rodenticide Act, as amended, 7 U.S.C. 136 et seq., and the Safe Drinking Water Act, as amended, 42 U.S.C. 300f et seq.

B. VANDALISM. The holder shall take reasonable measures to prevent and discourage vandalism and disorderly conduct and when necessary shall contact the appropriate law enforcement officer.

C. PESTICIDE USE.

1. Authorized Officer Concurrence. Pesticides may not be used outside of buildings in the permit area to control pests, including undesirable woody and herbaceous vegetation (including aquatic plants), insects, birds, rodents, or fish without prior written concurrence of the authorized officer. Only those products registered or otherwise authorized by the U.S. Environmental Protection Agency and appropriate State authority for the specific purpose planned shall be authorized for use within areas on National Forest System lands.

2. Pesticide-Use Proposal. Requests for concurrence of any planned uses of pesticides shall be provided in advance using the Pesticide-Use Proposal (form FS-2100-2). Annually the holder shall, on the due date established by the authorized officer, submit requests for any new, or continued, pesticide usage. The Pesticide-Use Proposal shall cover a 12-month period of planned use. The Pesticide-Use Proposal shall be submitted at least 60 days in advance of pesticide application. Information essential for review shall be provided in the form specified. Exceptions to this schedule may be allowed, subject to emergency request and approval, only when unexpected outbreaks of pests require control measures which were not anticipated at the time a Pesticide-Use Proposal was submitted.

3. Labeling, Laws, and Regulations. Label instructions and all applicable laws and regulations shall be strictly followed in the application of pesticides and disposal of excess materials and containers. No pesticide waste, excess materials, or containers shall be disposed of in any area administered by the Forest Service.

D. ARCHAEOLOGICAL-PALEONTOLOGICAL DISCOVERIES. The holder shall immediately notify the authorized officer of all antiquities or other objects of historic or scientific interest, including but not limited to historic or prehistoric ruins, fossils, or artifacts discovered in connection with the use and occupancy authorized by this permit. The holder shall follow the applicable inadvertent discovery protocols for the undertaking provided in an agreement executed pursuant to section 106 of the National Historic Preservation Act, 54 U.S.C. 306108; if there are no such agreed-upon protocols, the holder shall leave these discoveries intact and in place until consultation has occurred, as informed, if applicable, by any programmatic agreement with tribes. Protective and mitigation measures developed under this clause shall be the responsibility of the holder. However, the holder shall give the authorized officer written notice before implementing these measures and shall coordinate with the authorized officer for proximate and contextual discoveries extending beyond the permit area.

E. NATIVE AMERICAN GRAVES PROTECTION AND REPATRIATION ACT (NAGPRA). In accordance with 25 U.S.C. 3002(d) and 43 CFR 10.4, if the holder inadvertently discovers human remains, funerary objects, sacred objects, or objects of cultural patrimony on National Forest System lands, the holder shall immediately cease work in the area of the discovery and shall make a reasonable effort to protect and secure the items. The holder shall follow the applicable NAGPRA protocols for the undertaking provided in the NAGPRA plan of action or the NAGPRA comprehensive agreement; if there are no such agreed-upon protocols, the holder shall as soon as practicable notify the authorized officer of the discovery and shall follow up with written confirmation of the discovery. The activity that resulted in the inadvertent discovery may not resume until 30 days after the forest archaeologist certifies receipt of the written confirmation, if resumption of the activity is otherwise lawful, or at any time if a binding written agreement has been executed between the Forest Service and the affiliated Indian tribes that adopts a recovery plan for the human remains and objects.

F. PROTECTION OF THREATENED AND ENDANGERED SPECIES, SENSITIVE SPECIES, AND SPECIES OF CONSERVATION CONCERN AND THEIR HABITAT.

1. Threatened and Endangered Species and Their Habitat. The location of sites within the permit area needing special measures for protection of plants or animals listed as threatened or endangered under the Endangered Species Act (ESA) of 1973, 16 U.S.C. 1531 et seq., as amended, or within designated critical habitat shall be shown on a map in an appendix to this permit and may be shown on the ground. The holder shall take any protective and mitigation measures specified by the authorized officer as necessary and appropriate to avoid or reduce effects on listed species or designated critical habitat affected by the authorized use and occupancy. Discovery by the holder or the Forest Service of other sites within the permit area containing threatened or endangered species or designated critical habitat not shown on the map in the appendix shall be promptly reported to the other party and shall be added to the map.

2. Sensitive Species and Species of Conservation Concern and Their Habitat. The location of sites within the permit area needing special measures for protection of plants or animals designated by the Regional Forester as sensitive species or as species of conservation concern pursuant to FSM 2670 shall be shown on a map in an appendix to this permit and may be shown on the ground. The holder shall take any protective and mitigation measures specified by the authorized officer as necessary and appropriate to avoid or reduce effects on sensitive species or species of conservation concern or their habitat affected by the authorized use and occupancy. Discovery by the holder or the Forest Service of other sites within the permit area containing sensitive species or species of conservation concern or their habitat not shown on the map in the appendix shall be promptly reported to the other party and shall be added to the map.

G. CONSENT TO STORE HAZARDOUS MATERIALS. The holder shall not store any hazardous materials at the site without prior written approval from the authorized officer. This approval shall not be unreasonably withheld. If the authorized officer provides approval, this permit shall include, or in the case of approval provided after this permit is issued, shall be amended to include specific terms addressing the storage of hazardous materials, including the specific type of materials to be stored, the volume, the type of storage, and a spill plan. Such terms shall be proposed by the holder and are subject to approval by the authorized officer.

H. CLEANUP AND REMEDIATION.

1. The holder shall immediately notify all appropriate response authorities, including the National Response Center and the authorized officer or the authorized officer's designated representative, of any oil discharge or of the release of a hazardous material in the permit area in an amount greater than or equal to its reportable quantity, in accordance with 33 CFR Part 153, Subpart B, and 40 CFR Part 302. For the purposes of this requirement, "oil" is as defined by section 311(a)(1) of the Clean Water Act, 33 U.S.C. 1321(a)(1). The holder shall immediately notify the authorized officer or the authorized officer's designated representative of any release or threatened release of any hazardous material in or near the permit area which may be harmful to public health or welfare or which may adversely affect natural resources on federal lands.

2. Except with respect to any federally permitted release as that term is defined under Section 101(10) of CERCLA, 42 U.S.C. 9601(10), the holder shall clean up or otherwise remediate any release, threat of release, or discharge of hazardous materials that occurs either in the permit area or in connection with the holder's activities in the permit area, regardless of whether those activities are authorized under this permit. The holder shall perform cleanup or remediation immediately upon discovery of the release, threat of release, or discharge of hazardous materials. The holder shall perform the cleanup or remediation to the satisfaction of the authorized officer and at no expense to the United States. Upon revocation or termination of this permit,

the holder shall deliver the site to the Forest Service free and clear of contamination.

VI. LAND USE FEE AND DEBT COLLECTION

A. LAND USE FEES. The use or occupancy authorized by this permit is exempt from a land use fee or the land use fee has been waived in full pursuant to 36 CFR 251.57 and Forest Service Handbook 2709.11, Chapter 30.

VII. REVOCATION, SUSPENSION, AND TERMINATION

A. REVOCATION AND SUSPENSION. The authorized officer may revoke or suspend this permit in whole or in part:

1. For noncompliance with federal, state, or local law.

2. For noncompliance with the terms of this permit.

3. For abandonment or other failure of the holder to exercise the privileges granted.

4. With the consent of the holder.

5. For specific and compelling reasons in the public interest.

Prior to revocation or suspension, other than immediate suspension under clause VII.B, the authorized officer shall give the holder written notice of the grounds for revocation or suspension and a reasonable period, typically not to exceed 90 days, to cure any noncompliance.

B. IMMEDIATE SUSPENSION. The authorized officer may immediately suspend this permit in whole or in part when necessary to protect public health or safety or the environment. The suspension decision shall be in writing. The holder may request an on-site review with the authorized officer's supervisor of the adverse conditions prompting the suspension. The authorized officer's supervisor shall grant this request within 48 hours. Following the on-site review, the authorized officer's supervisor shall promptly affirm, modify, or cancel the suspension.

C. APPEALS AND REMEDIES. Written decisions by the authorized officer relating to administration of this permit are subject to administrative appeal pursuant to 36 CFR Part 214, as amended. Revocation or suspension of this permit shall not give rise to any claim for damages by the holder against the Forest Service.

D. TERMINATION. This permit shall terminate when by its terms a fixed or agreed upon condition, event, or time occurs without any action by the authorized officer. Examples include but are not limited to expiration of the permit by its terms on a specified date and termination upon change of control of the business entity. Termination of this permit shall not require notice, a decision document, or any environmental analysis or other documentation. Termination of this permit is not subject to administrative appeal and shall not give rise to any claim for damages by the holder against the Forest Service.

E. RIGHTS AND RESPONSIBILITIES UPON REVOCATION OR TERMINATION WITHOUT ISSUANCE OF A NEW PERMIT. Upon revocation or termination of this permit without issuance of a new permit, the holder shall remove all structures and improvements, except those owned by the United States, within a reasonable period prescribed by the authorized officer and shall restore the site to the satisfaction of the authorized officer. If the holder fails to remove all structures and improvements within the prescribed period, they shall become the property of the United States and may be sold, destroyed, or otherwise disposed of without any liability to the United States. However, the holder shall remain liable for all costs associated with their removal, including costs of sale and impoundment, cleanup, and restoration of the site.

VIII. MISCELLANEOUS PROVISIONS

A. MEMBERS OF CONGRESS. No member of or delegate to Congress or resident commissioner shall benefit from this permit either directly or indirectly, except to the extent the authorized use provides a general benefit to a corporation.

B. CURRENT ADDRESSES. The holder and the Forest Service shall keep each other informed of current mailing addresses, including those necessary for billing and payment of land use fees.

C. SUPERIOR CLAUSES. If there is a conflict between any of the preceding printed clauses and any of the following clauses, the preceding printed clauses shall control.

D. Invasive Species Prevention and Control (R9-D1). The holder shall be responsible for the prevention and control of noxious weeds and invasive species arising from the authorized use. For the purpose of this clause, noxious weeds and invasive species include those species recognized as such by the Ottawa National Forest. When determined to be necessary by the authorizing officer, the holder shall develop a plan for noxious weed and invasive species prevention and control. Such plans must have prior written approval from the authorizing official and upon approval, shall be attached to the permit as an appendix.

E. Suspension of Privileges (R9-E1). Use of this authorization may be suspended by the Forest Service in whole or in part for breach of any stipulation contained within. Continued use of the authorized area, privileges, or facilities thereon during suspension may result in termination or revocation of the authorization.

F. Operation and Management Plans (R9-X2). The attached operation and/or management (maintenance) plan (**Exhibit B**), when currently approved by the authorized officer will become a part of this authorization. Its terms and conditions are binding on the permittee/grantee.

G. Removal and Planting of Vegetation and Other Resources (D-5). This permit does not authorize the cutting of timber or other vegetation. Trees or shrubbery may be removed or destroyed only after the Authorized Officer or the Authorized Officer's designated representative has approved in writing and marked or otherwise identified what may be removed or destroyed. Timber cut or destroyed shall be paid for at current stumpage rates for similar timber in the Ottawa National Forest. The Forest Service reserves the right to dispose of the merchantable timber to those other than the holder at no stumpage cost to the holder. Unmerchantable material shall be disposed of as directed by the Authorized Officer. Trees, shrubs, and other plants may be planted within the permit area with prior written approval of the Authorized Officer.

H. Signs (X-29). Signs or advertising devices erected on National Forest System lands shall have prior approval by the Forest Service as to location, design, size, color, and message. Erected signs shall be maintained or renewed as necessary to neat and presentable standards, as determined by the Forest Service.

I. Submit Reports (X-87). The holder shall provide the Authorized Officer with a copy of all reports and publications resulting from the project including theses, dissertations, articles, monographs, and so forth. The final report on work performed shall be submitted in one copy to the Forest Service no later than 1 year following the completion of the research.

THIS PERMIT IS ACCEPTED SUBJECT TO ALL ITS TERMS AND CONDITIONS.

BEFORE ANY PERMIT IS ISSUED TO AN ENTITY, DOCUMENTATION MUST BE PROVIDED TO THE AUTHORIZED OFFICER OF THE AUTHORITY OF THE SIGNATORY FOR THE ENTITY TO BIND IT TO THE TERMS AND CONDITIONS OF THE PERMIT.

ACCEPTED:

[redacted]

DAN BEYER	(SIGNATURE)	DATE

APPROVED:

[redacted]

BARBARA VAN ALSTINE District Ranger Ottawa National Forest	(SIGNATURE)	DATE

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0596-0082. The time required to complete this information collection is estimated to average one hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

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To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, 1400 Independence Avenue, SW, Washington, DC 20250-9410 or call toll free (866) 632-9992 (voice). TDD users can contact USDA through local relay or the Federal relay at (800) 877-8339 (TDD) or (866) 377-8642 (relay voice). USDA is an equal opportunity provider and employer.

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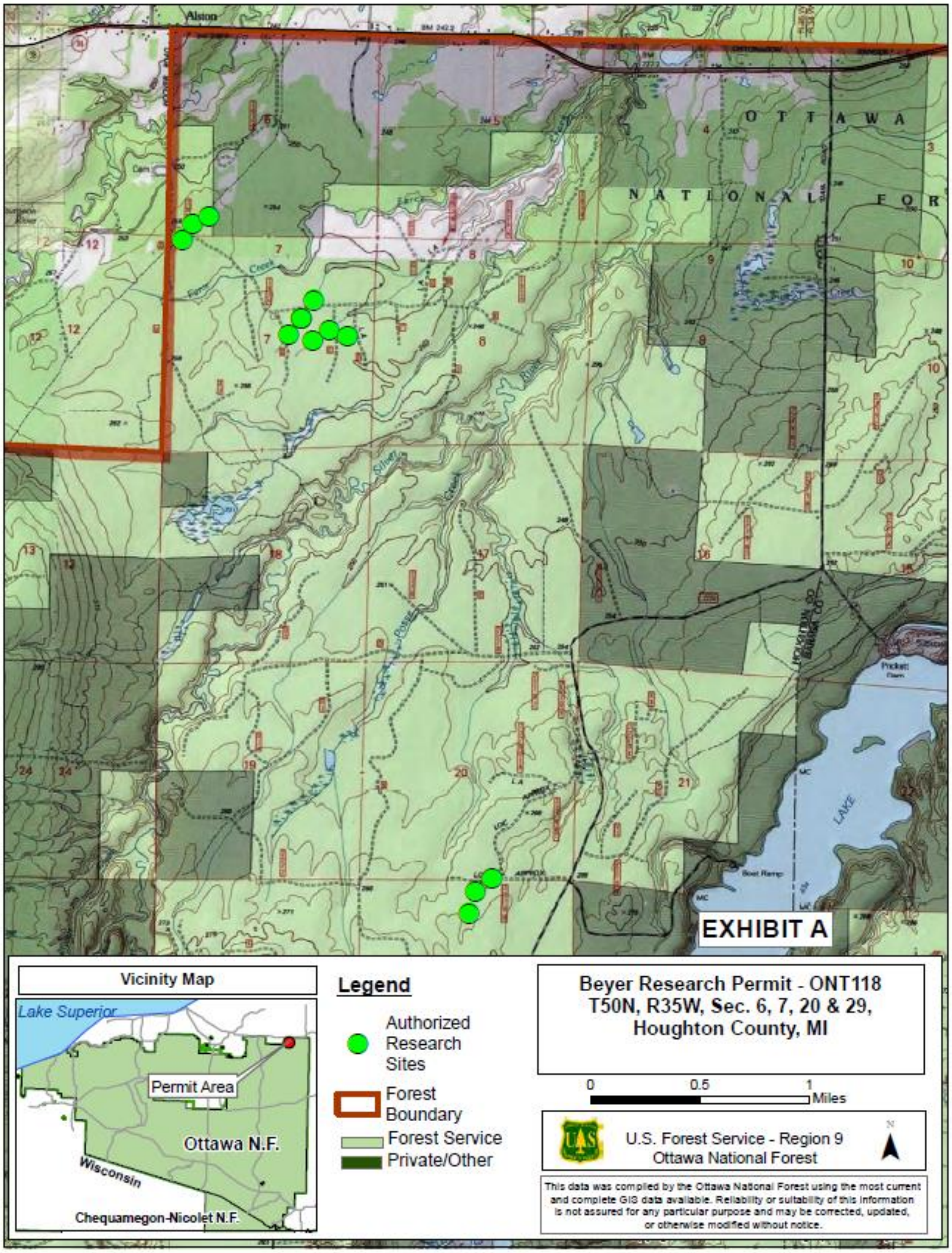


EXHIBIT A
O T T A W A
N A T I O N A L
F O R
Vicinity Map
Lake Superior
Permit Area
Ottawa N.F.
Wisconsin
Chequamegon-Nicolet N.F.
Legend
Authorized Research Sites
Forest Boundary
Forest Service
Private/Other
Beyer Research Permit - ONT118
T50N, R35W, Sec. 6, 7, 20 & 29,
Houghton County, MI
0
0.5
1
Miles
U.S. Forest Service - Region 9
Ottawa National Forest
This data was compiled by the Ottawa National Forest using the most current and complete GIS data available. Reliability or suitability of this information is not assured for any particular purpose and may be corrected, updated, or otherwise modified without notice.

PLAN OF OPERATION

RESEARCH PERMIT – Dan Beyer, ONT118
Ontonagon Ranger District, Ottawa National Forest

1. Ensure all equipment and field worker clothing are visually free of soil, seeds, vegetative matter or other debris that could contain or hold seeds prior to transport to the research sites to prevent the spread of non-native invasive plants (NNIP). Avoid parking in patches of NNIP along roadsides when the species are in seed, to prevent their movement.

2. Retain native vegetation in and around project activity to the maximum extent possible.

3. The holder shall protect, in place, all public land survey monuments, private property corners, and Forest boundary markers and soil survey plot markers.

4. Installations and monitoring shall be made in a manner that does not attract attention to or damage the environment. Any visible equipment left on site (e.g. if PVC pipe extends out of soil), should be signed with the Holder's contact information, including name and phone number, and that it is "Under permit by the U.S. Forest Service".

5. If proposed locations of the authorized improvements change, the new latitude/longitude will be provided to and approved by the Forest Service, prior to installation.

6. Comply with the Ottawa Motorized Vehicle Use Map when accessing research sites. Paper copies of the MVUM are available at any Forest Service office on the Ottawa and are available electronically at: https://www.fs.usda.gov/main/ottawa/maps-pubs.

7. All equipment (PVC tubes, tree tags, rebar, etc.) will be removed from the National Forest System land within 6 months of the permits' expiration, or at the conclusion of the research, whichever is earlier.

8. Incident Notification.

1. Contact the Authorized Officer as soon as practicable after the following incidents occur on National Forest System lands covered by a special use authorization:
   a) An incident resulting in death, permanent disability, or personal injuries that are life-threatening or that are likely to cause permanent disability;
   b) A structural, mechanical, or electrical malfunction or failure of a component of a facility designed for passenger transport or any operational actions that impair the function or operation of such a facility in a way that could affect public safety;
   c) A search and rescue operation to locate a person; or
   d) Any incident that has high potential for serious personal injury or death or significant property, environmental, or other natural resource damage, including avalanches, landslides, flooding, fire, structural failures, and release of hazardous materials.

2. Method of Notification. Contact the Permit Administrator by phone or email following an incident on National Forest System lands covered by a special use authorization.
   Michelle Holland, Realty Specialist
   Email: michelle.m.holland@usda.gov
   Office: 906-358-4021

EXHIBIT B

3. Contents of Notification. Specify when, where, and how the incident occurred and who was present or affected by the incident.

EXHIBIT B

A.4 Resin Collection and KCl Extraction Procedure

Protocol for Ecosystem Laboratory, Oak Ridge National Laboratory

Title: Potassium Chloride Extraction of Ion-Exchange Resins

Page 107 of 123

Written by: Gloria Jimenez **Date: 6 December 2007**
Updated by: Colleen Iversen **Date: 11 March 2010**

Purpose:

This protocol describes the procedure for extraction of WECSA ion-exchange resin balls with 2 M potassium chloride solution to obtain inorganic N (NH_4^+ and NO_3^-) for later analysis on the Lachat autoanalyzer.

References:

http://www.wecsa.com/SoilMon/Monitor.htm

Materials and Reagents List:

Material / Reagent	Source	Description	Catalog number	Size	Ea / case
Potassium chloride	Fisher		P217-3 or P217-500	3 kg bottle or 500 g bottle	1 bottle 1 bottle
Wide-mouth plastic bottles w/ screw caps				~ 60 ml	
Whatman #1 filters	Fisher		1001-150	15 cm diameter	100 / pack
Funnels	Fisher	Long-stem analytical funnels	10-500-14	50 ml capacity	12 / pack
Funnel racks	Fisher		30587-000		Ea
Specimen cups	Fisher	Polypropylene, non-sterile, white cap	14-375-148	4 oz.	500 / case
Lachat tubes	Fisher	Polystyrene culture tubes with screw cap	14-956-4A	13 mm × 100 mm	1000 / case
Amber bottle	Fisher	Amber glass jug	02-912-271	80 oz.	6 / case
Repipettor	Fisher		03-692-181	1 to 50 ml	Ea
Reciprocating shaker	Fisher	Eberbach 6010 Heavy Duty Shaker, Two Speed	14-265-10	115 V, 60 Hz	Ea

Personal Protective Equipment:

- Nitrile or other rubber gloves (to protect samples)
- Lab coat (to protect clothing)

1.0 Collect and replace ion-exchange resins.

1.1 Put two unused resins in a small plastic baggie in the fridge, and label with the date.

Note: These will be extracted later when you collect this resin set; these serve as "blanks" in case the resins from the manufacturer are contaminated with a small amount of nutrient.

1.2 Bring necessary number of unused resins to the field in a clean plastic bag.

1.3 Wearing gloves, remove inner tube from each access tube and unscrew resin holder at bottom. Remove resin ball and place in small plastic baggie.

Note: You should always wear gloves when handling the resins so that nutrients on your skin do not get adsorbed to the resin. Also, make sure each resin ball is placed in its own plastic bag.

1.4 Replace incubated resin ball with new resin ball, holding resin by edges of mesh as much as possible to avoid any contamination of the resin with dirt that will inevitably be on your gloves from the incubated resin ball.

Note: If your gloves get really dirty, make sure you change them at least between treatments to avoid cross-contamination of nutrients.

1.5 Upon return to the laboratory, while wearing new gloves, rinse resins well with deionized or distilled water until all visible soil is removed. You can rub the resin with your clean gloved hand if necessary to remove particles. A squirt bottle over a plastic bin works well for this. Do the same to the blanks that have been stored in the refrigerator.

Note: The soil removal is to avoid contamination of your samples with nutrients adsorbed to the soil.

1.6 Place newly cleaned resin ball in individual acid-washed, dry, wide-mouth plastic bottle. Do not cap the bottle.

Note: Bottle mouth should be wide enough to put in and remove resin ball, but narrow enough that the resin ball will not fall out while pouring.

1.7 Allow the resins within the plastic bottles to air-dry for approximately one week in a location where they will not be disturbed. Make sure you label the samples with the date and your name. Loosely cover tops of bottles with paper towel or plastic wrap to allow evaporation but prevent dust particles from contaminating the resins.

Note: Drying will prevent the dilution of your nutrient extractions with excess water, which could vary among resin balls.

2.0 Make 2 M potassium chloride (KCl) solution

2.1 Dissolve 2892 g of potassium chloride in 5 to 10 L of distilled or deionized water in a 20 L carboy.

2.2 Place carboy on shaker at low speed for 30 minutes (or until sample is dissolved). Add more distilled / deionized water to bring solution to 20 L. Shake by hand until well-mixed.

Note: It is easiest to make the solution if you first add 20 L of distilled / deionized water to the carboy and mark the line. You can then fill the solution to the line without having to measure 20 L of water in 1 L increments. The carboy should be acid-washed before the first use.

3.0 Calibrating re-pipettor

3.1 Attach the 50-ml re-pipettor to an acid-washed 2 L glass amber bottle that you have filled with distilled / deionized water. Set the control to 20 ml. Tare a specimen cup on a balance and add 20 ml to the cup. Re-weigh the cup. The re-pipettor is considered calibrated at 20 ± 0.01 g.

3.2 Once calibrated, remove the re-pipettor from the amber bottle containing water, and pump out any water remaining in the re-pipettor over the sink.
Note: You need to remove excess water from the re-pipettor to avoid diluting the KCl solution on the first sample.

3.3 Place re-pipettor on 2 L glass amber bottle containing 2 M KCl solution (make sure you agitate solution in carboy before removing 2 L for samples).
Note: Be sure to remove the re-pipettor from the bottle when you are finished with the experiment, and flush well with water. KCl is a salt, and will gum up just about anything it comes in contact with.

4.0 Resin extraction

4.1 Add 20 ml of 2 M KCl solution to each wide-mouth bottle containing a resin using the calibrated re-pipettor, and screw cap tightly on bottle.

4.2 Add 20 ml to two additional acid-washed wide-mouth bottles containing resin blanks.

4.3 Place bottles in a box on shaker so that resin will shake the long way. Pack tightly to avoid unbalancing the shaker and also to prevent spills.

4.4 Shake bottles on low speed for 30 minutes.

5.0 Filtering extractant

5.1 Label one specimen cup and one Lachat tube for each sample.

5.2 Wearing clean gloves, set up acid-washed funnels on funnel racks.

Note: Make sure you are able to access both sides of funnel rack. It is easier to clean up if you place pre-cut cardboard or cellulose paper at the bottom of each funnel rack to catch any excess KCl solution.

5.3 Wearing clean gloves, place one Whatman #1 filter paper in each funnel. Fold each filter paper into quarters so that it fits nicely into the conical structure of the funnel.

5.4 Place a “catch cup” (a previously used specimen cup that has been rinsed well with distilled / deionized water) underneath each funnel.

5.5 Place labeled specimen cup and Lachat tube at each funnel.
Note: It is easiest if you place cups and Lachat tubes in rational order because you will be returning to them three times.

5.6 Pour distilled / deionized water through each filter into catch cups, making sure that each filter is completely wetted.
Note: This is to leach any potential nutrient contamination from the filter papers.

5.7 Empty water from catch cups into large beaker, and pour down drain. Replace empty catch cups under each funnel.

5.8 Remove bottles from first extraction from shaker and place each bottle next to corresponding specimen cup / funnel.

5.9 Carefully pour extractant through funnel, being careful not to pour out the resin ball.
Note: Pour as slowly as you need to prevent splashing the extractant onto other filter papers. Note: If the resin breaks within the bottle, you may need to count this sample as a loss.

5.10 Once first few drops have gone through filter into catch cup, quickly switch out catch cup for the clean, labeled specimen cup.
Note: The catch cup is to catch the first few drops of extractant that may be diluted by the water you have used to leach the filter paper.

5.11 Repeat the extraction procedure (steps 4.1 through 4.4) two more times, for a total of 60 ml of sample in each specimen cup.

5.12 Once each sample has finished filtering, swirl the extractant in the specimen cup to mix, and carefully pour off 20 ml sample into labeled Lachat tube and cap the tube. Leave approximately 1 finger width at top of tube to allow for expansion during freezing.

5.13 Place Lachat tubes in test tube rack, and place entire rack in paper bag before freezing at -20 °C. The samples will be ready to thaw and analyze on the Lachat at any time.
Notes: The samples need to be frozen to prevent volatilization of NH_4^+. Placing tubes in paper bags prevents extra fast freezing that might lead to bursting of the Lachat tubes.

6.0 Clean up and disposal

6.1 Use forceps to remove resins from wide-mouth bottles. Place used resins in "non-hazardous" waste container.

6.2 Wipe down lab bench thoroughly—the salt solution makes a big mess if you don't clean it up right away.

6.3 Dispose of excess KCl solution remaining in specimen cups by pouring down the drain with excess water (our lab has a variance for this).

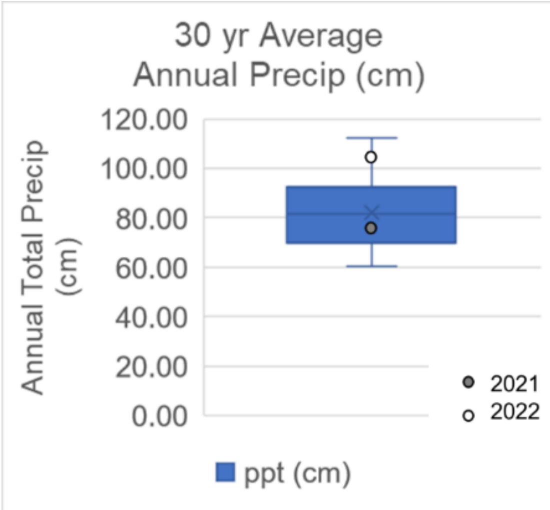
6.4 Throw away filter paper and used specimen cups. Keep catch cups to use in next extractions.

6.5 Soak funnels, wide-mouth bottles, and catch cups in warm water.
Note: Soaking the containers before acid-washing will help to remove excess salt solution.

6.6 Rinse catch cups well with distilled water and place at back of sink.

6.7 Acid-wash funnels and wide-mouth bottles. Dry upside-down.
Note: If necessary, use ethanol to remove the sharpie label from the bottles before acid-washing.

A.5 Weather History



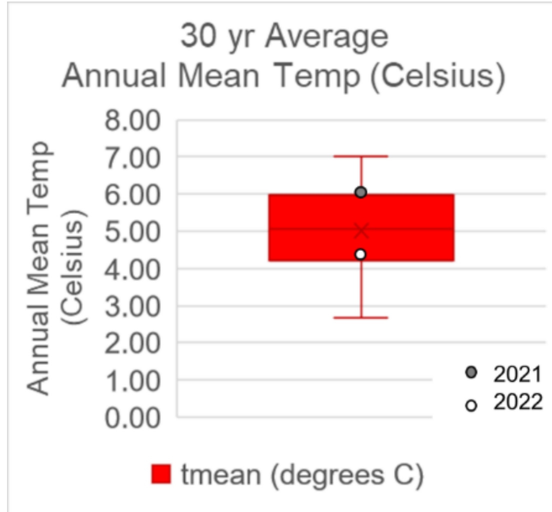


Figure V.1.1 and Figure V.1.2. Previous 30-year weather data (1991-2020) for precipitation (cm) and temperature (Celsius) with study years (2021, 2022) noted with overlaid dots. Values were obtained from monthly PRISM data. Precipitation represents the total precipitation for the time period while temperature represents the average temperature. Precipitation is shown in blue on the left (Figure V.1.1) and temperature is shown in red on the right (Figure V.1.2).

Table V.1. Study site weather history, summary values for weather history of 2021 study year, 2022 study year, and previous 30-year weather averages (1991-2020) for precipitation and temperature. Values were obtained from monthly PRISM data. Precipitation represents the total precipitation for the time period while temperature represents the average temperature.

	Precip (cm)	**T_{mean} (deg C)**
2021	74.12	6.06
2022	103.96	4.33
30yr Average (1991-2020)	81.98	5.02

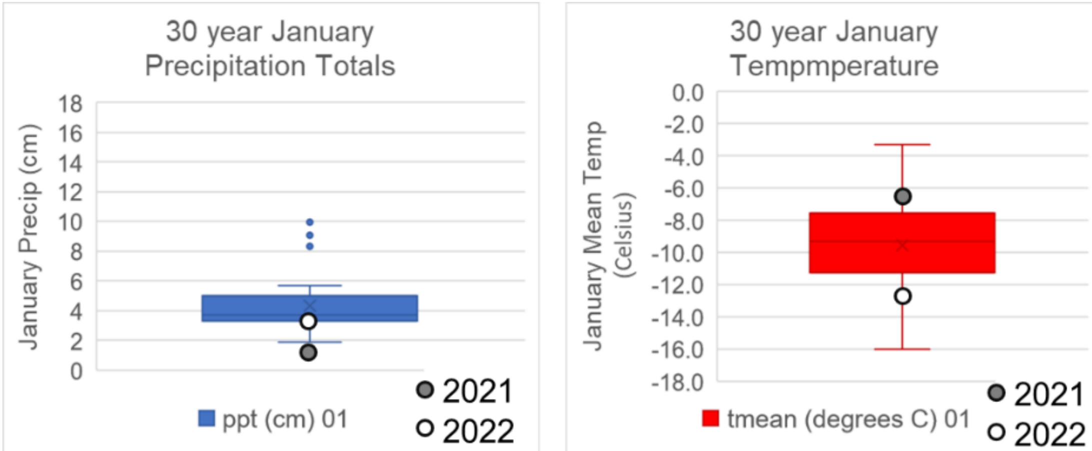
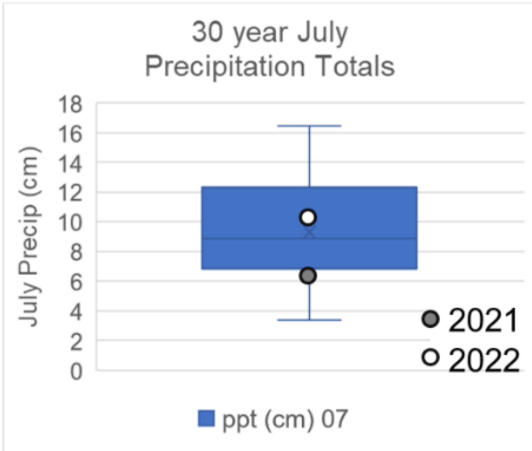


Figure V.2.1 and Figure V.2.2. Previous 30-year weather data (1991-2020) for January precipitation (cm) and January temperature (Celsius) with study years (2021, 2022) noted with overlaid dots. Values were obtained from monthly PRISM data for January. Precipitation represents the total precipitation for the time period while temperature represents the average temperature. Precipitation is shown in blue on the left (Figure V.2.1) and temperature is shown in red on the right (Figure V.2.2).

Table V.2. Study site January weather history summary values for weather history for January 2021 study year, January 2022 study year, and previous 30-year weather data (1991-2020) for January for precipitation and temperature. Values were obtained from monthly PRISM data. Precipitation represents the total precipitation for the time period while temperature represents the average temperature.

	Precip (cm)	**T_{mean} (deg C)**
Jan 2021	1.495	-6.4
Jan 2022	3.379	-12.7
30yr Average (1991-2020)	4.348	-9.7



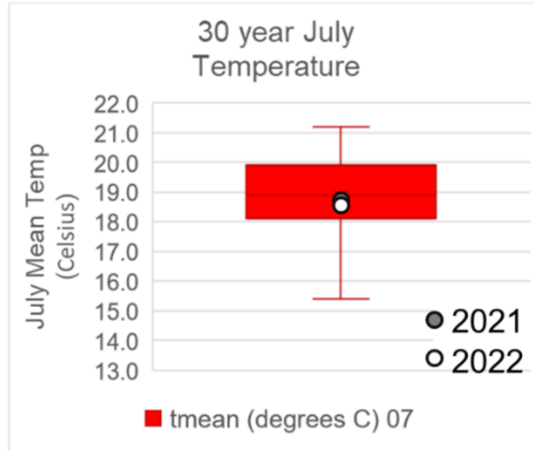


Figure V.3.1 and V.3.2. Previous 30-year weather data (1991-2020) for July precipitation (cm) and July temperature (Celsius) with study years (2021, 2022) noted with overlaid dots. Values were obtained from monthly PRISM data for July. Precipitation represents the total precipitation for the time period while temperature represents the average temperature. Precipitation is shown in blue on the left (Figure V.2.1) and temperature is shown in red on the right (Figure V.2.2).

Table V.3. Study site July weather history summary values for weather history for July 2021 study year, July 2022 study year, and previous 30-year weather data (1991-2020) for July for precipitation and temperature. Values were obtained from monthly PRISM data. Precipitation represents the total precipitation for the time period while temperature represents the average temperature.

	Precip (cm)	**T_{mean} (deg C)**
July 2021	6.042	18.9
July 2022	10.111	18.7
30yr Average (1991-2020)	93.232	18.9

B Copyright documentation

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