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Bronte Hoefer
hoeferbe@hollins.edu

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The Role of Edge Effects on Emerald Ash Borer Infestation and Forest Responses

Bronte Hoefer, *Hollins University*

The Role of Edge Effects on Emerald Ash Borer Infestation and Forest Responses

Bronte E. Hoefer

Under the direction of Dr. Elizabeth R. Gleim

For Consideration as a Senior Honors Thesis in Environmental Studies

Hollins University

Committee

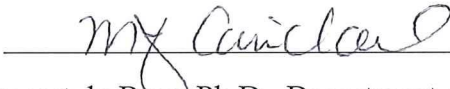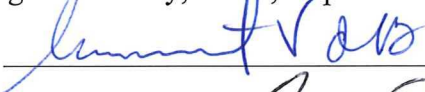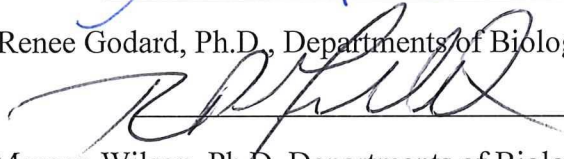Elizabeth Gleim, Ph.D., Departments of Biology & Environmental Studies, Thesis Advisor

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Mary Jane Carmichael, Ph.D., Departments of Biology & Environmental Studies
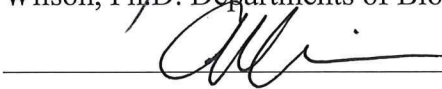
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Margaret du Bray, Ph.D., Department of Environmental Studies

_____

Renee Godard, Ph.D., Departments of Biology & Environmental Studies

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Morgan Wilson, Ph.D. Departments of Biology & Environmental Studies

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Abstract

The emerald ash borer (EAB; *Agrilus planipennis*) is a buprestid beetle native to Asia, classified as an invasive species in North America. EAB infests trees of genus *Fraxinus* (ash) and has spread to 35 states since its introduction in the early to mid-1990s. Notably, EAB has the potential to functionally extirpate all native ash species in North America. Our study aims to characterize the ecological impacts of EAB infestation in the Roanoke Valley of Southwest Virginia, to quantify the impact of edge effects on EAB forest invasion and mortality of ash trees, as well as investigate the role that forest edge effects play in forest regeneration post-ash tree mortality. In 2017, twelve forested study sites, six with ash trees and six without (e.g. controls), were established in the Roanoke Valley. Data were collected annually through 2020 on tree species composition and growth, as well as understory woody species composition. Signs of EAB infestation and ash mortality were tracked via a dieback scoring system at the ash sites across all study years. Significant increases in dieback scores of large (>12-cm DBH) and small (<12-cm DBH) trees across all years were documented, indicating progressive mortality of ash trees. Large trees had significantly higher dieback scores than small trees indicating more rapid progression of mortality, particularly in 2019 and 2020. Finally, while there were significantly less ash seedlings at the edge of ash sites (8.04 +/- 0.98) than in the core (20.20 +/- 2.27), there were no significant changes over time in the mean number of ash seedlings in the edge or core of ash sites. This may be due to harsher microclimatic conditions in the edge leading to lower seed production and/or recruitment. Because percent cover of invasive vine species was significantly higher at the edge of ash sites (14.22 +/- 3.02%) than in the core (6.51 +/- 3.27%), the survival of ash seedlings at the forest edge may be suppressed and thus survival of ash in these forests may be dependent upon the survival of ash seedlings in the core.

Introduction

Natural History of the Emerald Ash Borer

The emerald ash borer (EAB, *Agrilus planipennis*) is a buprestid beetle native to Asia and invasive in North America. EAB infests all species in the genus *Fraxinus* (ash) and since its detection and identification in the U.S. in 2002, EAB has caused widespread devastation of ash throughout the eastern portion of North America. However, in its native range of eastern Russia, northeastern China, Korea, Mongolia, Japan, and Taiwan, healthy trees of all native ash species are not killed by EAB due to a co-evolutionary history with the beetle that has resulted in genetic resistance to EAB (Rebek et al. 2008, Herms and McCullough 2014).

Adult beetles target stressed ash trees via chemical markers, but will also readily infest and kill healthy ash trees not native to Asia as small as 2.5-cm diameter at breast height (DBH) (Herms and McCullough 2014). As with other host-specific insect herbivores, EAB likely selects host plants based off of a variety of stimulatory and inhibitory chemicals emitted by host plants. For example, in a study by Crook et al. (2008), artificially girdled *Fraxinus pennsylvanicca* (green ash) trees had elevated sesquiterpene levels, and six of the elevated compounds produced antennal responses of both male and female EAB. Mated female EAB elicited more frequent antennal responses than either virgin females or males, demonstrating that volatile cues from sesquiterpenes may play an important role in oviposition site selection (Crook et al 2008). Notably, the isolated sesquiterpene compounds that produced antennal activity in adult EAB, when used in baited traps, caught significantly more adult EAB than non-baited control traps (Crook et al. 2008).

In its native range, EAB reportedly has the ability to colonize other genera apart from *Fraxinus*, including *Ulmus* (elm), *Juglans* (walnut), and *Pterocarya* (wingnut) (Anulewicz et al. 2008). Closely related congeners of these genera are common in forested landscapes in much of

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North America, and as such there was some concern over threat to additional tree species and/or their ability to facilitate the spread of EAB here in North America (Anulewicz et al. 2008). However, in southeast Michigan, field studies utilizing freshly cut logs of *Fraxinus* spp. and congener species such as *Ulmus americana* (American elm), *Celtis occidentalis* (hackberry), *Juglans nigra* (black walnut), *Carya ovata* (shagbark hickory), and *Syringa reticulata* (Japanese tree lilac) found that adult EAB occasionally landed and oviposited upon non-ash logs (Anulewicz et al. 2002). While EAB larvae developed normally upon ash logs, larvae failed to survive and develop in non-ash logs (Anulewicz et al. 2008), thus suggesting that the non-ash target species native to North America are not susceptible to EAB. One exception has been identified, *Chionanthus virginicus* (white fringetree); however, it exhibits greater tolerance to EAB in addition to reduced rates of infestation in comparison to native *Fraxinus* species (Ellison 2012).

The lifecycle of EAB is completed in 1-2 years, depending on climatic and environmental conditions and tree stress (Haack et al. 2002, Herms and McCullough 2014). Adult beetles emerge from early May to July and live for 3-6 weeks, feeding on small amounts of ash foliage but causing insignificant defoliation. Eggs are laid on ash bark and hatch within two weeks of deposition (Haack et al. 2002). When larvae hatch, they bore into the tree and feed on the cambium and phloem of the ash tree, effectively girdling the tree over time as infestation progresses, thus disrupting nutrient and water transport throughout the tree (Haack et al. 2002, Herms and McCullough 2014). Larvae feed from mid-summer through fall, completing four larval instars (Chamorro et al. 2012), and typically overwintering in the prepupal fourth instar stage within the outer bark or sapwood (Cappaert 2005). Larvae undergo pupation in the middle

to late spring, with adult beetles emerging soon after, leaving a D-shaped exit hole 2-3 mm in width in the bark of the tree.

An ash tree exhibits numerous signs of infestation as mortality of the tree progresses. Infestation typically begins at the top of the tree and progresses steadily downward toward the base of the tree. Initial signs of infestation include progressive canopy thinning and dieback. As top-down tree mortality progresses, an infested ash tree will frequently produce epicormic sprouts in response to canopy death in order to continue to conduct photosynthesis. These epicormic sprouts are produced by epicormic buds which lie, typically dormant, under the bark of the trunk of the tree. Epicormic sprouts typically emerge progressively lower on the trunk of a tree as mortality progresses (Haack et al. 2002).

Other signs of EAB infestation commonly include the peeling away of bark by woodpeckers in order to feed on developing larvae within the growth layer of the tree, commonly referred to as "flecking." As tree mortality becomes more severe, bark may also begin to peel from the inner sapwood of the tree, revealing characteristic frass-filled, S-shaped larval galleries created by EAB larvae as they feed on the cambium and phloem of the tree from mid-June to mid-October (Haack et al. 2002, Herms and McCullough 2014). Finally, as newly formed EAB adults emerge, the tell-tale D-shaped exit holes can be observed on the trunk of the tree. Ultimately, full tree mortality generally occurs 2-5 years following initial infestation (Haack et al. 2002, Herms and McCullough 2014).

History of Emerald Ash Borer in the United States

EAB was first discovered in North America in 2002 near Detroit, Michigan. Subsequent dendrochronological analysis has shown that EAB was introduced in southeastern Michigan in the early to mid-1990's (Siegert et al. 2014), likely through infested shipping crates or packing

material (Haack 2002, Smith 2006). Since its establishment, EAB has spread across the eastern portion of North America, and as of August 2021 is present in 35 states from Maine, south to Georgia, and as far west as Colorado (Emerald Ash Borer Information Network, n.d.). If left unchecked and ash nears 100% mortality in infested areas, some scientists predict that EAB has the potential to functionally extirpate all native ash species within North America (Haack et al. 2002, Smith 2006, Klooster et al. 2014).

The spread of EAB throughout the U.S. occurred via both natural and human-facilitated movement of the beetle. EAB spreads relatively short distances via natural dispersal (adult migration). Specifically, adult beetles typically fly in short spurts of 8 to 12 meters, although mated females can fly distances of over one kilometer (Haack et al. 2002). Additionally, satellite populations were initially established due to human transport of infested ash wood which rapidly increased the spread of EAB (Herms and McCullough 2014).

Many species of ash, particularly white and green ash, are commercially important timber species. Ash wood is used for a variety of applications due to its superior strength, including the production of baseball bats, tool handles, furniture, and flooring, and as such is an extremely important timber resource (MacFarlane and Meyer 2005, Bonner and Karrfalt 2008). Forest ash in the United States has an estimated compensatory value (an economic estimation of the cost of replacement of those trees as a structural asset in urban areas) of $282.3 billion and comprises approximately 7.5% of hardwood sawtimber value. Therefore, the loss of forest ash as a timber resource would be economically devastating (Poland and McCullough 2006).

Furthermore, ash is also a common landscaping tree and an important component to many urban forests in North America. Ash constitutes over 20% of total trees in many North American municipalities (Kovacs et al. 2009) and much of the economic impact of EAB

devastation will ultimately be attributed to the costs of treating and removing high-risk trees in urban areas (Kovacs et al. 2009, Herms and McCullough 2014). Specifically, removal of all urban ash trees in the United States would cost an estimated $20-60 billion, not including replacement (Poland and McCullough 2006). Beyond the economic impacts, mature trees also offer a large variety of ecosystem services to urban areas including reduction of the urban heat island effect, controlling runoff and erosion, improving airborne pollutant levels by offsetting emissions, as well as increasing property values and quality of life (Wolf 2007, Herms and McCullough 2014, United States Forest Service 2015, Livesley et al. 2016).

Eradication and Control Efforts

In response to the disastrous threat that EAB poses to municipalities and ecosystems in North America, many eradication and control methods have been explored and implemented. Early eradication efforts following initial EAB detection in Michigan and Ontario focused on containing infestations in the target areas as well as eliminating localized "outlier" satellite populations (Herms and McCullough 2014). Unfortunately, these efforts were not successful and due to the lack of effective, practical eradication methods, the focus of the EAB management program lead by the United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA APHIS) has shifted from eradication to integrated pest management (APHIS 2015).

Biological control has been extensively studied as a means to control EAB infestation and spread through an integrated pest management approach. Examined biological control measures include predators, pathogens, and parasitoids native to North America as well as parasitoids that target EAB in their native range (Bauer et al. 2003, Cappaert 2009, Herms and McCullough 2014, Gould et al. 2020). For example, North American woodpecker species

regularly prey on larval EAB, and studies found that they represent the largest source of naturally-caused EAB mortality in forested systems here in the U.S. (Lindell 2008, Duan et al. 2010). In addition, a number of parasitoids, pathogenic fungi, and predatory insects native to North America that target EAB have been observed but generally result in low EAB mortality (Bauer et al. 2003, Duan et al. 2010).

Upon the identification of EAB parasitoid wasps native to its host range (*Oobius agrili* [Encyrtidae]*, Tetrastichus planipennisi* [Eulophidae]*, Spathius agrili,* and *Spathius galinae* [Braconidae]), an APHIS facility in Michigan was established for the purpose of rearing and releasing adult wasps in order to control EAB populations. This facility presently holds permits to release all four studied parasitoid species in every state where EAB is currently established (APHIS 2015, Gould et al. 2020). However, none of these biological control agents have been capable of eradicating or dramatically reducing EAB populations, although some have been shown to control population growth (Gaudon and Smith 2020).

Finally, insecticidal control targeting both adult and larval EAB as a means to control EAB spread and individual ash mortality has been extensively studied. Unlike biological control, insecticides have demonstrated effectiveness at reducing EAB populations below damaging thresholds (Herms 2009, Bick et al. 2018). While these treatments are expensive and must be maintained over a period of years and thus are not a viable control option in natural, forested settings, they are used within urban settings for individual high-value trees. Indeed, in urban settings the costs of preventative treatment may be more economically viable than the subsequent costs of removal, replacement, and lost ecosystem services (Vannatta et al. 2012). Thus, this approach has been implemented by some homeowners, businesses, and municipalities (Vannatta et al. 2012, Herms and McCullough 2014).

Known Ecological Impacts of Emerald Ash Borer

Fraxinus spp. comprise a significant portion of North American forests. There are nine species of *Fraxinus* native to North America, with white and green ash (*F. americana* and *F. pennsylvanica* respectively) having the widest distribution (Bonner and Karrfalt 2008). White ash is found from Nova Scotia, south to Florida, and as far west as Texas and Minnesota, while green ash is found from Cape Breton Island, Nova Scotia, and Alberta, Canada, south to Texas and northwest Florida (Bonner and Karrfalt 2008). In an atlas ranking 80 common tree species found in the eastern contiguous United States, white ash and green ash ranked number 17 and 12, respectively, in average importance value (how dominant a species is in comparison to other species within a localized area) (Iverson et al. 1999). Four ash species are native to Virginia, including white and green ash, black ash (*Fraxinus nigra*), and pumpkin ash (*Fraxinus profunda*), and of these species, white and green ash are native to the Roanoke Valley.

As ash nears 100% mortality in localized areas and with no control measures identified that would result in eradication of EAB, it has been suggested that ash in North America could be functionally extirpated due to EAB (Klooster et al. 2013, Herms and McCullough 2014). Therefore, understanding the ecological impacts of the loss of ash trees is important. The persistence of ash in forests that have been infested with EAB ultimately hinges upon the ability of ash to regenerate following mortality, and whether EAB populations persist in areas in which all or most ash trees are dead.

Importantly, EAB is capable of infesting ash trees well before they have reached reproductive maturity. For example, *F. americana* and *F. pennsylvanica* do not produce seeds until they are 20 years old, i.e. when a tree is 8-10-cm DBH, whereas EAB can infest and kill ash trees as small as 2.5-cm DBH (Klooster et al. 2013). Thus, the storage of ash seeds within the

soil of a given ecosystem, i.e. the seed bank, will be essential to both the survival of *Fraxinus* as a genus in North America, as well as how forests naturally regenerate following ash mortality (Brunet and Von Oheimb 1998, Taiwo et al. 2018).

Ash persistence in a forest is typically maintained through seed drops during mast years which occur up to every 5 years for *F. pennsylvanica* and *F. americana* (Bonner and Karrfalt 2008). Seed bank composition for a given species can be classified as transient or persistent, wherein transient seed banks are composed of seeds that only remain viable for one germination cycle (typically one calendar year), and persistent seed banks remain viable through at least the second germination season. Furthermore, persistent seed banks can be classified as either having short or long-term persistence, with suggested viability thresholds at two and six germination seasons, respectively (Walck et al. 2005). Ecosystems generally maintain both transient and persistent seed banks. Although ash seeds exhibit complex dormancy and can be artificially stored for periods of years (no loss in viability was noted for green ash seeds stored for 7 years in regulated conditions) (Bonner and Karrfalt 2008), ash usually germinate in the spring following their dispersal, and as such do not form a persistent seed bank (Taylor 1972, Kashian 2016).

Regardless of whether ash species are able to persist via their seedbank, the loss of mature canopy dominant ash trees will result in canopy gaps in the forest. Forest canopy loss can result in a disruption and redistribution of a variety of ecosystem resources, including moisture, nutrient levels, and light exposure (Ford et al. 2011). Much of the prior research done examining canopy gap dynamics has focused upon more sudden canopy gap formation, typically associated with windthrow or logging. Such gaps cause photodamage (Jones and Thomas 2004), disruptions to microclimate, increased disturbance to understory and soil (Schaetzl et al. 1988, Gray et al.

2002), and often leave the forest more vulnerable to weather events such as storms (i.e. more susceptible to damage and less resilient) (Garber et al. 2010).

However, ash death from EAB is slow, and dead standing trees may remain in forests for years which results in a more gradual gap formation (Davis et al. 2017). This slower gap formation associated with EAB may lessen the effects typically associated with suddenly formed canopy gaps. Few studies have focused on gradual gap formation. Of the studies that have examined this, none of which were specific to EAB, gradual gaps created by standing dead trees were found to create only a limited increase in resource availability, such as light or access to bare soil, in comparison to sudden gaps (Schaetzl et al. 1988, Gray et al. 2002). In a study of second-growth forests of the southern Appalachians, gradual gaps were shown to be less effective at promoting long-term tree species diversity and seedling regeneration with the exception of *Acer rubrum* (red maple) seedlings whose densities did increase following gap formation (Beckage et al. 2000). While sudden canopy gaps create greater disturbance to the understory, such as increased mineral soil exposure and light availability, this may be less severe during the formation of gradual gaps (Schaetzl et al. 1988, Gray et al. 2002). Furthermore, the regeneration response of woody species in the understory following gap formation can be actively suppressed by an herbaceous or shrub layer, particularly due to the lack of severe understory disturbance during gradual gap formation that may be important for woody seedling germination and recruitment (Beckage et al. 2000, Davis et al. 2017).

The gradual gap formation caused by emerald ash borer mortality can result in differential forest responses based upon the species composition of the forest. A study analyzing growth rates of forests dominated by green ash in northwestern Ohio from 1990-2010 showed that the species second-most abundant to ash, *Acer* spp. [*A. rubrum* and *Acer saccharinum* (silver

maple)], responded to ash mortality with an increase in radial growth and an advancement of crown class, particularly in suppressed (receiving no direct sunlight) and intermediate (just below the general canopy, receiving little sunlight) trees (Costilow et al. 2017). This conclusion is in support of a growth response hypothesis suggesting immediate and positive effects on the growth rates of the existing forest sub-canopy in response to the creation of canopy gaps (Jones and Thomas 2004, Hart and Grissino-Mayer 2008).

Because tree mortality as a result of invasive insects such as EAB creates canopy gaps, some hypothesize that infestation can facilitate the spread of invasive plant species (Smith 2006, Eschtruth and Battles 2009, Gandhi and Herms 2010, Hausman 2010). Specifically, because many invasive plant species are shade intolerant, they are frequently found in areas where light is less limited by interspecific competition (Knapp and Canham 2000). Thus, invasive species may be more likely to colonize under canopy gaps. Relaxed resource competition, aggressive invader species characteristics (e.g. high fecundity and rapid growth rates), high propagule pressure (defined as the number and distribution of arriving plant propagules) due to seed dispersal facilitated by proximity of edge to transportation corridors, and forest stand age also contribute to the abundance and spread of invasive plant species (Flory and Clay 2006, Eschtruth and Battles 2009, Dillon et al. 2018).

Therefore, when canopy gaps become more abundant as a result of tree mortality, the potential of the spread of gap-obligate, invasive plant species may increase (Knapp and Canham 2000, Eschtruth and Battles 2009). One study examining this found that ash decline due to EAB was associated with increases in the radial growth of invasive *Lonicera maackii* (Amur honeysuckle) and greater abundance of *L. maackii* seedlings (Hoven et al. 2017). Therefore, in

locations where invasive species are already present and established, ash death may result in further establishment and spread of invasive plant species.

Beyond the threat of invasive plants, it is also important to understand how native species and overall species composition might change post-EAB. Indeed, the decline of endemic dominant tree species has historically altered forest community composition significantly. For example, the successional response following *Tsuga canadensis* (eastern hemlock) mortality due to *Adelges tsugae* (hemlock woolly adelgid) infestations favored *Quercus velutina* (black oak), *A. rubrum* (red maple), *Sassafras albidum* (sassafras), *Betula lenta* (black birch), *Betula alleghaniensis* (yellow birch), and *Fagus grandifolia* (American beech) depending on pre-existing forest characteristics (Small et al. 2005, Gandhi and Herms 2010). Several studies have investigated the successional response and subsequent changes in forest community structure as a result of EAB-induced ash mortality. One study found that although no increase in small woody stems was found following an EAB simulation treatment in Michigan, individual plant species densities did change, such as an increase in *A. rubrum* and *B. alleghaniensis* densities (Davis et al. 2017).

Edge Effects

A forest edge is defined as a transitional zone between a mature forest and a clear-cut or open area. This transitional zone represents a vastly different habitat and microclimate when compared to the interior (core) of the forested ecosystem (Chen and Franklin 1992). Abiotic differences at the forest edge include an increase in wind, sun, and exposure to weather events and storms (Laurance et al. 2007). Meanwhile, biotic differences between edge and core include differential plant species composition, increased abundance of invasive plant species, and increased stress and mortality of edge trees (Chen and Franklin 1992, Hunter and Mattice 2002,

Gandhi and Herms 2010). These different conditions experienced along the forest's edge are called edge effects. The impact that such edge effects have on the interior of the forest steadily decreases as distance from the edge increases (Chen and Franklin 1992).

Previous studies have speculated that edge effects could impact forest responses to EAB-induced ash mortality, but to date no studies have directly quantified these effects (Orwig and Foster 1998, Herms et al. 2008, Gandhi and Herms 2010). Furthermore, because *Agrilus spp.* have consistently been shown to preferentially colonize stressed trees (McCullough et al. 2009, Siegart et al. 2010, Tluczek et al. 2011) and appear to prefer high-light environments (Anderson 1944, Smith 2006), it is possible that they may preferentially infest trees in the edge of forests.

Furthermore, the impact of EAB and its ecological consequences has not been thoroughly studied outside of Ohio and Michigan where the infestation originated. However, the abundance of ash and tree species composition varies in other parts of the country. It is therefore important to understand the impacts and forest responses to EAB in other regions of the country, as differences in plant community composition may result in differential forest responses long-term.

To that end, EAB infestation was first observed in Roanoke, VA in 2015 and officially confirmed in 2016 (O'Bryan 2016, Virginia Department of Forestry 2019). The ultimate purpose of our study was to 1) document the impacts of EAB and forest responses in the Roanoke Valley of Virginia, and 2) quantify the impact of edge effects on forest invasion of EAB, subsequent mortality of ash trees, as well as the role edge effects play in forest regeneration post-EAB. With regards to general forest impacts, it was hypothesized that as ash mortality progressed, 1) there would be a measurable increase in the woody understory as regeneration occurs, and 2) the number of ash seedlings would decrease over time. In terms of edge effects, we hypothesized that 1) ash dieback and mortality would be quickest and most severe at the forest edge, and 2) as

canopy cover declined due to ash mortality, there would be an increase in invasive species, particularly within the forest edge.

Methodology

Study Area & Design

Twelve study sites were located on both public and private lands within the counties of Roanoke, Montgomery, Botetourt, and Bedford Counties, Virginia (Figure 1). All relevant permits and permissions were acquired for all sites. All sites were mixed hardwood forests. As with most forested areas in the southern Appalachians, these sites were secondary successional forests, and the most abundant species on average across all sites were *Nyssa sylvatica* (black gum), *Prunus spp.* (*P. avium* and native *P. serotina*; cherry spp.), *Acer saccharum* (sugar maple), *Fraxinus spp.*, *Acer rubrum* (red maple), *Carya glabra* (pignut hickory), *Quercus alba* (white oak), *Oxydendrum arboreum* (sourwood), *Juniperus virginiana* (eastern red cedar), and *Ailanthus altissima* (invasive; tree of heaven) (Table 1).

Additionally, all study sites were selected based on their location on the forest edge (e.g. bordering pastures, open fields, or powerline corridors). Of the twelve sites, six were sites with ash trees and six were sites with no ash trees (i.e. control sites). The purpose of control sites were primarily to ensure that changes in variables such as canopy cover and/or woody vine cover could be attributed to loss of ash trees, and not due to other phenomena such as weather events. While species composition was inherently different due to differences in ash versus non-ash forests, efforts to ensure that the control sites were similar otherwise were made. Specifically, control sites were all deciduous forests and the transects at each site had similar average basal areas to the ash sites. Specifically, ash site transects averaged 1.52 +/- 0.10 m^2 in basal area and control site transects averaged 1.37 +/- 0.15 m^2 in basal area. Furthermore, the average elevation at ash versus control sites was similar with ash sites on average being at 452.9 +/- 36.8-m and

control sites on average being at 467.2 +\- 35.8-m. At each study site, 2-3, 10-m x 50-m belt transects were established (three transects per site were established whenever possible), extending from the forest edge into the core (Figure 2). As in Klooster et al. (2013), all transects were established a minimum of 20-m apart from adjacent transects, with two exceptions in which transects were placed 10-m apart due to the length of the edge of the forest stand.

Because the length that edge habitat extends into a forest varies from location to location, the border between "edge" and "core" habitat of each transect (Figure 2) was determined based on the following habitat characteristics: reduced (edge) versus full (core) canopy height, greater presence of trees <12 cm DBH (edge), and the general presence (edge) versus absence (core) of herbaceous ground cover. Importantly, the end of the 50-m transect in the forest core was required to be a minimum of 50-m from any other edges, thus precluding smaller forest fragments from this study. Finally, since EAB is known to be able to infest trees ≥2.5-cm DBH (Herms and McCullough 2014), ash sites were required to have at least one ash tree ≥2.5-cm DBH within both the edge and the core of each transect, whereas control sites were characterized as having no ash trees ≥2.5-cm DBH within each transect.

Finally, four 1-m by 1-m microplots were established in each transect (Figure 2) and marked with survey stakes so as to be able to survey the same spot each year. Specifically, within each transect at control sites, two microplots were located in the middle of the transect (widthwise) at 0-m and 50-m (Figure 2). The other two microplots were placed in the middle of the transect (widthwise) halfway into the edge and halfway into the core, respectively. In order to ensure that changes in canopy and/or ash seedling numbers were captured at ash sites, an identical scheme was followed excepting whichever microplot in both the edge and core was closest to an ash tree; this microplot was placed 1-m away from the base of the ash tree. If both microplots initially

appeared to be equidistant from ash trees in either the edge or the core, one microplot was moved away from the ash tree in order to clear the ash canopy cover.

Site Surveys

Sites were surveyed, in June-September (prior to leaves beginning to drop) annually from 2017-2020[1]. Specifically, within the transects, all trees ≥2.5-cm DBH were identified and their DBH measured. Tree location by distance along the transect was noted so that individual trees could be tracked across all years. Trees were classified as "small" (2.5-12.5-cm DBH) or "large" (>12.5-cm DBH). For all ash trees, regardless of size classification, any signs of EAB infestation were noted: D-shaped exit holes, leaf thinning or dieback, epicormic shoots, bark flecking, and S-shaped larval galleries. Canopy dieback was quantified using a 1-5 dieback scale as outlined in Smith (2006) (Table 2, Figure 3). To ensure that microplot data could be compared from year-to-year, in addition to marking the locations with permanent stakes, orientation of the 1-m by 1-m quadrat was always aligned to the cardinal directions. For microplots, all woody seedlings <2.5-cm were identified, counted, and classified as >30.5-cm or <30.5-cm. Woody vines were also quantified based off of estimated percent cover and classified based on size (>30.5-cm or <30.5-cm). Canopy cover directly above the center of each microplot was measured using a spherical crown densiometer, with values taken at each of the four cardinal directions and averaged.

Data Analyses

Relative abundance was calculated for each species by dividing the total number of individuals of a species by the total number of trees at a transect. This value was averaged across all sites for both size classes in order to characterize species abundance across sites. The four initial canopy cover values taken at each microplot were averaged and multiplied by a factor of

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[1] Dr. Elizabeth Gleim collected data across all study years. Catherine Kirkpatrick collected data from 2017-2018, Madison Simms Hill collected data from 2018-2019, and Bronte Hoefer collected data from 2019-2020.

1.04 and divided by 100 to result in the percent canopy cover at each microplot (Forestry

Suppliers, Inc.).

Shannon Diversity Index (SDI) was calculated for all transects at both ash and control

sites, using the equation:

$$H = -\sum_{i=1}^{S} p_i \, lnp_i$$

Where H = SDI value, s = the total number of species in the community (species richness), p_i =

the proportion of species i to the total number of species, and ln = the natural logarithm. Notably,

to capture changes in biodiversity due to mortality of ash trees for any given year, individual ash

trees that had a dieback score of 5 were considered "removed" from the transect and thus

excluded from these calculations, along with any trees of other species that had died during the

course of the study.

Further, to calculate Shannon's equitability (E_H), e.g. species evenness, the maximum

possible H value at each transect was calculated using the same equation assuming equal

abundance of all species, and this value was deemed H_{max}. A proportion of H to H_{max} was

calculated to determine E_H, and thus the resultant value fell within a normalized range of 0-1,

with a value closer to 0 indicating lesser species evenness, and a value closer to 1 indicating

higher species evenness.

For all analyses, variables were examined to determine whether they were normally

distributed by examining histograms and normal probability plots of residuals. Dieback score

data were normally distributed. Data for canopy cover, SDI, Shannon's equitability, woody vine

species, and ash seedlings were not normally distributed. However, canopy cover, SDI and

Shannon's equitability data were successfully transformed using a Johnson transformation. Ash seedlings and vine data could not be successfully transformed.

For dieback scores and the transformed canopy cover, SDI, and Shannon's equitability data, a mixed effects linear regression model with random effect for site was run (Minitab 19, 2019). This model was utilized as it accounted for repeated measures and clustering by site. In the case of canopy cover, SDI, and Shannon's equitability, the model was specifically used to examine differences between ash and control sites and between edge and core across all years. For dieback scores, this model was used to examine differences between years and between large and small trees at the edge and core of ash sites. For instances in which interactions between variables were identified in the initial model, the specific nature of the interactions was determined with mixed effects linear models which examined just the two interacting variables(Minitab 19, 2019).

Much of the woody vine cover was attributable to native species, and *Rubus* spp. were only identified on a genus-level and not distinguished between native and non-native species. Thus, data for non-native and native woody vine species, both which exhibit invasive qualities, were pooled for analyses and averaged by site. Due to differences in sampling estimation in 2017, data for woody vine species were only analyzed across study years 2018-2020. Because data was not normally distributed, they were categorized into three groups: 1) sites at which woody vine cover was <1%, 2) sites at which woody vine cover was 1-10%, and 3) sites at which woody vine cover was >10%. The categorized groups were then analyzed using a mixed ordinal logistic regression model with a random effect for site in order to compare woody vine cover in the edge versus core at ash versus control sites over time (StataCorp, 2021). This model was selected as it was appropriate for ordinal response data while also still accounting for repeated

measures and clustering by site. The model was built in a step-wise fashion in which an initial model was built that included main effect terms and all possible two-way interactions between variables. Non-significant two-way interactions were then removed one at a time, always removing the least significant variable, until only significant variables and main effect terms for variables of interest remained.

Data for number of ash seedlings present at ash site microplots over time were averaged by site. Since data violated assumptions of normality and could not be successfully transformed, a mixed effects negative binomial regression with a random effect for site was used to determine differences in the mean number of ash seedlings at ash sites between edge and core across time (StataCorp, 2021). This model was selected because it accounted for the high number of zeros in this data set while also accounting for repeated measures and clustering by site. Step-wise model selection was done as previously described in order to determine significance of a two-way interaction between edge vs. core and year, retaining main effect terms regardless of significance.

Results

Canopy Cover

Ash sites had significantly higher mean canopy cover (90.84 +/- 0.53%) than control sites (83.56 +/- 1.61%) ($F_{1.00, 27.29} = 9.28$, $p = 0.005$) (Figure 4). A significant interaction between edge versus core and year was also found ($F_{3.00, 29.31} = 8.38$, $p < 0.000$; Figure 5). There was significantly lower conditional mean canopy cover in the edge (84.58 +/- 2.82) compared to the core (93.91 +/- 0.50) in the year 2017 ($p = 0.014$), but this difference was not found to be significant for any other study year (Figure 5). No significant changes in mean canopy cover in the edge were noted over time (Figure 5). Mean canopy cover in the core fluctuated throughout

the study, having significantly lower cover in 2018 (87.52 +/- 2.65, $p = 0.002$) and 2020 (88.87 +/- 1.07, $p = 0.005$), but significantly higher in 2017 (93.91 +/- 0.50 $p = 0.000$) in comparison to the other study years (Figure 5).

Dieback Scores

Trees in the core had significantly higher mean dieback scores (2.82 +/- 0.18) than trees in the edge (2.26 +/- 0.17) ($F_{1.00, 59.39} = 13.13$, $p = 0.001$) (Figure 6). There was a significant interaction between size class and year ($F_{3.00, 59.00} = 3.55$, $p = 0.020$) wherein large trees had significantly lower mean dieback scores in 2017 (2.09 +/- 0.30, $p = 0.001$) and 2018 (2.24 +/- 0.42, $p = 0.021$) in comparison to 2020 (3.80 +/- 0.25, $p = 0.001$), but not in 2019 (3.31 +/- 0.36, $p = 0.088$) (Figure 7). The increase in mean dieback score over time for small trees was not significant for any year. The mean dieback score of large trees did not significantly differ from small trees during any study year except 2020, in which large trees (3.80 +/- 0.25) had significantly higher dieback scores than small trees (2.40 +/- 0.32, $p = 0.028$). Notably, small trees appear to be progressing in mortality much more gradually than large trees. For example, mean scores of small trees progressed from 1.99 (+/- 0.31) in 2017 to 2.40 (+/- 0.32) by 2020, while average scores of large trees progressed from 2.09 (+/- 0.30) in 2017 to 3.80 (+/- 0.25) by 2020.

Shannon Diversity Index and Shannon Equitability

Ash sites had significantly higher mean SDI scores (2.21 +/- 0.07) than control sites (1.79 +/- 0.06) ($F_{1.00, 42.87} = 60.99$, $p = 0.000$). No significant differences in conditional means were noted between study years ($F_{3.00, 36.85} = 0.12$, $p = 0.950$) nor was there a significant interaction between ash and control and year ($F_{3.00, 36.85} = 0.09$, $p = 0.965$) (Figure 8).

Conversely, there were no significant differences between the mean E_H scores of ash (0.039 +/- 0.43) and control sites (-0.052 +/- 0.43)($F_{1.00, 9.99} = 0.05$, p = 0.825) nor was a significant interaction between ash and control and year found ($F_{3.00, 28.99} = 0.54$, $p = 0.662$). However, there were significant changes in E_H scores over time. A significant decrease in the transformed mean equitability value was noted between 2018 (0.11 +/- 0.311) and 2019 (-0.15 +/- 0.311)($F_{3.00, 28.99} = 4.11$, $p = 0.015$) (Figure 9).

Woody Vine Cover

There were no significant changes in woody vine cover over time. However, the edge of the forest did have significantly higher woody vine cover (14.22 +/- 3.02%) compared to the core (6.51 +/- 3.27%) [OR = 22.58, $z = 3.93$ (95% CI: 4.77, 106.814), $p = 0.000$] (Figure 10). Furthermore, ash sites had significantly higher mean percent woody vine cover (20.72 +/- 8.17%) as compared to control sites (7.13 +/- 3.67%) [$z = 2.89$ (95% CI: 0.119, 0.626) $p = 0.004$].

Ash Seedlings

The edge of ash sites did have significantly lower mean number of ash seedlings (8.04 +/- 0.98) than the core (20.2 +/- 2.27) (Table 3, Figure 11). Although year was not significantly associated with the average number of ash seedlings at ash sites, there was a steady decline in the average number of ash seedlings at ash sites over time with the difference between the average number of ash seedlings in 2017 (20.6 +/- 2.46) and 2020 (10.5 +/- 1.35) approaching significance (Table 3).

Discussion

To date, all previous studies examining the ecological impacts of EAB invasion have occurred near the epicenter of invasion in Ohio and Michigan (Smith 2006, Klooster et al. 2014,

Kashian 2016, Costilow et al. 2017, Davis et al. 2017). Therefore, this is the first study examining ecological impacts of EAB outside of the initial epicenter and thus provides valuable insight on EAB invasion in other ecosystems with different plant community composition and climate. This is also the first study to examine the role of edge effects in EAB infestation dynamics and forest responses.

Mean dieback scores of 1.99 and 2.09 in small and large trees, respectively, in 2017 indicate that both large and small trees were either un-infested or in the very early stages of EAB infestation at the beginning of the study. This, in conjunction with the fact that dieback scores have increased over time, indicates that we were able to begin monitoring trees early on in EAB invasion in the Roanoke Valley and thus have been able to document EAB infestation dynamics and forest responses as ash tree mortality progressed.

The mean dieback score of large trees was significantly higher than in small trees only in 2020, supporting prior research indicating that larger trees are likely to progress in mortality more rapidly than do small trees (Robinett and McCullough 2019). Indeed, Robinett and McCullough (2018) found a negative relationship between average DBH and white ash survival rate. Specifically, they hypothesized that larger, older white ash may exhibit less vigor than smaller, younger trees (MacFarlane and Meyer 2005), thus attracting ovipositing female EAB adults who prefer stressed trees (McCullough et al. 2009, Siegart et al. 2010). Furthermore, EAB has been shown to prefer to feed on leaves grown in the sun, perhaps due to higher total protein concentration in such foliage (Chen and Poland 2009). Because larger trees are higher in the canopy and thus receive more sunlight, they may be preferable to smaller trees which receive less sunlight. Additionally, it has been suggested that EAB use visual cues to locate host trees

(McCullough et al 2009). Therefore, smaller trees may simply be obscured by the thick canopy of surrounding foliage whereas canopy-dominant mature ash are not.

Interestingly, while the mean dieback score increased somewhat for small trees, the change was not significant. Conversely, the mean dieback score for large trees was significantly higher and progressed more rapidly over time. This rapid decline may be due to the increase in stress and subsequent decrease in tree vigor as EAB infestation progresses in a tree. Importantly, progression of mortality is based upon a number of factors - for example, large trees may reach peak infestation earlier due to being infested first (Robinett and McCullough 2019). Large ash trees may also exhibit lower vigor, such as ability to respond to pests and ability to survive in less-favorable environmental conditions, in comparison to smaller trees (Marshall et al. 2009, Porter 2009). Furthermore, several studies have noted that trees with rougher bark are more suitable oviposition sites for adult EAB females (Anulewicz et al. 2006, Marshall et al. 2009). The deeper bark furrowing characteristic of mature ash trees may be preferable to the shallower, smoother bark of young trees, thus potentially leading to earlier colonization and/or higher larval loads.

Contrary to our hypothesis that trees would die more rapidly in the edge, trees in the core had significantly higher mean dieback scores than trees in the edge. One might postulate that perhaps higher densities of mature trees in the core were attracting higher densities of EAB. However, there were no differences between the density of large trees in the edge versus the core of ash sites (data not shown). Furthermore, previous research has shown that EAB infestation is not host density-dependent (Smith 2006). An alternative hypothesis to explain this phenomenon may be that ash trees are experiencing higher competition with surrounding overstory species in the core, and subsequently less-ideal growing conditions as compared to the edge where there is

increased light availability. Indeed, increased competition with overstory trees has been previously suggested to cause higher mortality for small trees in these conditions (Robinett and McCullough 2019). It is also possible that the dispersal of EAB through the core of the forest rather than along the edges was preferable, although additional research would be needed to explore this hypothesis.

In terms of the length of time it took for ash trees to die, past studies have indicated that large and small ash trees generally reach full mortality within 2-5 years following initial infestation (Smith 2006, Herms and McCullough 2014, Klooster et al. 2014). However, small trees in our study appear to be progressing in mortality much more gradually than large trees. Notably, *Fraxinus pennsylvanica* and *F. americana*, the species present at our study sites, have been found to exhibit varying rates of mortality. For example, some studies have documented little-to-no resistance and thus mass mortality within 2-5 years (Gandhi and Herms 2010, Klooster et al. 2014), while other studies have noted extended morbidity lasting beyond 5 years for large trees, with extremely variable survival rates by location (Knight et al. 2013, Robinett and McCullough 2019). In these studies, it was postulated that survivability may be related to larval densities in trees and the size of the tree, as well as the high density of smaller ash in forest stands. Therefore, the more gradual decline seen in small ash trees may be attributable to size of tree EAB adults prefer, differential habitat characteristics based off of the size and relative age of the ash tree, biological differences between older and younger trees, or a combination of these factors.

Mean percent canopy cover was significantly higher at ash sites in comparison to control sites. This is likely due to the ecological conditions characterizing the sites – the edge of several ash sites tended to be remarkably more overgrown than control sites, and thus the dense mid-

story of these edges may have artificially increased canopy cover readings as any cover over waist-height would have been included in canopy cover estimations. Notably, a denser mid-story is often indicative of an area that has been more recently or more frequently disturbed. It is unclear whether this difference is characteristic of ash-dominant forests in the region, or if this is a random occurrence. It is of note, however, that because of the tendency of ash species to opportunistically regenerate in canopy gaps with increased sunlight, ash has tended to be an early-successional species that progresses to canopy dominance in secondary forests following logging (Clebsch and Busing 1989) and other forest pest events (Smith 2006).

While overall change in canopy cover over time was found to be significant, no significant differences in canopy cover at the edge of our sites was noted over time. Mean canopy cover was significantly lower in the core of our sites in 2018 and 2020, but significantly higher in 2017, and therefore no consistent significant upward or downward trend in canopy cover was noted across all study years. While this could indicate expansion and contraction of the canopy over time, this trend could more plausibly be attributed to differences in sampling estimation by researchers between study years.

While ash sites did exhibit significantly higher mean SDI scores than control sites, there was no significant difference in mean E_H scores between ash and control sites. Further, while no noticeable change in SDI occurred over time, mean E_H score decreased significantly between study years 2018 and 2019. This difference may be attributable to the sharp increase in ash tree mortality observed between the two study years, lowering relative evenness as ash trees reach total mortality and were subsequently removed from calculations. It is plausible to hypothesize that loss of biodiversity may occur in a longer time frame as ash continues to progress toward total mortality and its presence as a genus is gradually extirpated. Furthermore, changes in

overall species evenness may occur should proportions of species change in response to canopy gap formation. However, patterns in species evenness with succession are poorly understood due to their inherent complexity (Schaetzl et al. 1988, Beckage et al. 2000). Regardless, high levels of biodiversity and even distribution of species are both important to maintaining ecosystem resiliency with regards to secondary-successional trajectories post-disturbance (Connell 1978, Derroire et el. 2016). Even with loss of ash as a genus in these ecosystems due to EAB, the ecosystems may remain resilient due to their high biodiversity and relative evenness.

With regards to ash regeneration through seed germination and seedling survival, while the current study did not document significant declines in the number of ash seedlings over time, consistent declines were observed and approached significance by the end of the study. This is in line with previous studies which have found that ash do not form a persistent seed bank (Taylor 1972, Kashian 2016). Accordingly, as reproductively mature ash continue to progress to full mortality, the ash seedbank ceases regeneration and number of ash seedlings decreases (Klooster et al. 2014).

Furthermore, the edge of ash sites had a lower presence of ash seedlings than the core (Clebsch and Busing 1989, Smith 2006). This difference was consistent across study years prior to widespread mortality of reproductively mature ash trees. This difference between the edge and core is perhaps due to increased environmental stress and competition present at the forest edge.

Notably, there was significantly higher woody vine cover present in the edge of ash sites than the core. Furthermore, invasive woody vine species were also significantly more abundant at ash sites. Because these trends were present from the beginning of the study, the presence of these invasive species is unlikely to be related to the invasion of EAB. Invasive woody plant species are often associated with forest edges because they tend to be adapted to disturbance, and edge

spaces provide an optimal "point of invasion" for propagules and seeds (Flory and Clay 2006).

However, the presence of these invasive species does have implications for, 1) why fewer ash

seedlings are present in the forest edge, and 2) the forest successional response to ash mortality.

Notably, the increased vine cover at the edge may mitigate some microclimatic alterations in soil

conditions associated with canopy gap formation. However, because invasive woody plant

species are extremely disturbance-adapted, it is possible that they may outcompete ash seedlings

observed in the edges of study sites over time. As ash in the edge continue to progress towards

total mortality and thus cease seedbank regeneration, ash seedling recruitment and survival could

cease entirely, resulting in the potential extirpation of ash in forest edges. Therefore, as is

consistent with a number of previous studies, it is expected that the potential for survival of ash

as a genus at these sites may largely hinge upon the ability of currently established ash seedlings

in the forest core to successfully compete with other seedlings (Davis et al. 2017) and evade low-

density EAB populations in the future (Klooster et al. 2014).

Conclusions

Increased environmental stress at the forest edge does not appear to impact ash mortality

over time. Consistent with the findings of prior research, larger trees, regardless of proximity to

the forest edge, are infested first and increase in mortality more rapidly than smaller trees

(Robinett and McCullough 2018). As dieback of reproductively mature ash trees progresses,

mean number of ash seedlings, although not yet statistically significant, is steadily declining.

Because invasive woody vine species are almost exclusively present in the edge of ash sites in

conjunction with potentially lower ash seed production at the edge, ash seedlings may decline

and ultimately be extirpated over time at the forest edge. Therefore, the survival of ash within

these ecosystems may hinge upon the ability of ash seedlings in the core to successfully

outcompete other species and evade EAB infestation in the future. In areas in which substantial loss due to EAB has not yet occurred, future studies may be warranted to examine ash seed production and ash seed banks at the edge and core of forests in order to better understand these trends.

Finally, a previous study by Costilow et al. (2017) conducted in northwest Ohio demonstrated that *Acer* spp. surrounding ash tended to increase in crown class and radial growth rate following EAB infestation and subsequent mortality. Therefore, the use of increment core sampling as per Costilow et al. (2017) to further investigate the potential radial growth response of small and large trees surrounding dead ash as a differential forest response to gap formation is warranted. Additional analyses should be explored in order to forecast which species are likely to replace ash in forests in southwest Virginia.

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Tables and Figures

Table 1: Top ten species for mean species abundance value across all study sites (ash and control) and all size classes (small and large).

Species	Mean Species Abundance +/- SE
N. sylvatica	13.83 +/- 20.48%
Prunus spp.	9.90 +/- 11.86%
A. saccharum	5.13 +/- 6.65%
*Fraxinus spp.**	5.54 +/- 6.57%
A. rubrum	4.93 +/- 2.56%
C. glabra	4.26 +/- 3.73%
Q. alba	3.89 +/- 2.02%
O. arboreum	3.80 +/- 6.24%
J. virginiana	3.32 +/- 5.54%
A. altissima	3.10 +/- 2.22%

* As study sites were selected based off of either presence or lack of *Fraxinus spp.,* their mean species abundance is likely skewed. At ash sites, mean abundance of *Fraxinus spp.* was 11.07+/- 0.02%.

Table 2: Dieback scoring (1-5) parameters used in the current study as per Smith (2006).

Dieback Score	Canopy Characteristics
1	Healthy; no infestation
2	Minor thinning, no substantial dieback
3	10-49% dieback
4	50-99% dieback
5	100% dieback; complete mortality

Table 3: Mixed effects negative binomial regression model for the prediction of ash seedling counts at ash sites.

Variable	Coefficient (SE)	IRR (95% CI)	P
Location			0.002
Edge	Reference	Reference	
Core	-0.694 (0.227)	0.50 (0.32, 0.78)	
Year			0.299
2017	Reference	Reference	
2018	-0.281 (0.296)	0.75 (0.42, 1.35)	0.342
2019	-0.357 (0.297)	0.70 (0.39, 1.25)	0.230
2020	-0.571 (0.302)	0.56 (0.31, 1.02)	0.059
Constant	2.954 (0.371)	NA	< 0.001

SE = Standard error; IRR = Incidence rate ratio; NA = Not applicable
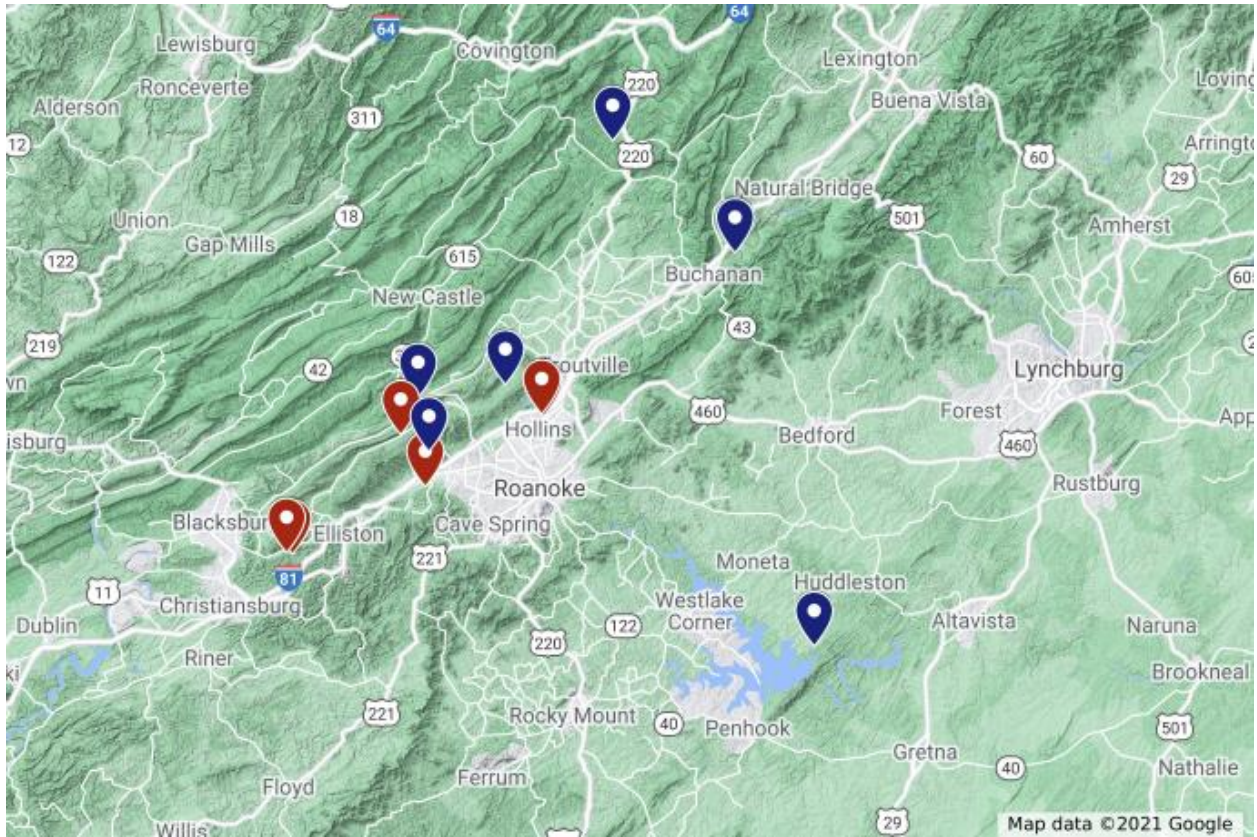
Figure 1: Map of study site locations in the Roanoke Valley of Southwest Virginia. Blue markers represent control sites, red markers represent ash sites.
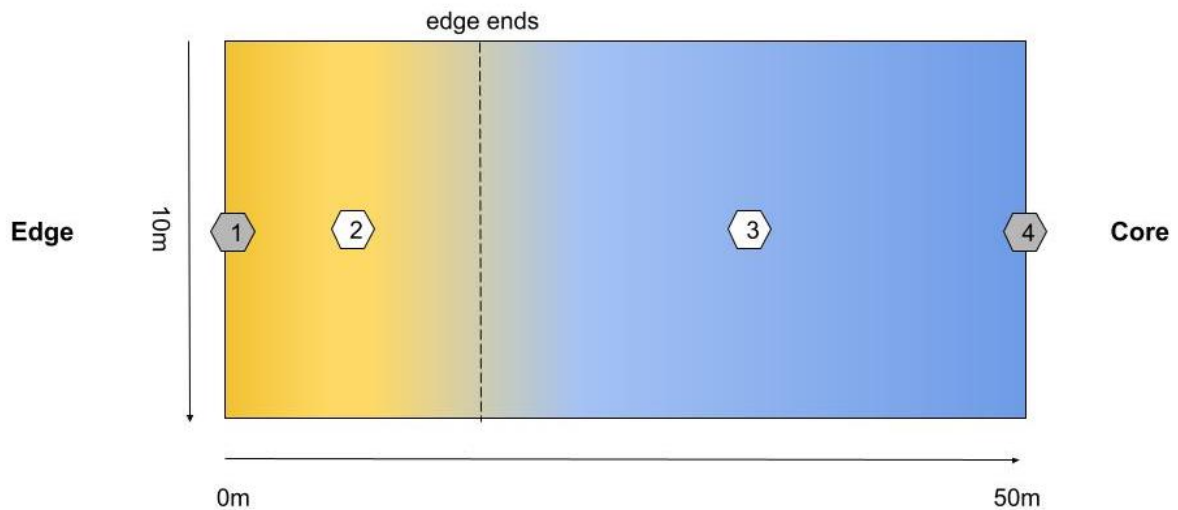

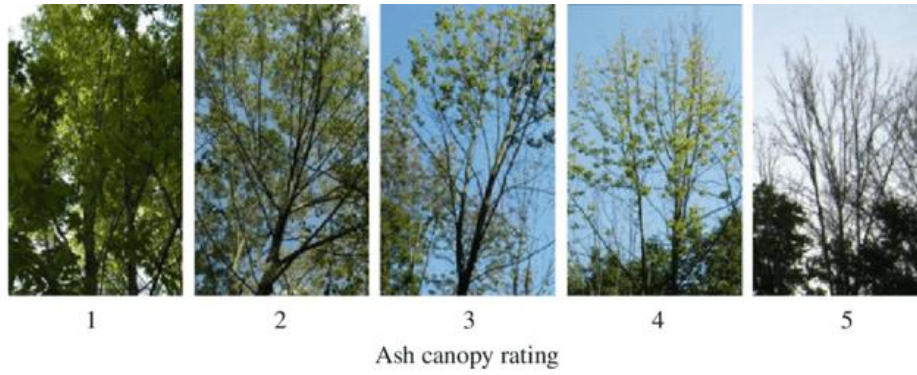**Figure 2**: General transect layout at study sites. Numbered hexagons represent microplots.

Figure 3: A visual representation of the 1-5 dieback canopy rating scale, where a score of 1 indicates a healthy tree with no visible signs of infestation, and a score of 5 indicates a tree with 100% mortality (Image Credit: Klooster et al. 2013).
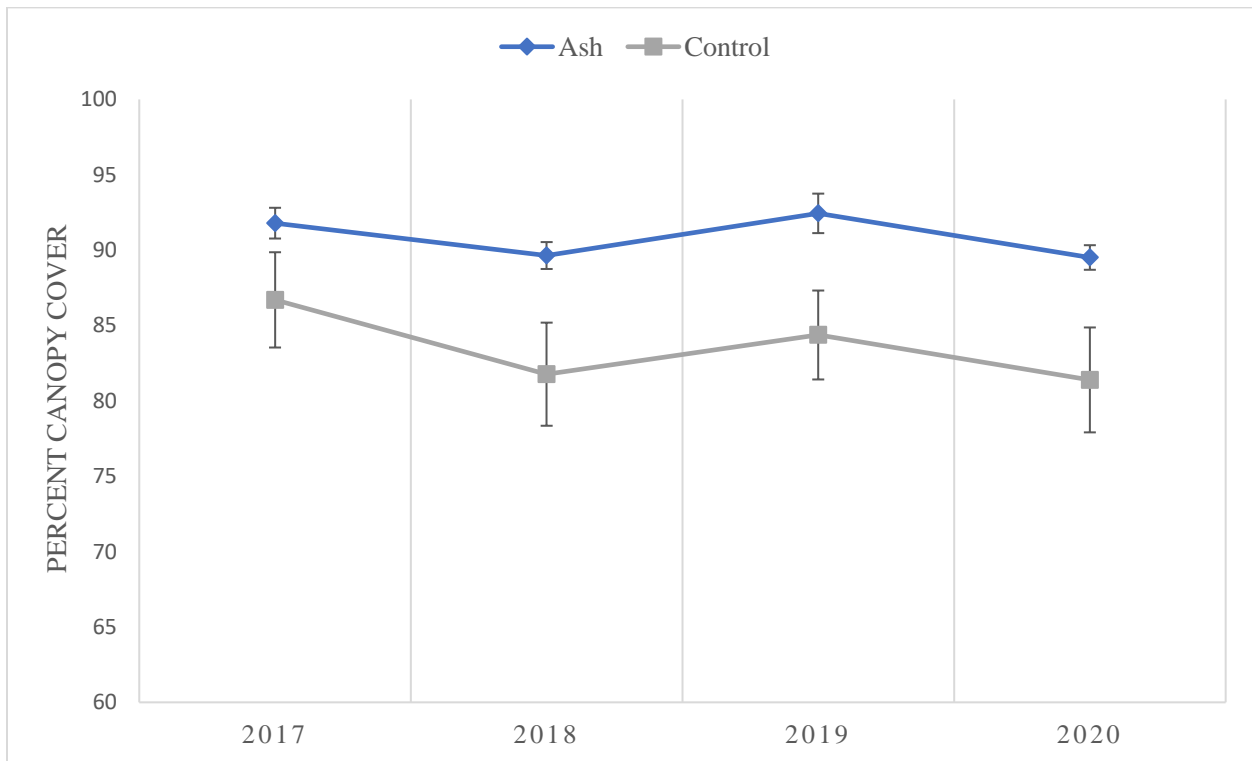

Figure 4: Mean canopy cover at ash and control sites across study years. Error bars denote standard error.
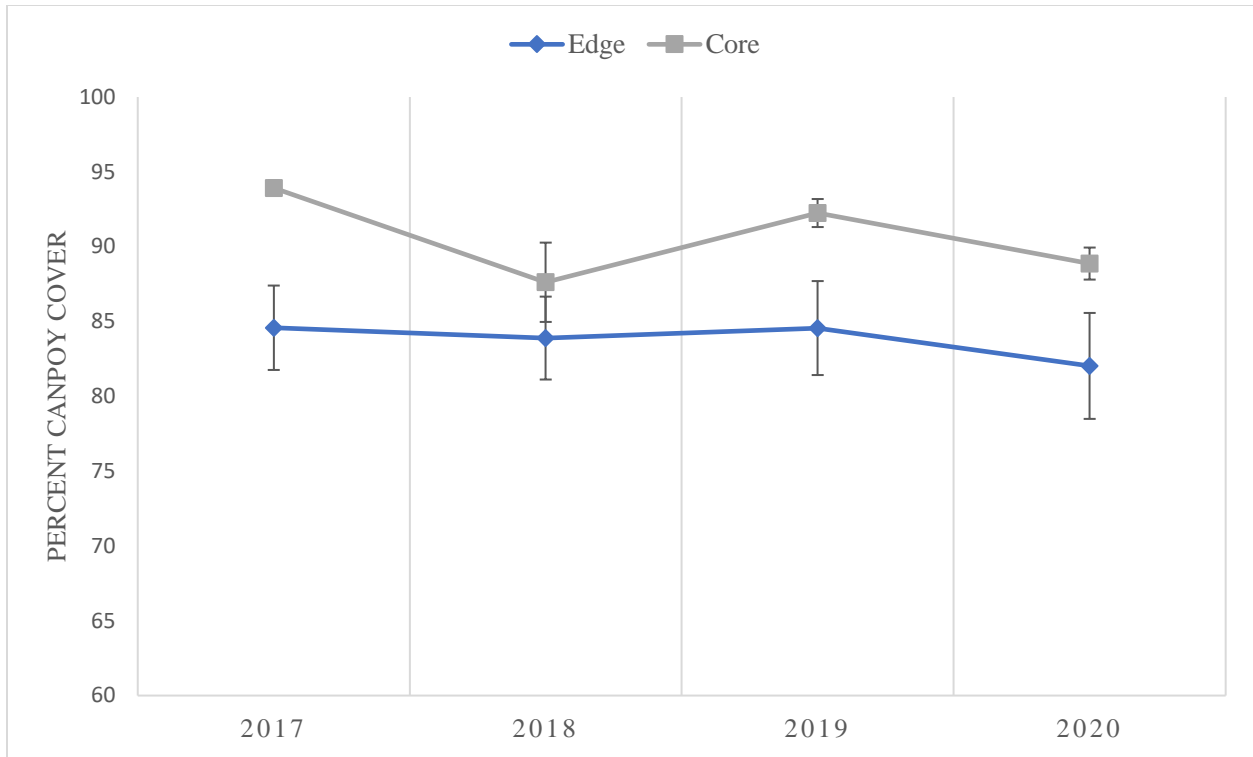
Figure 5: Mean canopy cover between edge and core from study years 2017-2020. Error bars denote standard error of the mean.
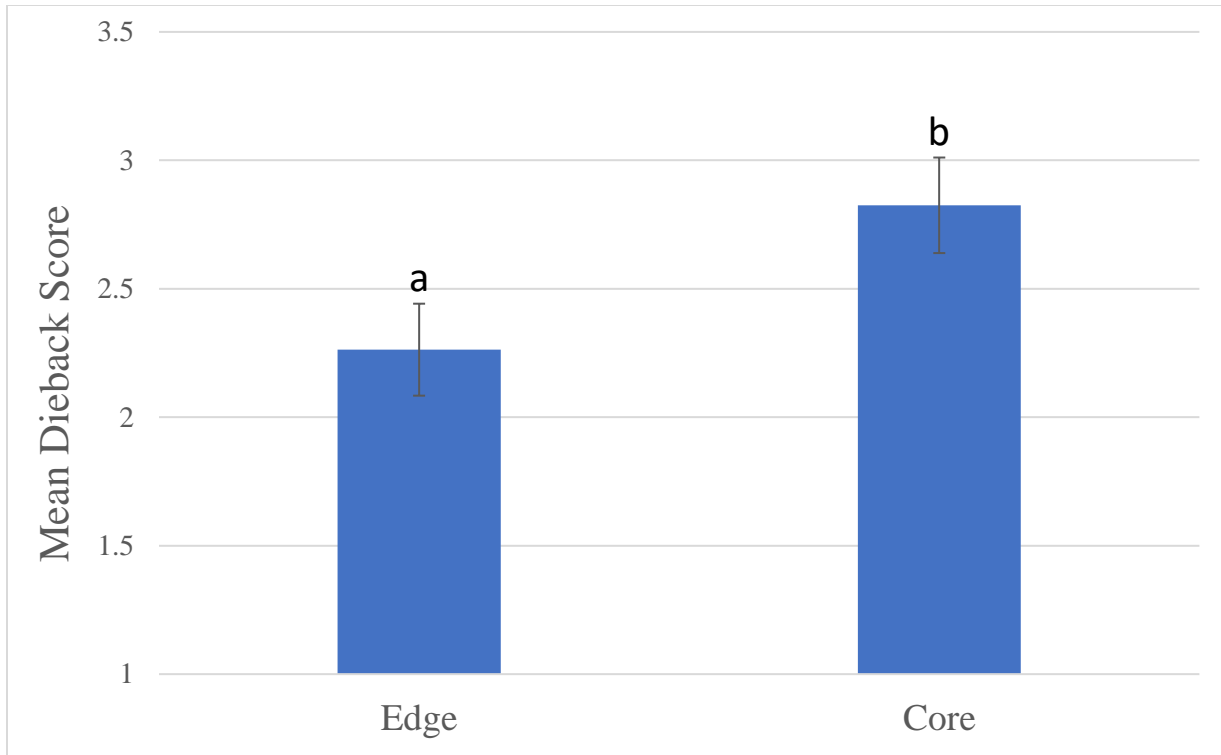
Figure 6: Mean dieback score of ash trees at the edge and core of transects. Different letters denote values that are significantly different ($p < 0.05$). Score of 1 indicates no infestation and score of 5 indicates a completely dead tree.
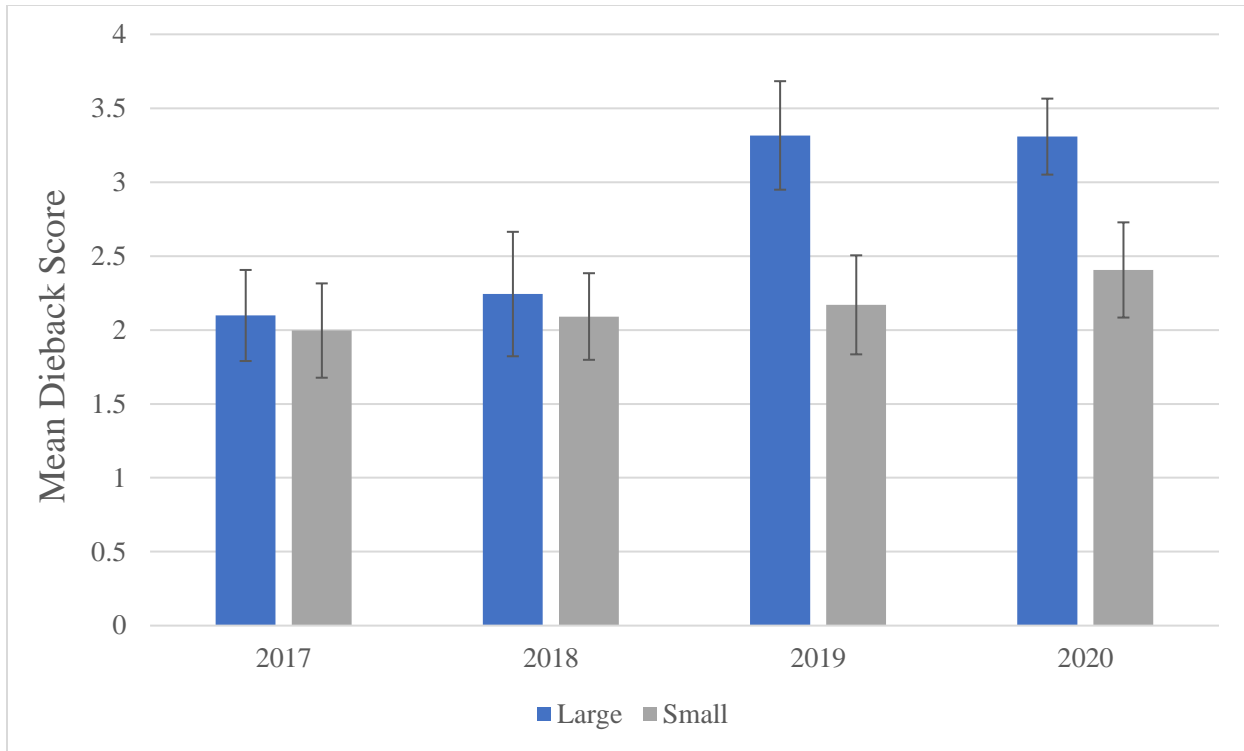
Figure 7: Mean dieback score of large (>12-cm DBH) and small (≥2.5-cm <12-cm DBH) ash trees during study years, where a score of 1 indicates no infestation and score of 5 indicates a completely dead tree. Error bars denote standard error.
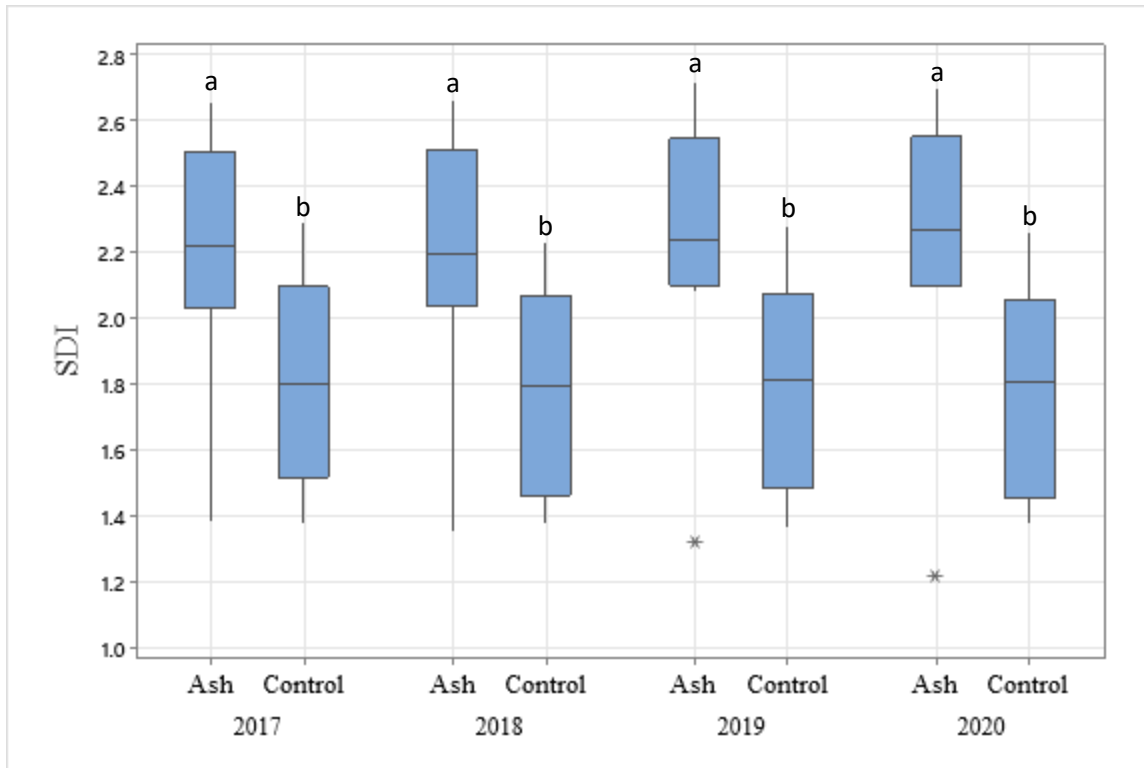
Figure 8: Shannon Diversity Index (SDI) values of tree species at ash and control sites across all study years. Asterisks denote outlier values. Whiskers denote upper and lower quartiles. Data shown in graph is untransformed data – data was transformed for analyses using a Johnson Transformation. Bars sharing the same letter are not significantly different (p > 0.05), and significance refers to transformed values.
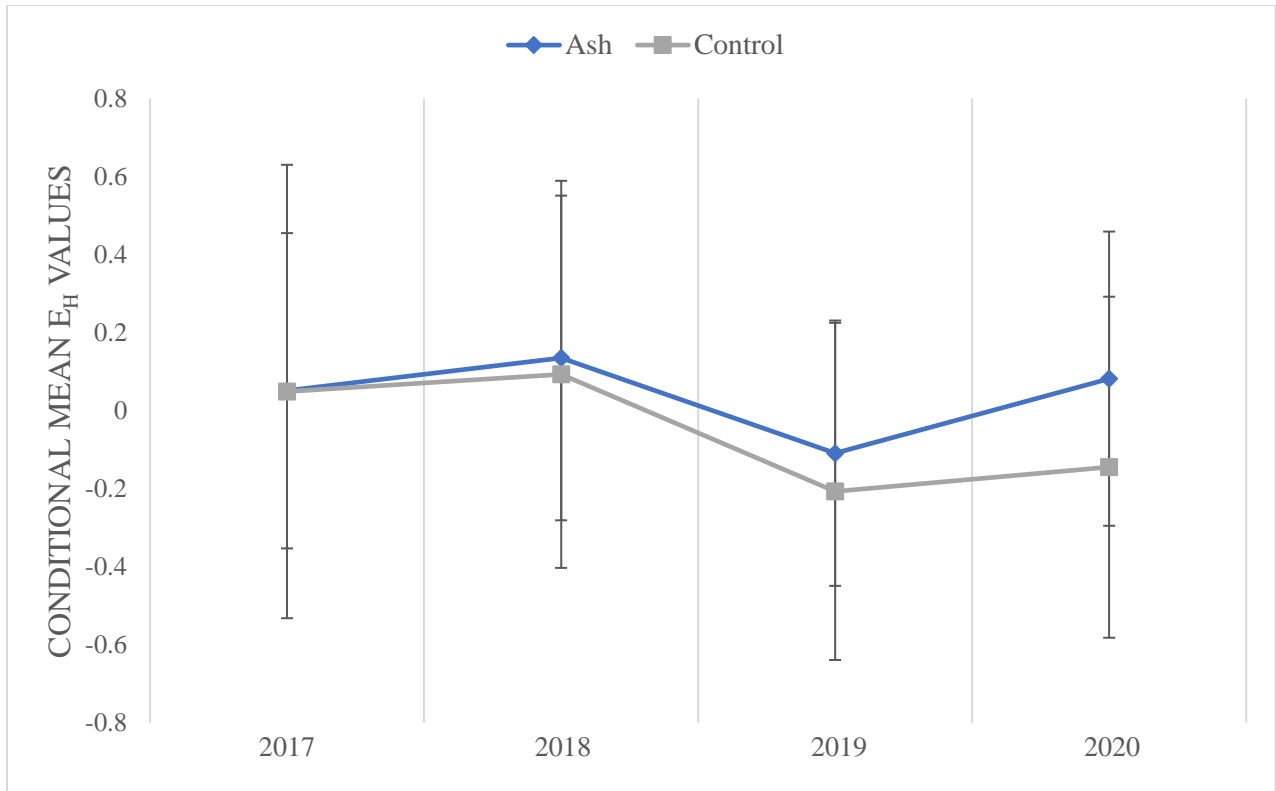
Figure 9: Conditional mean Shannon Equitability (E_H) values for tree species at ash (A) and control (C) sites across all study years. Data transformed using a Johnson Transformation. Error bars denote standard error.
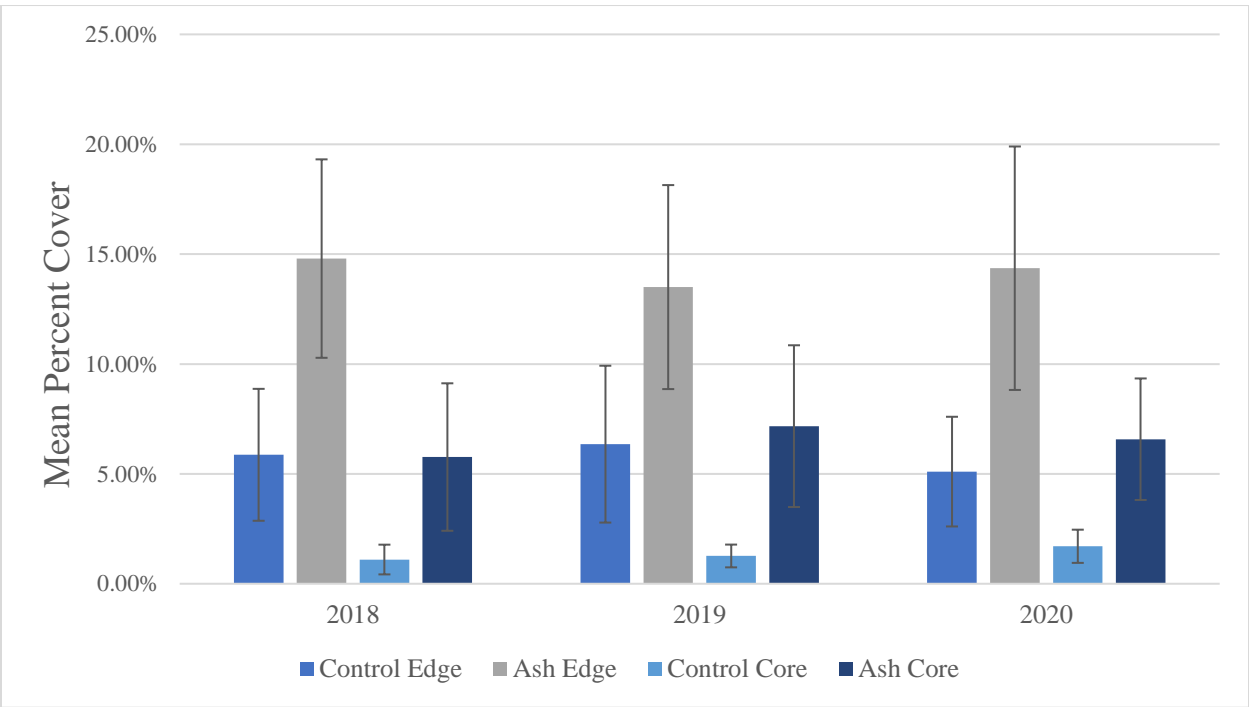
Figure 10: Mean percent cover of woody vine species (non-native and native species) over time at ash and control microplots between edge and core. Note that 2017 data were recorded in different units than data from following years and were thus excluded from data analyses. Error bars represent standard error of the mean.
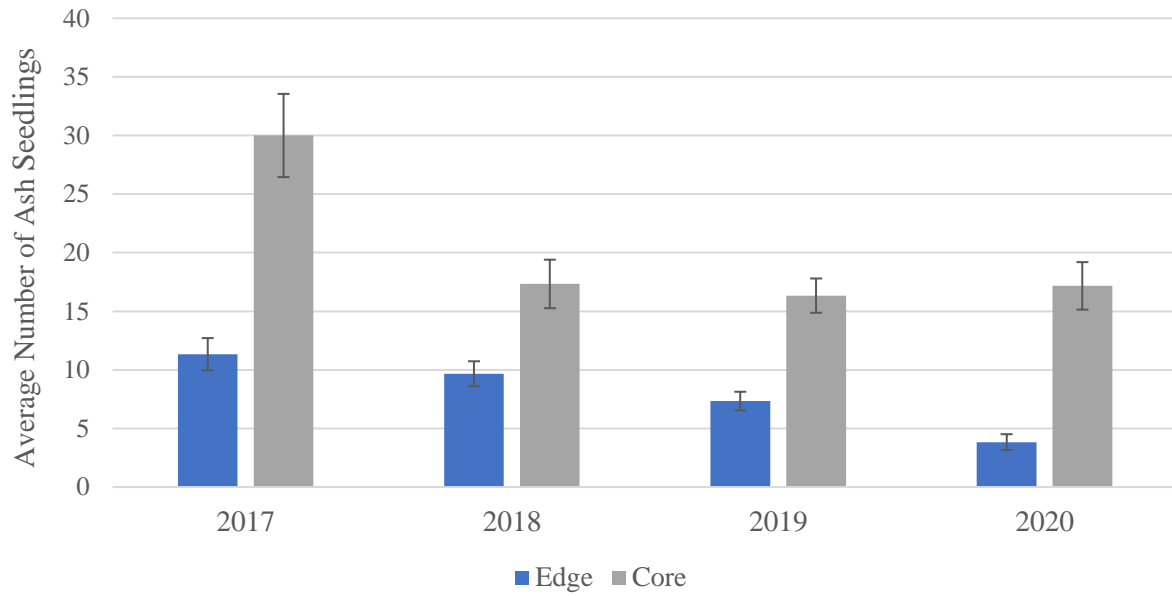
Figure 11: Mean number of ash seedlings over time between edge and core microplots at ash sites. Error bars denote standard error of the mean.