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**VEGETATION DYNAMICS AND NITROGEN CYCLING RESPONSES  
TO SIMULATED EMERALD ASH BORER INFESTATION IN  
FRAXINUS NIGRA-DOMINATED WETLANDS OF UPPER  
MICHIGAN, USA**

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VEGETATION DYNAMICS AND NITROGEN CYCLING RESPONSES TO  
SIMULATED EMERALD ASH BORER INFESTATION IN *FRAXINUS NIGRA*-  
DOMINATED WETLANDS OF UPPER MICHIGAN, USA

By

Joshua C. Davis

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In Forest Science

MICHIGAN TECHNOLOGICAL UNIVERSITY

2016

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This dissertation has been approved in partial fulfillment of the requirements for the Degree of DOCTOR OF PHILOSOPHY in Forest Science.

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## **Preface**

This dissertation has been composed as a collection of three articles, each of which is intended for future journal publication for which I will be the primary author. For all chapters, I led the study design, data collection, and data analysis. The PIs on the grant proposal that funded the majority of this research were Drs. Thomas G. Pypker, Randall K. Kolka, Tony D’Amato, Andrew Storer, Steve Sebestyen, Brian Palik, Mark Fedora, and Joseph Wagenbrenner. Overall study design was guided by Joseph Shannon and Drs. Thomas G. Pypker and Randall K. Kolka. Chapter 2 has been accepted pending revision by the *Canadian Journal of Forest Research* and is in the process of being revised. Co-authors on the submission – Joseph Shannon, Nicholas Bolton, Thomas G. Pypker, and Randall K. Kolka – provided significant feedback on the study design and original draft of the article. Joseph Shannon and Nicholas Bolton additionally assisted with field data collection. In addition to the co-authors who contributed to Chapter 2, Chapters 3 and 4 benefited from the data collection provided by Dr. Matthew Van Grinsven. The members of my committee – Drs. Thomas G. Pypker, Randall K. Kolka, Andrew J. Burton, Rodney A. Chimner, and Bradley H. Baltensperger provided important feedback on the proposed research and initial drafts of the entire dissertation.



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my dog Gus, who contributed nothing, but whose company while up late at night reading was always welcome.

## Dissertation Abstract

Throughout many North American forests, the invasive emerald ash borer (EAB, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)) is a significant threat to biodiversity and ecosystem processes. The fate of *Fraxinus nigra* Marshall (black ash) is of particular concern, as it is frequently a dominant canopy species across much of its range. A multiyear manipulative study was conducted to investigate the potential impacts of EAB on vegetation dynamics and nitrogen (N) cycling in nine *F. nigra*-dominated wetlands of Upper Michigan, USA. The effects of early- and late-stage infestations were simulated by girdling ('Girdle') or felling ('Ash-Cut') all ash stems greater than 2.5 cm in diameter.

Vegetation responses were limited to lower canopy layers and herbaceous species. No change in growth rate of residual overstory species was observed, while sapling growth rate of non-*F. nigra* species increased in the 'Girdle' treatment. Species composition of the seedling layer is shifting to co-dominant species, as densities of *Acer rubrum* L. (red maple) and *Betula alleghaniensis* Britton (yellow birch) increased following treatment. The strongest response was exhibited in the herbaceous community, the areal cover of which nearly doubled by the third post-treatment growing season. Though this expansion did not appear to as yet inhibit regeneration of woody species, the potential to impact future forest recovery remains a concern.

Despite reduced demand for nitrogen by the dominant overstory species, soil N availability did not increase following treatment, though an increasing trend in soil  $\text{NO}_3^-$  was observed in the 'Girdle' treatment. These responses are potentially due to increased microbial activity and biomass, leading to increased N immobilization, which may influenced growth rate responses in retained species. Biochemical and morphological changes observed in overstory foliage were thus driven predominantly by aboveground conditions, as leaves acclimated to shade were replaced with sun-acclimated leaves. Succession to *A. rubrum* or *B. alleghaniensis*, the litter from which has lower N content than *F. nigra*, may lead to slower but more complete litter decomposition, potentially affecting future soil carbon storage and nutrient cycling rates.

All examined forest canopies were net sinks for atmospheric N deposition. However, this sink strength was reduced by the ‘Girdle’ and ‘Ash-Cut’ treatments and led to increased deposition of  $\text{NO}_3^-$  to the forest floor in the ‘Ash-Cut’ treatment. Although these effects were relatively minor in these forests, the observed response has important implications in regions with greater N deposition. In forests of these regions, the reduction in retention following the loss of *Fraxinus* species may exacerbate the impacts of N pollution.

These results highlight the interconnected nature of ecosystem processes and suggest potential consequences of EAB invasion in this forest type. In the near term, *F. nigra* mortality may result in increased export of  $\text{NO}_3^-$  from the ecosystem, which may be amplified by reducing the canopy sink strength for atmospherically deposited N. In the long term, the recovery of woody vegetation is uncertain, due to competition with herbaceous species. Should recovery of a closed forest canopy occur, the foliar and litter characteristics of the replacement species may reduce carbon storage and site fertility, through feedback mechanisms between the canopy and forest floor. Understanding these potential responses is critical to developing mitigation strategies to reduce the impacts of EAB on ecosystem processes and the provisioning of ecosystem services.

## 1.0 Introduction

Invasive insects are a serious threat to forests across North America (Lovett et al. 2006). The effects of introduced insects and associated pathogens have resulted in widespread alteration to the structure of these forests and ecosystem processes (Gandhi and Herms 2010). In addition to these ecological consequences, the estimated economic costs associated with control and mitigation of the negative effects of invasive insects alone range into the billions of US dollars (Pimentel et al. 2005).

Among the recently discovered invasive insects in North America, the emerald ash borer (EAB, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)) has received considerable attention for the breadth, depth, and severity of its potential impacts (Herms and McCullough 2014). First collected near Detroit, Michigan in 2002 (Haack et al. 2002), this invasive wood-boring beetle has likely been present in the United States since the early- to mid-1990s (Siegert et al. 2014). Despite efforts to control the rate and extent of dispersal (Cappaert et al. 2005, Herms and McCullough 2014), EAB has since been discovered in 27 US states and two Canadian provinces (Emerald Ash Borer Info, 2016). All eastern North American ash (genus: *Fraxinus*) species are considered to be potential hosts (Rebek et al. 2008). Infestations occur in stems as small as 2.5 cm in diameter, with mortality often occurring within three to four years of the initial infestation, as the phloem-feeding larvae effectively girdle the host tree (Cappaert et al. 2005, McCullough et al. 2015). Mortality of *Fraxinus* trees in infested stands may exceed 99% (Herms and McCullough 2014).

Concern over the potentially profound effects of EAB on forest composition and ecosystem processes stem from the ubiquity of the genus *Fraxinus* across central and eastern North America (Cappaert et al. 2005). Members of the genus are common in a wide variety of forest types throughout the region, as well as in urban environments (MacFarlane and Meyer 2005). Dramatic economic losses and expenditures related to EAB across such a wide geographic range and diversity of habitats have made EAB the



most costly invasive species in the US to date (Aukema et al. 2011), and these costs may soon total in excess of 10 billion US dollars (Kovacs et al. 2010).

The potential fate of *Fraxinus nigra* Marshall (black ash) is of particular concern (Kashian and Witter 2011). Though other *Fraxinus* species are widespread throughout the eastern half of the continent, they often tend to comprise relatively minor proportions of the canopy (MacFarlane and Meyer 2005). In contrast, *F. nigra* is frequently a major component of deciduous and deciduous-conifer forested wetlands (Wright and Rauscher 1990) and occurs in nearly pure stands in portions of its range (Erdmann et al. 1987), where it has been described as a foundation species (Telander et al. 2015). Within the Lower Peninsula of Michigan, *Acer rubrum* L. (red maple) is often the most common overstory species of the *F.nigra-Ulmus americana* L (American elm)-*A. rubrum* forest type. However, this community tends to be dominated by *F. nigra* in the Upper Peninsula of Michigan, where in areas with very poor drainage and organic soils, a nearly pure *F. nigra* overstory may be found (Erdmann et al. 1987). The current composition of these forests may be due, in part, to the loss of *Ulmus* species to Dutch elm disease during the early and mid-20<sup>th</sup> century (Barnes 1976, Erdmann et al. 1987).

The loss of a foundation species – a single abundant species that contributes significantly to the maintenance of ecosystem processes and provisioning of services (Ellison et al. 2005) – can have dramatic implications for ecosystem function. Foundation species are currently threatened in a number of forest types throughout North America, including *Tsuga canadensis* (L.) Carrière (eastern hemlock) and *Pinus albicaulis* Engelm (whitebark pine), the decline of which can be at least partly attributed to phytophagous insects (Orwig et al. 2002, Ellison et al. 2005). In the case of *T. canadensis*, the threat is posed by the hemlock wooly adelgid (HWA, *Adelges tsugae* Annand), which is one example of the many invasive insect species currently affecting forest ecosystems. Invasive insects frequently have profound direct and indirect effects on ecosystem structure and function (Gandhi and Herms 2010), including impacts on vegetation dynamics and successional trajectory (Lovett et al. 2006), nutrient cycling (Lovett et al.

2002), and canopy interactions with precipitation and nutrient deposition (Guswa and Spence 2012, Lovett et al. 2002). Investigations into the effects of both native and invasive insects and pathogens provide a useful framework from which to examine the potential effects of EAB on North American forests. Much insight can also be derived from the extraordinary body of literature regarding the effects of both natural and anthropogenic disturbance on forest processes (Sousa 1984, Rogers 1996), and the feedbacks between disturbance agents (Turner 2010, Peters et al. 2011).

Mortality or removal of trees in the forest canopy creates gaps that are critical to the development of forest structure and biodiversity (Runkle 1982, Sousa 1984). Retained canopy trees frequently benefit from the increased resource availability that follows gap creation, resulting in increased growth rates (Lorimer and Frelich 1989, Nowacki and Abrams 1997). Lower vegetation layers can similarly exhibit positive responses to canopy openings (Sharma et al. 2006). However, though mortality resulting from insect invasions has been demonstrated to benefit unaffected species (e.g. Nuckolls et al. 2009), the types of gaps created by insects and disease are fundamentally different from those created by fallen trees or harvest (Krasny and Whitmore 1992, Krasny and DiGregorio 2001). As a result, the scope or scale of response from remaining vegetation may differ from other disturbance types (Beckage et al. 2000). Additionally, the sudden change in growing conditions to which surviving trees are exposed can induce a stress response that reduces growth responses in overstory trees (Harrington and Reukema 1983, Jones and Thomas 2004), while understory vegetation can be subject to increased competition from herbaceous vegetation (Roberts 2004, Elliot et al. 2015).

The effects of disturbance on forest biogeochemical processes have been extensively studied. Mortality due to invasive insects may increase soil nutrient availability (e.g. Jenkins et al. 1999) through the interaction of a number of processes (Hunter 2001). These nutrients may then subject to export from the system (Cessna and Nielson 2012), as is frequently the case following forest harvest (Likens et al. 1970, Martin et al. 1986), or may instead be redistributed through the ecosystem (Lovett et al. 2002), potentially

with positive effects on plant nutrition (Griffin et al. 2011). Concurrently, aboveground tissues adapt morphologically and biochemically to the new environmental conditions (Naidu and DeLucia 1997 and 1998, Jones and Thomas 2007) which may, in turn, have important feedback effects on soil properties and processes (Prescott 2002). The fundamentally interconnected nature of above- and belowground processes highlights the importance of considering potential cascading effects in biogeochemical cycles (Bardgett et al. 1998), and the difficulty in applying previously observed response patterns to new disturbance or ecosystem types (Hunter 2001).

The upper forest layers are further linked with the forest floor through the interaction of the canopy with incoming precipitation and nutrient deposition (Parker et al. 1983, Levia and Frost 2006). Precipitation that passes through the forest canopy, termed throughfall, can be enriched or depleted in nutrients, depending on the constituent of interest, forest type, and geographical or landscape position (Lovett 1994, Weathers et al. 2006). Canopy disturbance can alter the enhancement or uptake of nutrients by reducing the strength of interactions with incoming deposition, and may also alter the physical structure of the canopy in such a way as to result in increased deposition of nutrients below retained vegetation (Weathers et al. 2001, De Schrijver et al. 2007). Insect damage may increase the quantity of nutrients delivered below the forest canopy, by enhancing leaching losses (Hollinger 1986, Stadler et al. 2005), increasing contributions from insect exudates (Orwig et al. 2008), or by resulting in defoliated conditions that reduce canopy nutrient uptake (Houle et al. 1999). By altering the quantity and distribution of nutrient deposition to the forest floor, disturbances may improve soil nutrient status (Orwig et al. 2008) or create “hotspots” of nutrient deposition that may positively influence co-occurring vegetation (De Schrijver et al. 2007, Tulloss and Cadenasso 2015).

The interacting effects of the residual vegetation response, soil process impacts, foliar morphological and biochemical acclimation, and effects on incoming precipitation or nutrient deposition combined are likely to produce a response to disturbance that may differ considerably by ecosystem type. Importantly, much of research into disturbance

impacts on forest processes, particularly biogeochemical cycles, has been conducted in upland ecosystems, and as such it is unknown the extent to which similar responses can be expected in forested wetlands. Additional factors, such as disturbance severity, site hydrology, and anthropogenic influences contribute further complexity. As a result, while consideration of similar types of disturbance may be useful in considering the range of potential impacts EAB may have on these forest processes, it remains difficult to predict the magnitude and perhaps even direction of response to infestation in a specific forest type.

The following chapters present the results of a multiyear manipulative experiment into the potential effects of EAB on vegetation and biogeochemical processes in *F. nigra*-dominated wetlands of Upper Michigan, USA. The growth, recruitment, and regeneration responses of residual woody and herbaceous vegetation are presented in Chapter 2, and implications for future restoration of forested conditions are explored. Chapter 3 examines the impacts of *F. nigra* mortality on soil nutrient status and acclimation of aboveground tissues to altered growing conditions, and how these effects may influence short- and long-term vegetation recovery, site fertility, and carbon cycling. In Chapter 4, the interactions of the forest canopy with precipitation and nutrient deposition are investigated, and the consequences of *Fraxinus* decline on canopy uptake and soil nutrient status considered. Finally, Chapter 5 discusses the interactions of these responses, and the potential trajectory of vegetation and biogeochemical response following EAB infestation.

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## **2.0 Vegetation responses to simulated emerald ash borer infestation in *Fraxinus nigra*-dominated wetlands of Upper Michigan, USA<sup>1</sup>**

### **2.1 Abstract**

The invasive emerald ash borer (EAB, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)) is a significant threat to biodiversity and ecosystem processes in North American forests. Of particular concern is the fate of *Fraxinus nigra* Marshall (black ash) which, unlike most members of the genus, is frequently a dominant canopy species across much of its range. To investigate the potential vegetation response to the loss of this foundation species, EAB infestation was simulated in nine *F. nigra*-dominated wetlands in the Upper Peninsula of Michigan, USA. No growth response of residual canopy species occurred over the course of three growing seasons, while a significant increase in non-*Fraxinus* sapling growth rate was observed. Mortality of *F. nigra* did not impact total stem recruitment or regeneration, though species composition is shifting towards *Acer rubrum* L. (red maple) and *Betula alleghaniensis* Britton (yellow birch) in the seedling layer. The herbaceous community exhibited the greatest response, nearly doubling in areal cover by the end of the study. Importantly, this expanded cover was not associated with decreased establishment of new woody seedlings, suggesting that increased competition between these functional groups has not yet impacted the potential for future recovery of woody vegetation in these forests.

### **2.2 Introduction**

Invasive insects are a serious threat to forests across North America (Lovett et al. 2006). The effects of introduced insects and associated pathogens have resulted in widespread alteration to the structure of these forests and ecosystem processes (Gandhi and Herms 2010). Among the more recently discovered invasive insects in North America, the emerald ash borer (EAB, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)) has

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<sup>1</sup> Material contained Chapter 2 has been accepted pending revision by the *Canadian Journal of Forest Research*.

received considerable attention for the breadth, depth, and severity of its potential impacts (Herms and McCullough 2014). Concerns over the potentially profound effects of EAB on forest composition and ecosystem function stem from the ubiquity of the genus *Fraxinus* (ash) across eastern North America (Cappaert et al. 2005). Members of the genus are common in a wide variety of forest types throughout the region, though they often tend to comprise relatively minor proportions of the canopy (MacFarlane and Meyer 2005). In contrast, *Fraxinus nigra* Marshall (black ash) is frequently a major component of deciduous and deciduous-conifer forested wetlands (Wright and Rauscher 1990) and occurs in nearly pure stands in portions of its range (Erdmann et al. 1987). In the Upper Peninsula of Michigan, USA, the *F. nigra*-*Ulmus americana* L. (American elm)-*Acer rubrum* L. (red maple) forest type tends to be dominated by *F. nigra*, particularly in areas with very poor drainage and organic soils (Erdmann et al. 1987). As a result, the potential fate *F. nigra* of is of particular concern, owing to the potential severity of impacts that may result from the mortality of a significant portion of the forest canopy (Kashian and Witter 2011).

The EAB-induced mortality of a substantial proportion of both the overstory and understory within these forests may have extensive impacts on successional dynamics (Looney et al. 2015). Disturbance resulting from invasive phytophagous insects and other agents alters the forest environment in many ways, resulting in both positive and negative impacts on surviving individuals and co-occurring species (Gandhi and Herms 2010). The removal of overstory vegetation, both through forestry practices or by disturbances such as windthrow or disease, is well-studied and is generally assumed to have a positive and relatively immediate effect on growth rates of remaining overstory vegetation, presumably due to reduced competition (Lorimer and Frelich 1989, Nowacki and Abrams 1997). Both natural mortality processes and insect invasions can create gaps that have positive effects on growth rates of surviving stems (Pederson and Howard 2004, Nuckolls et al. 2009), which frequently extend to subcanopy layers (Poage and Peart 1993).

However, the effect of overstory mortality is not uniformly positive. Gap creation can result in increased mortality of overstory stems near the affected area (Sprugel and Bormann 1981), resulting from changing environmental conditions that increase stress on remaining individuals. Reductions in growth rates of residual trees, termed “thinning shock,” can also occur following sudden exposure and may persist for more than a decade (Harrington and Reukema 1983). Additionally, a positive response to gap formation may lag the opening of the canopy by many years (Jones and Thomas 2004, Latham and Tappeiner 2002). Finally, positive effects may not be exhibited equally across all stem sizes or classes, potentially resulting in little to no response from dominant overstory trees (Poage and Peart 1993, Latham and Tappeiner 2002), and stronger responses in subdominant stems (Sharma et al. 2006, Jones and Thomas 2004).

Beyond the effect on existing stems, gap creation can also impact seedling establishment. Canopy gaps have long been recognized for their importance in the process of forest regeneration (Runkle 1982, Bormann and Likens 1994), with gap creation frequently resulting in increased seedling establishment in a number of forest types (Chen et al. 1992, Beckage et al. 2000, Coates 2002), though the effects can vary by species, gap size, and competition with other woody species (Runkle 1982, Kneeshaw and Bergeron 1998, Beckage et al. 2000). Recently, the importance of the herbaceous layer has received increased attention for the role it plays in the establishment, growth, and survival of woody plant species. The density of herbaceous layer cover may increase following disturbance (Roberts 2004, Elliot et al. 2015), resulting in competition with woody plant species that may diminish regenerative capacity, thus altering successional dynamics (Royo and Carson 2006, Gilliam 2007).

The type of mortality created by EAB and other phytophagous insects differs fundamentally from other types of disturbance, in that mortality is generally slower and standing dead trees may persist in these forests for many years (Gandhi and Herms 2010, Krasny and DiGregorio 2001). The canopy gaps that occur following EAB-induced mortality may thus be termed “gradual” gaps, as opposed to the “sudden” gaps that result

from windthrow or logging (Krasny and Whitmore 1992) that are likely to cause more rapid changes in microclimate and disruption to the forest floor, exposing mineral soil. As a consequence, gradual gaps may induce a vegetation response that differs from that expected following sudden gap creation (Kneeshaw and Bergeron 1998, Beckage et al. 2000).

In *F. nigra*-dominated wetlands, the potential hydrologic response is a further consequence of EAB-induced mortality that must be considered. Removal of significant proportions of woody vegetation may result in increased discharge from streams draining affected areas (Bosch and Hewlett 1982), or rising water tables (Riekirk 1983, Slesak et al. 2014) and increased overland flow (Beasley and Granillo 1988) in forests with poorly drained soils. Flooding has the potential to impact woody plants at nearly all life stages, resulting in a multitude of deleterious effects (Kozlowski 1997). Erdmann et al. (1987) reported increased water table height following clearcutting of *F. nigra*-dominated wetlands in the Great Lakes region, which was associated with increased herbaceous layer growth and decreased tree regeneration.

To assess these potential interactive effects on *F. nigra*-dominated wetlands, a multiyear manipulative study was established on the Ottawa National Forest, Michigan, USA. Emerald ash borer-induced mortality of *F. nigra* was simulated across a series of stands to emulate the effects of early- and late-stage infestation on growth and survival of co-occurring species, woody regeneration and recruitment, and herbaceous layer cover. Our objective was to determine the effects of *F. nigra* mortality on successional dynamics to understand how the loss of the dominant overstory species will affect the future species composition and fate of these stands. Additionally, the inclusion of both early- and late-stage simulations of infestation may provide insight into the responses of vegetation to sudden and gradual gaps within this forest type.

## 2.3 Methods and Materials

### 2.3.1 Study site description

Study sites were located on the Ottawa National Forest (ONF) in the western Upper Peninsula of Michigan, USA (Figure 2.1). The regional climate is continental, with average monthly temperatures ranging from a minimum of -11.3 °C in January to a maximum of 18.2 °C in July (1981-2010 normals data from the Bergland Dam, MI station, UTM Zone 16N 304840 5162412, Arguez et al. 2010). Over the same period, mean annual precipitation was 1010 mm year<sup>-1</sup>, with the majority occurring from June through November (Arguez et al. 2010). During this study, surface water in these wetlands typically persisted from the onset of snowmelt until mid-summer and remained near the soil surface throughout the growing season (Van Grinsven 2015). Surface soils consisted of woody peat histosols ranging in depth from 40 to greater than 690 cm, typically underlain by a layer of clay or clay loam.

Nine wetlands were selected based upon overstory composition, size, and landscape position. All nine study sites contained *F. nigra* as the most abundant overstory species, ranged in size from 0.20 to 1.25 ha, and were located in isolated depressions within first-order watersheds. Study sites were divided into three blocks based upon geographical location, in which a ‘greedy’ algorithm was used to create blocks with minimized in-block spatial variation, using the blockTools package (Moore 2011) for the R statistical environment (R Core Team 2015). To assign treatments within each block, scaled and weighted values for initial estimates of percent *F. nigra* basal area (BA), total site area, and average depth of organic soil were calculated. These values were then used to assign treatments such that among-treatment variation in these values was minimized. Each block contained one site of each treatment type, which consisted of ‘Control,’ ‘Girdle,’ and ‘Ash-Cut,’ where ‘Girdle’ and ‘Ash-Cut’ treatments were intended to mimic early- and late-stage EAB infestations, respectively. Within ‘Girdle’ sites, all *F. nigra* stems  $\geq$  2.5 cm in diameter at breast height (DBH, 1.37 m) were hand-girdled with a drawknife to a depth sufficient to sever all phloem and cambium tissue. All *F. nigra* stems  $\geq$  2.5 cm DBH within ‘Ash-Cut’ sites were felled by chainsaw and allowed to remain onsite. Both



treatment types were applied in the winter of 2012/2013. The lower diameter limit for treatment of *F. nigra* was based on reports that EAB-induced mortality is typically confined to ash stems > 2.5 cm DBH (McCullough et al. 2015). No additional treatment of epicormic sprouting that occurred following treatment was undertaken.

### 2.3.2 Vegetation data collection

Vegetation surveys were conducted annually from 2012 to 2015 in late July and early August. Surveys were timed to coincide with the peak of total herbaceous species cover. At each site, at least three 0.04 ha (11.3 m radius) permanent vegetation monitoring plots were established, centered on randomly generated coordinates (ArcGIS, ESRI, Redlands CA). Additional monitoring plots were added in accordance with each site's area, such that at least 15% of the total site area was surveyed. Each monitoring plot contained a set of three nested subplots, located at 120° intervals from an initial random bearing, at 5.5 m from plot center (Figure 2.2). Plots and subplots were used to quantify classes of vegetation as follows: *overstory* (woody stems  $\geq 10$  cm DBH; plot radius 11.3 m; plot area 400 m<sup>2</sup>), *saplings* (woody stems 2.5 cm > DBH < 10 cm; plot radius 4 m; three 50 m<sup>2</sup> subplots), *large woody stems* (stems  $\leq 2.5$  cm DBH and > 50 cm in height; plot radius 1.5 m; three 7 m<sup>2</sup> subplots), and *small woody stems* (stems of potential overstory species  $\leq 50$  cm in height, and stems of non-overstory species 15 to 50 cm in total height; plot radius 0.56 m; three 1 m<sup>2</sup> subplots).

Overstory stems and saplings were identified to species and DBH was measured to the nearest 0.5 mm using a diameter tape. Large woody stems and small woody stems were identified and counted. Ocular estimates of the percent cover of herbaceous species and non-canopy woody species < 15 cm in height were conducted within the small woody stem subplot. Estimates were assigned using the ten cover classes established for the North Carolina Vegetation Survey as follows: trace, 0-1%, 1-2%, 2-5%, 5-10%, 10-25%, 25-50%, 50-75%, 75-95%, and >95%, where “trace” is defined as solitary individuals that comprise very little cover (Peet et al. 1998). Herbaceous vegetation was typically identified to species, with the exception of graminoids, as subplots often contained

multiple morphological leaf forms which overlapped and interspersed such that estimation of cover beyond the genus level was prone to unacceptable potential levels of error. The percent cover values of the two *Dryopteris* species found in these sites, *D. carthusiana* (Vill.) H. P. Fuchs (spinulose woodfern) and *D. intermedia* (Willd.) A. Gray (intermediate woodfern) were combined for analyses, due to occasional damage (e.g. missing or broken pinnules) that masked diagnostic characters and the potential for hybridization (Reznicek et al. 2011). Ocular estimates were always performed by at least two observers, and at least one observer was present at all surveys across the four years of study.

### 2.3.3 Analytical methods

Measurements of DBH were used to calculate per-stem BA, which were then used to estimate BA ( $\text{m}^2 \text{ ha}^{-1}$ ) at the plot (overstory) or subplot (saplings) level. Counts of stems within plots and subplots were used to calculate stem density ( $\text{stems ha}^{-1}$ ). Relative dominance for each species was calculated as the sum of BA from all plots for that species divided by the total BA of all species within a given site. Relative density for each species was calculated as the sum of the stem count from all plots for that species divided by the total number of stems of all species within a given site. Importance values (IV) were calculated following Iverson and Prasad (1998) as  $((100 \times \text{relative density}) + (100 \times \text{relative dominance}))$ , though final IVs were divided by 2 so that values could be compared to Forest Inventory and Analysis (FIA) estimates presented in Figure 2.1 (Prasad and Iverson 2003). Estimates of aboveground biomass were calculated using the generalized biomass equations presented in Chojnacky et al. (2014), which require DBH and species only as inputs. Relative growth rates (RGR) were calculated as a single value over the course of the study as the change in calculated biomass at the plot (overstory) or subplot (sapling) level over three growing seasons, normalized by initial biomass. Stems that died or recruited from lower vegetation strata over the course of the study were excluded from RGR calculations.

Mixed effects models were fit to the collected data with the R package lme4 (Bates et al. 2015), using year and treatment as fixed effects. Woody basal area models incorporated plot (overstory) or subplot (sapling) nested within site as random effects. Large and small woody stem count data from subplot clusters were summed to yield a single value per plot, which was included in models as a nested random effect within site. Similarly, herbaceous cover data from subplots were averaged to yield a single cover value per plot, which was included as a random effect nested within site. Growth rate models included treatment and site only as the fixed and random effects, respectively. Basal area, growth rate, and percent cover data were square-root transformed before fitting linear models to resolve violations of non-normality and non-constant variance assumptions. Though the use of generalized linear models using Poisson or negative binomial distributions for count data has recently been suggested as a preferred technique (O'Hara and Kotze 2010), models fit using these methods performed poorly with the stem count data in this study. As such, linear models were fit to stem count data that were transformed as  $\ln(x + 1)$ . Visual examination of residual plots from fitted models did not reveal significant violations of the assumptions of normality or homogeneity of variance, though minor heteroskedasticity in the distribution of residuals of stem count and herbaceous cover data for individual species was observed, as would be expected given that these data were zero-inflated. Pairwise comparisons were made using the package lsmeans (Lenth 2016), using the Tukey-HSD method to adjust  $p$ -values. A significance level of  $p \leq 0.05$  was used for all comparisons. Standard errors for presentation in figures were calculated using a method of accounting for within-subjects designs presented in Morey (2008).

## 2.4 Results

### 2.4.1 *Pre-treatment forest composition and response to treatment*

Overstory summary statistics for all nine study sites are shown in Table 2.1. Within a block, overstory characteristics were similar, though differences in *F. nigra* BA and density between blocks were observed. Within Block 1, which is in the northeast portion of the ONF, basal area and density of *F. nigra* were lowest, which is in agreement with

the patterns in IV from FIA data (Figure 2.1). Importance values for *F. nigra* overstory and saplings calculated for 20 km by 20 km cells range from 3-13 on the ONF, and from 5-11 for the cells that include these study sites (Figure 2.1, Prasad and Iverson 2003). Within these wetlands, mean pre-treatment *F. nigra* IV for these strata was considerably higher than FIA IVs calculated across broader geographic scales in the region (Table 2.2).

Following treatment application, abundant epicormic branching was observed in both the ‘Girdle’ and ‘Ash-Cut’ treatment. Though some of these sprouts died from browsing or other unknown causes over the course of the study, many continued to grow vigorously, in some cases reaching heights of more than 6 m by the third year post-treatment (JCDavis, *personal observation*). In the ‘Girdle’ treatment, foliage in the upper canopy was produced in the first year post-treatment (see Chapter 3), but none was produced during subsequent growing seasons. No instances of phloem closure over the girdles were observed.

#### 2.4.2 Overstory

Prior to treatment application, *F. nigra* comprised greater than 66% of the total overstory basal area, while the remainder consisted of a mix of species, dominated by *Betula alleghaniensis* Britton (yellow birch) and *A. rubrum* (Table 2.2). Though basal area of non-*F. nigra* species increased in both the ‘Control’ and ‘Girdle’ sites over the study period ( $10.66 \pm 0.19$  to  $11.29 \pm 0.40$  m<sup>2</sup> ha<sup>-1</sup> and  $8.36 \pm 0.16$  to  $8.63 \pm 0.20$  m<sup>2</sup> ha<sup>-1</sup>, respectively) and decreased in the ‘Ash-Cut’ sites ( $8.56 \pm 0.41$  to  $8.47 \pm 0.18$  m<sup>2</sup> ha<sup>-1</sup>), these differences were not significant. Basal area of individual species also did not change significantly. Similarly, overstory RGR was not significantly affected by the mortality of *F. nigra* in these sites, for either all non-*F. nigra* stems combined (‘Control’:  $0.024 \pm 0.004$  g g<sup>-1</sup> yr<sup>-1</sup>, ‘Girdle’:  $0.036 \pm 0.007$  g g<sup>-1</sup> yr<sup>-1</sup>, ‘Ash-Cut’:  $0.035 \pm 0.004$  g g<sup>-1</sup> yr<sup>-1</sup>), or individual species. Growth rates by 10 cm increment DBH size class were also calculated and showed no difference in response both between treatments for a given size class, as well as within a treatment between size classes (data not shown).

### 2.4.3 Saplings

The sapling layer was comprised primarily of *F. nigra*, but relative dominance was low compared to the overstory (Table 2.2). *Abies balsamea* (L.) Mill. (balsam fir) was more dominant in the sapling layer than in the overstory, whereas *B. alleghaniensis*, despite occupying a similar proportion of the basal area as in the overstory, was exceeded by both *A. balsamea* and *A. rubrum* in relative sapling dominance.

No significant change in sapling basal area was observed (Figure 2.3a), however, total non-*F. nigra* sapling RGR was significantly greater ( $p = 0.039$ ) in the ‘Girdle’ sites compared to ‘Control,’ representing a nearly three-fold greater RGR over three growing seasons (Figure 2.3b). Sapling RGR was not significantly different in the ‘Ash-Cut’ treatment compared to either the ‘Girdle’ or ‘Control’ treatments. While the RGR of individual species followed similar patterns, differences were not statistically significant.

### 2.4.4 Large and small woody stems

Large *F. nigra* stems had the highest relative density, followed closely by *U. americana* and *A. rubrum*, though mean absolute density of *U. americana* across all sites was relatively low (Table 2.2). *Alnus incana* (L.) Moench (gray alder) stems were also abundant in this layer. In contrast to upper canopy layers, *B. alleghaniensis* abundance was very low. Small woody stems were strongly dominated by *F. nigra* and *A. rubrum*, which together comprised 73.6% of the small woody stems in these sites. Note that in some sites these strata contained one or very few species of which abundance was relatively low, which lead to apparent discrepancies between absolute and relative densities (Table 2.3), as was the case for *U. americana* (often high relative density due to a lack of regeneration of other species, but low absolute density).

Compared to the first post-treatment growing season (2013), large *A. incana* stems increased in the ‘Ash-Cut’ treatment by the third post-treatment growing season compared to the first pre-treatment growing season (2015,  $p = 0.010$ ), yet the 2015 tally

was not greater than the pre-treatment value (Figure 2.4a). No other significant differences within treatments were observed, however the increase in *F. nigra* large woody stems within ‘Ash-Cut’ sites from 2012 to 2015 approached significance ( $p = 0.085$ )

Compared to pre-treatment, small *F. nigra* stems increased significantly within the ‘Control’ sites in the third post-treatment growing season (2015,  $p < 0.001$ ), though no change was observed in either the ‘Girdle’ or ‘Ash-Cut’ treatments (Figure 2.4b). *A. rubrum* stems in the ‘Ash-Cut’ treatment increased by the second year post-treatment (2012 to 2014,  $p = 0.009$ ), while small *B. alleghaniensis* stems increased in the ‘Girdle’ sites over the same period ( $p = 0.003$ ).

#### 2.4.5 Herbaceous species

The most common herbaceous vascular plant species present in these wetlands were members of the genus *Carex*, which occurred in 67% of study plots in the pre-treatment study period and was the taxa with the greatest mean percent cover (Table 2.3). Fern species, including *Osmunda cinnamomea* L. (cinnamon fern), *Onoclea sensibilis*, L. (sensitive fern), *Athyrium filix-femina* (L.) Roth (common ladyfern), *D. carthusiana*, and *D. intermedia* were common and tended to comprise a large proportion of the herbaceous cover. Facultative wetland species (FACW, Reed 1988) dominated the herbaceous cover in pre-treatment surveys (Figure 2.5a).

Following treatment, cover of all herbaceous vascular (VASC, Figure 2.5a) plants increased by 101% and 75% in the ‘Girdle’ and ‘Ash-Cut’ sites, respectively (2012 to 2015,  $p < 0.001$ ), while no change was observed in the ‘Control’ sites. When species were examined by wetland indicator status, non-graminoid obligate wetland species (OBL, Figure 2.5a) increased in both ‘Girdle’ and ‘Ash-Cut’ treatments (2012 to 2015,  $p = 0.004$  and  $p < 0.001$ ), and by 2015 occupied a similar proportion of the herbaceous cover as the FACW, despite much greater cover of FACW species in the pre-treatment

surveys. Neither the FACW nor the combined facultative and facultative upland (FAC+FACU) groups responded significantly to treatment.

Among individual taxa, *Carex* spp. exhibited the strongest treatment response, reaching percent cover values of 321% and 413% of the pre-treatment cover in the ‘Girdle’ and ‘Ash-Cut’ treatments, respectively (Figure 2.5b, 2012 to 2015  $p < 0.001$ ). A number of additional species exhibited positive responses to treatment, including *Scutellaria lateriflora* L. (blue skullcap), *Lycopus uniflorus* Michx. (northern bugleweed), and *Caltha palustris* L. (yellow marsh marigold), though the effect varied in magnitude and by treatment type. While representing a substantial proportion of the pre-treatment cover, none of the fern species present in these sites responded to treatment.

## 2.5 Discussion

### 2.5.1 Overstory

Jones and Thomas (2004) put forth a set of three hypotheses that suggest potential responses to the residual forest canopy following gap creation. The first, and that typically assumed by dendroecological studies (e.g. Lorimer and Frelich 1989, Nowacki and Abrams 1997), is that increased resource availability results in a positive growth response shortly after gap creation. The second, termed “thinning” or “gap” shock, predicts a decrease in growth of residual stems following gap creation, related to photodamage or other stresses. In the third hypothesis, these two processes operate in opposition to each other, with the effects of stress offsetting the increase in resource availability, resulting in a lagged response in growth rate such that positive effects are not observable for several growing seasons (Jones and Thomas 2004). A delayed response resulting from increased stress, despite a change in aboveground resource availability (see Chapter 3), may provide an explanation for the lack of observable positive effects on retained canopy trees during this study.

The ‘Ash-Cut’ treatment, while designed to mimic late-stage infestation in which dead *F. nigra* stems have begun to fall, represents a much more rapid change in the overstory

than is likely to be experienced during an actual EAB infestation. This treatment may then be more appropriately considered in light of other disturbances that create “sudden” gaps, such as windthrow or commercial thinning, rather than the “gradual” gaps that result from mortality due to insects or disease (Krasny and Whitmore 1992). Increased mortality following sudden gap formation has been attributed to a change in local environmental conditions that increase stress on remaining trees. Following mortality of overstory *A. balsamea* in the mountains of New England, downwind trees are subject to higher wind velocities, which may result in increased rime ice deposition, branch breakage, and desiccation in these trees, inducing the “wave-regeneration” characteristic of these forests (Sprugel and Bormann 1981). Changes in microclimate, including increased wind and decreased soil moisture, have been implicated in higher rates of mortality in residual trees following the application of silvicultural techniques that create gaps in coniferous forests of the Pacific Northwest (Chen et al. 1992, Garber et al. 2011). Mortality of overstory stems reduced total non-*F. nigra* basal area in the ‘Ash-Cut’ sites of this study, though these reductions were small and not statistically significant. Observable aboveground damage to retained trees resulting from treatment application in the ‘Ash-Cut’ sites was rare, and can only be directly implicated in the deaths of a small number of individuals.

By omitting mortality and recruitment from the analysis, the relative growth rates of stems present throughout the course of the study were compared. These comparisons, however, did not show any significant response to treatment. These results stand in contrast to those reported by Flower et al. (2013), which showed a positive growth effect in co-occurring species during EAB infestation in riparian forests of northern Ohio, although this study was conducted over six years. A positive effect of competitive release on growth rates is a commonly reported response to both selection harvests (Latham and Tappeiner 2002, Pederson and Howard 2004) and in the gradual gaps created by invasive insects and pathogens, such as beech bark disease (Twery and Patterson 1984, DiGregorio et al. 1999), hemlock wooly adelgid (*Adelges tsugae* Annand; Small et al. 2005, Ford et al. 2012), or gypsy moth (*Lymantria dispar* L.; Muzika and Liebhold 1999,



Jedlicka et al. 2004). However, nearly all of these studies examined growth rates over longer time frames (Latham and Tappeiner 2002, Pederson and Howard 2004, Twery and Patterson 1984, Small et al. 2005) or used more sensitive measurement techniques than those used in this study, such as increment cores (Muzika and Liebhold 1999) or dendrometers (Ford et al. 2012).

Surviving trees may have experienced increased stress resulting from the sudden change in environmental conditions (e.g. increased wind damage, desiccation, or snow and ice loading) or photodamage (Harrington and Reukema 1983, Lovelock et al. 1994, Jones and Thomas 2004). Stress-related symptoms of gap or thinning shock may be exhibited in a number of ways, including reduced growth, chlorotic foliage, and mortality (Sharma et al. 2006). Increased stress on residual stems has been implicated in the negative growth response observed in stands following commercial thinning, which may require an adjustment period of several years before a positive response is observed (Harrington and Reukema 1983, Sharma et al. 2006). Both basal-area reducing mortality and chlorotic foliage (see Chapter 3) were observed in the treated sites in the current study, suggesting an increase in stress of residual overstory trees which may have reduced the potential for a positive growth response.

### 2.5.2 *Saplings*

Release from suppression of understory trees through gap creation is a common response across nearly all closed-canopy forests and has played a significant role in the development of successional dynamics theories (Runkle 1982, Bormann and Likens 1994). The response of the sapling layer is important to the near-term future of these sites, as expansion of existing canopy crowns is unlikely to fill gaps of the size created in this study. The observed increased sapling growth rate (Figure 2.3b) suggests a positive effect of release resulting from mortality of the *F. nigra* overstory. This increased growth rate did not result in a significant increase in non-*Fraxinus* sapling basal area, though an increasing trend appears to be emerging in the ‘Girdle’ treatment (Figure 2.3a). Overall patterns of growth rate evident in the combined sapling data appear to repeat across the

other commonly co-occurring species; as such the lack of statistical significance can perhaps be attributed to relatively small samples sizes for each individual species (Figure 2.3b). That no significant response in sapling growth rate within ‘Ash-Cut’ sites was observed is notable, as these stems would theoretically be expected to receive the largest increase in available resources. However, they are also likely subject to the greatest change in environmental conditions, and thus the lack of response may be attributable to the balance between resource availability and abiotic stressors.

### 2.5.3 *Large and small woody stems*

As with saplings, the creation of gaps is often predicted to result in increased seedling establishment, an effect which has been reported in both experimentally created gaps (Gray and Spies 1996, Coates 2002) and those resulting from other invasive insects, such as the hemlock wooly adelgid (Orwig and Foster 1998, Ford et al. 2012). Though no increase resulting from treatment in total small woody stems was observed in this study, individual species densities did change, with increased *A. rubrum* and *B. alleghaniensis* density by the second post-treatment growing season, while *F. nigra* stem density remained unchanged (Figure 2.4b). The demographics of *F. nigra* stems in the treated sites is the expected result of the removal of seed sources (Klooster et al. 2014), as evidenced by the lack of new regeneration in the ‘Girdle’ and ‘Ash-Cut’ sites in 2015, while in the same year, new *F. nigra* seedlings were abundant in the ‘Control’ sites. Similar results have been reported in studies of EAB infestation elsewhere in the region. Kashian and Witter (2011) reported abundant capacity for potential *Fraxinus* regeneration within infested sites in the Lower Peninsula of Michigan, but a decline in newly germinated seedlings over time. In a separate study, *Fraxinus pennsylvanica* Marshall (green ash) below the size infested by EAB was common throughout southern Michigan, but new regeneration declined with time since EAB invasion (Burr and McCullough 2014). Klooster et al. (2014) described comparable results across Michigan and Ohio, labeling established *Fraxinus* seedlings an “orphaned cohort” resulting from mortality of all larger stems, the subsequent lack of seed source, and absence of a persistent seedbank for any *Fraxinus* species in the region. Though seed-bearing

epicormic sprouts have recently been reported from *F. pennsylvanica*-dominated wetlands (Kashian 2016), it is unknown if epicormic branching from other *Fraxinus* species will survive to reach reproductive maturity. As a result, it is unclear whether the abundant epicormic branching observed in the forests in this study will persist long enough to act as a seed source for future *F. nigra* regeneration.

In *F. nigra*-dominated wetlands of northern Minnesota exhibiting natural dieback, regeneration has been shown to be dominated by shrub species such as *A. incana*, prompting observers to suggest that this forest type may be eventually be succeeded by scrub-shrub wetlands (Palik et al. 2012). Though the number of *A. incana* large woody stems in the ‘Ash-Cut’ sites increased over the course of the post-treatment period (Figure 2.4a) this appears to result from increased recruitment from the small woody stem class, as no increase in *A. incana* seedlings was observed. Large woody stems of potential overstory species outnumbered *A. incana* across all sites, despite these increases. Additionally, while *A. incana* stems are frequently abundant in terms of absolute density, this does not appear to inhibit regeneration of other species, as mean relative density is relatively low (Table 2.2). This suggests that there is currently ample prospect for regeneration of potential overstory species, in contrast to the observations from *F. nigra* stands in Minnesota (Palik et al. 2012).

Prior to treatment, *B. alleghaniensis* comprised a small proportion of the large and small woody stems (Table 2.2), which may be ascribed to a lack of suitable substrate, primarily exposed mineral soil or down coarse woody debris (CWD), that is important to the regeneration of this species (Godman and Krefting 1960, Shields et al. 2007). Additionally, coniferous CWD tends to promote *B. alleghaniensis* establishment (Marx and Walters 2008, Bolton and D’Amato 2011) but is not abundant in these hardwood-dominated wetlands. Though increased *B. alleghaniensis* small woody stems were observed in both the ‘Girdle’ and ‘Ash-Cut’ sites in 2014, a similar pattern was observed in the ‘Control’ sites (Figure 2.4b), and thus may be attributable to a mast year for this species across the region, rather than an effect of treatment. The gradual gaps resulting

from EAB-mortality are unlikely to create favorable substrate conditions, such as exposed mineral soil, for *B. alleghaniensis* seedling establishment in the near-term. As a result, it appears unlikely that the canopy openings resulting from EAB alone are sufficient to promote future *B. alleghaniensis* overstory dominance equivalent to that currently present in these sites.

#### 2.5.4 Herbaceous species

The greatly increased cover of herbaceous species represents the most dramatic impact of simulated EAB infestation in the present study. Though the response of the herbaceous layer lagged the treatment application by two to three growing seasons, strong effects were observed across both treatments (Figure 2.5a). The differential response of obligate wetland species (OBL) versus facultative wetland species (FACW), suggests a response to reported changes in hydrology (Van Grinsven 2015), as the facultative wetland species did not respond to treatment, despite being the dominant wetland indicator class in the pre-treatment surveys. In addition to the significant increases in cover of species present in the pre-treatment surveys (Figure 2.5b), a number of obligate wetland species, including *Alisma triviale* Pursh (northern water-plantain) and *Cicuta bulbifera* L. (water hemlock), were not observed in any of the sites prior to treatment, but appeared in the latter years of the study. The single invasive species identified in these wetlands, *Solanum dulcamara* L., (climbing nightshade) did not increase in cover. A number of invasive plants, however, are potential threats to forested wetlands of the region (Weber et al 2007, Marlbor et al. 2014), and the potential for increased invasibility following EAB mortality in these forests should be considered likely (Davis et al. 2005).

The interaction between the herbaceous layer and the large and small woody stems may prove important in determining the future fate of woody species in these communities. Erdmann et al. (1987) describe the conversion of *F. nigra*-dominated wetlands to herbaceous or scrub-shrub wetlands following clearcutting, and warn that removal of significant portions of the overstory may result in a total loss of tree species, including established regeneration. Herbaceous species are known to compete strongly with woody

vegetation in some forest types (Royo and Carson 2005, Gilliam 2007), and may increase in cover following disturbance (Elliot et al. 2015, Roberts 2004). As has been observed in the ‘Girdle’ sites in these wetlands, even the types of gradual gaps associated with slow tree mortality and the retention of standing dead trees can result in greatly increased herbaceous cover (Figure 2.6). Similar effects have been seen in studies that examined the response of herbaceous species to *Tsuga canadensis* (L.) Carrière (eastern hemlock) decline resulting from hemlock wooly adelgid infestation (Yorks et al. 2003, Eschtruth et al. 2006, Ford et al. 2012).

Herbaceous cover has been implicated in the inhibition of woody regeneration in a number of ecosystem types. The effect of dense fern cover has received particular attention, and has been linked to reduced establishment, density, and growth of tree seedlings (Maguire and Forman 1983, George and Bazzaz 1999a, 1999b), which may influence the development rate and composition of a forest canopy following disturbance (Royo and Carson 2006, Gilliam 2007). Graminoid species, such as *Calamagrostis canadensis* (Michx.) P. Beauv. (bluejoint) have also been shown to influence future canopy development in boreal forests (Lieffers et al. 1993). Though fern species are among the most common herbaceous plants in these wetlands, they did not respond significantly to either treatment. Additionally, increased *Carex* cover, as well as other species, did not impact seedling survival by the third year post-treatment. As such, it is perhaps cause for cautious optimism that the recent increase in herbaceous cover has yet to reduce the potential for future woody vegetation recovery in these forests.

Erdmann et al. (1987) linked the increase in herbaceous cover and poor regeneration of woody species to observations of a higher water table following overstory removal, a change in hydrology also reported from a study in Minnesota similar to the present one (Slesak et al. 2014). However, within the wetlands studied here, mean growing season water table height was unaffected by either treatment, though a reduction in the rate of drawdown was observed, leading to significant differences in water table height late in the growing season (Van Grinsven 2015). This change in hydrology may explain the

strong response of obligate wetland species over other functional groups (Figure 2.5a) and may have contributed to stress in woody species that reduced the potential response to competitive release (Kozlowski 1997). Given the available data, the possibility for increases in water table height following disturbance in similar wetland types across the region persists, and the effects of such changes on vegetation should remain a concern.

## 2.6 Conclusion

Predicting the future fate of this forest type in the presence of EAB is, at best, an uncertain task. Based upon the results of this and other studies, the continued presence of *Fraxinus* species in forests throughout infested regions seems unlikely, due to high mortality rates and the lack of seed sources or a persistent seedbank. Surviving small stems are unlikely to reach reproductive maturity (Kurmis and Kim 1989, Klooster et al. 2014) before succumbing to infestation. Within *F. nigra*-dominated wetlands, overstory stems of co-occurring species appear likely to survive, and the increased growth rate of saplings may rapidly fill gaps resulting from *F. nigra* mortality. Should woody species remain abundant in these forests, it appears that the canopy will eventually be dominated by *A. rubrum* as occurs elsewhere in the region (Erdmann et al. 1987), though *F. nigra* mortality may improve otherwise poor regeneration and recruitment of *B. alleghaniensis* in the present forest. Though increased herbaceous cover has not inhibited seedling response in this study, the potential for intensified competition from herbaceous species to reduce future woody regeneration remains a concern. Continued work within this forest type, as well as investigations into potential means of maintaining forest cover in similar habitats of the region (Looney et al. 2015), are critical to determining how these forests may respond in the future and identifying means of mitigating the negative impacts of EAB.

## 2.7 Tables

**Table 2.1.** Pre-treatment (2012) overstory summary statistics by study site.

	Area (ha)	<i>F. nigra</i>				All non- <i>F. nigra</i> Species				
		DBH (cm)	Maximum DBH (cm)	BA (m <sup>2</sup> ha <sup>-1</sup> )	Density (stems ha <sup>-1</sup> )	DBH (cm)	Maximum DBH (cm)	BA (m <sup>2</sup> ha <sup>-1</sup> )	Density (stems ha <sup>-1</sup> )	Species Richness
‘Control’ 1	0.30	18.2 ± 1.0	34.3	6.2 ± 1.3	225 ± 90	20.1 ± 1.0	43.4	18.2 ± 2.2	500 ± 50	7
‘Girdle’ 1	0.29	20.7 ± 0.9	37.1	16.1 ± 3.6	442 ± 131	20.4 ± 1.0	39.1	12.3 ± 1.7	325 ± 52	8
‘Ash-Cut’ 1	0.61	14.7 ± 0.5	30.9	13.9 ± 2.5	758 ± 121	18.1 ± 0.9	47.7	17.8 ± 2.5	567 ± 94	9
‘Control’ 2	0.23	24.3 ± 0.8	37.6	23.8 ± 1.9	483 ± 58	16.4 ± 1.6	39.3	5.2 ± 1.3	200 ± 29	6
‘Girdle’ 2	0.33	21.9 ± 1.3	43.2	20.5 ± 3.6	458 ± 51	19.8 ± 2.1	33.8	4.5 ± 2.1	125 ± 38	5
‘Ash-Cut’ 2	0.35	20.9 ± 0.7	49.5	28.0 ± 2.0	742 ± 22	17.4 ± 1.2	30.8	4.8 ± 1.4	183 ± 30	4
‘Control’ 3	0.81	20.4 ± 0.9	62.3	27.5 ± 4.0	700 ± 46	17.8 ± 1.2	46.7	9.1 ± 1.2	300 ± 54	10
‘Girdle’ 3	0.61	19.0 ± 1.0	51.0	20.5 ± 3.8	608 ± 88	19.1 ± 1.2	41.8	8.4 ± 1.9	267 ± 118	7
‘Ash-Cut’ 3	1.19	17.4 ± 0.6	55.0	16.1 ± 2.6	590 ± 42	15.1 ± 0.9	45.1	5.3 ± 1.1	275 ± 58	4

**Table 2.2.** Pre-treatment (2012) basal area (BA) and density calculations (mean  $\pm$  standard error, calculated across all study sites ( $n = 9$ )) for the five species with highest BA or density within each stratum, sorted by overstory BA.

	Overstory				Saplings				Importance Value <sup>§</sup>
	BA (m <sup>2</sup> ha <sup>-1</sup> )	Relative Dominance (%) <sup>*†</sup>	Density (stems ha <sup>-1</sup> )	Relative Density (%) <sup>†‡</sup>	BA (m <sup>2</sup> ha <sup>-1</sup> )	Relative Dominance (%)	Density (stems ha <sup>-1</sup> )	Relative Density (%)	
<i>Fraxinus nigra</i>	19.2 $\pm$ 2.3	66.3 $\pm$ 6.8	556 $\pm$ 57	64.8 $\pm$ 5.0	1.000 $\pm$ 0.273	36.2 $\pm$ 8.1	332 $\pm$ 96	30.8 $\pm$ 7.6	59.7 $\pm$ 5.44
<i>Betula alleghaniensis</i>	3.2 $\pm$ 0.6	11.5 $\pm$ 2.3	91 $\pm$ 18	10.7 $\pm$ 2.2	0.325 $\pm$ 0.102	11.7 $\pm$ 2.6	129 $\pm$ 39	11.2 $\pm$ 2.0	11.3 $\pm$ 2.07
<i>Acer rubrum</i>	2.7 $\pm$ 0.8	9.9 $\pm$ 3.0	94 $\pm$ 23	11.0 $\pm$ 2.9	0.224 $\pm$ 0.077	14.4 $\pm$ 5.0	98 $\pm$ 39	14.7 $\pm$ 5.6	10.6 $\pm$ 3.0
<i>Thuja occidentalis</i> L.	0.9 $\pm$ 0.4	3.1 $\pm$ 1.5	29 $\pm$ 13	3.6 $\pm$ 1.8	0.031 $\pm$ 0.022	1.1 $\pm$ 0.7	7 $\pm$ 5	0.5 $\pm$ 0.4	3.0 $\pm$ 1.5
<i>Tsuga canadensis</i>	0.6 $\pm$ 0.3	2.2 $\pm$ 1.1	9 $\pm$ 5	1.1 $\pm$ 0.6	0.002 $\pm$ 0.002	0.2 $\pm$ 0.2	3 $\pm$ 3	0.7 $\pm$ 0.7	1.6 $\pm$ 0.8
<i>Abies balsamea</i>	0.6 $\pm$ 0.3	1.7 $\pm$ 0.8	35 $\pm$ 17	3.4 $\pm$ 1.4	0.522 $\pm$ 0.131	24.3 $\pm$ 5.0	257 $\pm$ 64	27.4 $\pm$ 5.4	6.3 $\pm$ 1.7
<i>Ulmus americana</i>	0.4 $\pm$ 0.3	1.6 $\pm$ 1.0	20 $\pm$ 9	2.8 $\pm$ 1.5	0.164 $\pm$ 0.077	6.3 $\pm$ 2.9	47 $\pm$ 20	5.3 $\pm$ 2.5	2.8 $\pm$ 1.2
<i>Acer saccharum</i> Marsh.	0.1 $\pm$ 0.1	0.3 $\pm$ 0.2	4 $\pm$ 2	0.6 $\pm$ 0.3	0.056 $\pm$ 0.037	2.3 $\pm$ 1.2	27 $\pm$ 14	3.1 $\pm$ 1.6	0.9 $\pm$ 0.4
<i>Alnus incana</i>					0.076 $\pm$ 0.057	1.7 $\pm$ 1.1	85 $\pm$ 62	3.9 $\pm$ 2.7	1.2 $\pm$ 0.9

\*Relative dominance was calculated as  $(BA(species) \div BA(all\ species)) \times 100$  for all plots summed in a given site.

†Species list was not comprehensive; as a result relative dominance and relative density in a column will not sum to 100.

‡Relative density was calculated as  $(Density(species) \div Density(all\ species)) \times 100$  for all plots summed in a given site.

§Importance value was calculated using combined overstory and sapling BA and Density as  $(Relative\ Dominance + Relative\ Density) \div 2$ .



**Table 2.2(continued).** Pre-treatment (2012) density calculations (mean  $\pm$  standard error, calculated across all study sites ( $n = 9$ )) for the five species with highest BA or density within each stratum, sorted by overstory BA.

	Large Woody Stems		Small Woody Stems	
	Density (stems ha <sup>-1</sup> )	Relative Density (%) †‡	Density (stems ha <sup>-1</sup> )	Relative Density (%)
<i>Fraxinus nigra</i>	1898 $\pm$ 1406	17.8 $\pm$ 7.7	21105 $\pm$ 13017	41.7 $\pm$ 11.4
<i>Betula alleghaniensis</i>	231 $\pm$ 172	1.9 $\pm$ 0.9	1975 $\pm$ 1358	3.5 $\pm$ 2.5
<i>Acer rubrum</i>	1055 $\pm$ 481	16.0 $\pm$ 7.6	21944 $\pm$ 12638	31.9 $\pm$ 11.3
<i>Thuja occidentalis</i> L.	17 $\pm$ 17	0.7 $\pm$ 0.7	370 $\pm$ 185	0.4 $\pm$ 0.4
<i>Tsuga canadensis</i>	N/A	N/A	1111 $\pm$ 786	2.3 $\pm$ 1.9
<i>Abies balsamea</i>	514 $\pm$ 192	10.4 $\pm$ 4.2	1462 $\pm$ 910	4.4 $\pm$ 3.0
<i>Ulmus americana</i>	345 $\pm$ 130	16.7 $\pm$ 10.8	1259 $\pm$ 469	6.6 $\pm$ 3.9
<i>Acer saccharum</i> Marsh.	270 $\pm$ 129	4.5 $\pm$ 2.4	1327 $\pm$ 845	2.4 $\pm$ 1.3

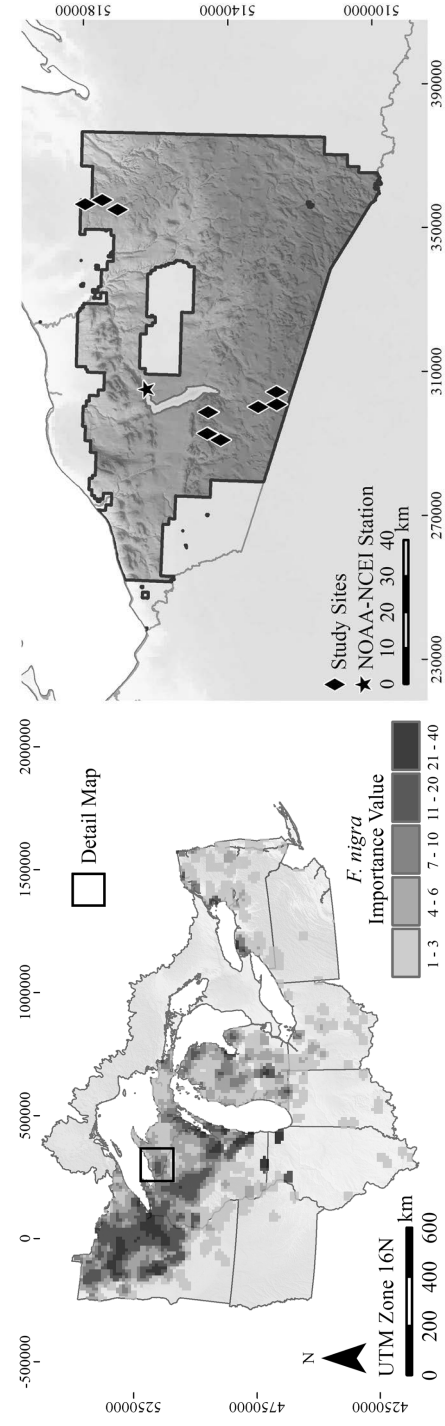
†Species list is not comprehensive; as a result relative density in a column will not sum to 100.

‡Relative density is calculated as (Density(species)  $\div$  Density(all species))  $\times$  100 for all plots summed at a given site.

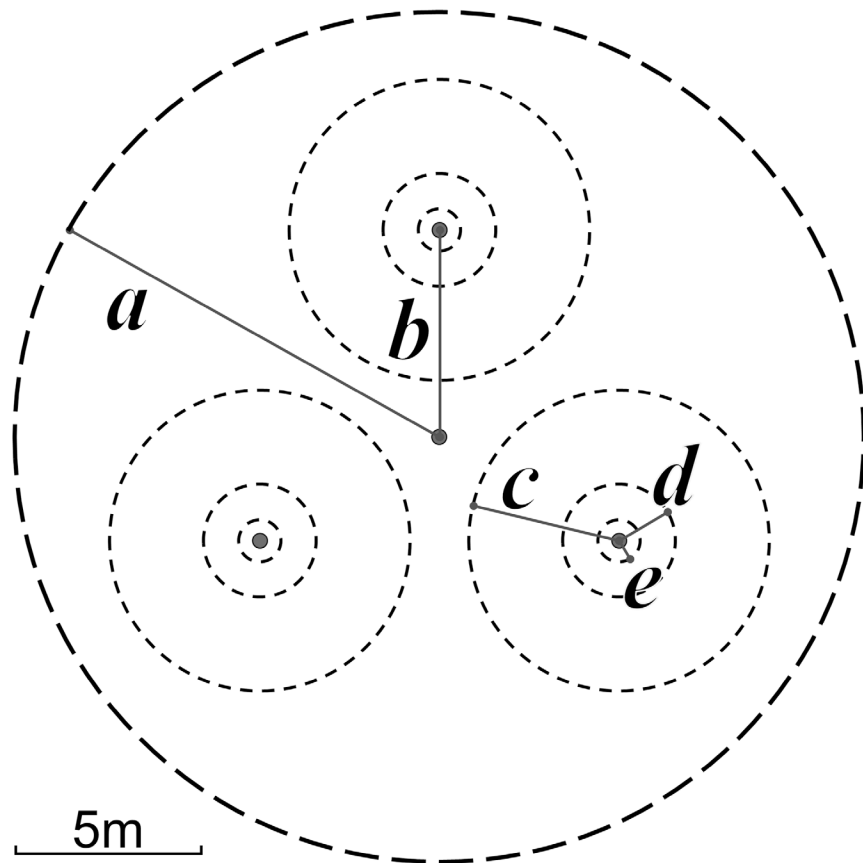
**Table 2.3.** Pre-treatment (2012) percent cover (mean  $\pm$  standard error, calculated across all study sites ( $n = 9$ )) of all non-woody vascular plant species that occur in  $\geq 10\%$  of herbaceous study plots.

	Frequency ( $n$ )	Percent cover
<i>Carex</i> spp. L.	60	8.6 $\pm$ 2.5
<i>Dryopteris</i> spp. Adans.	40	4.1 $\pm$ 1.3
<i>Onoclea sensibilis</i>	35	3.9 $\pm$ 1.5
<i>Rubus pubescens</i> Raf.	33	2.1 $\pm$ 1.1
<i>Scutellaria lateriflora</i>	32	0.4 $\pm$ 0.1
<i>Galium</i> spp. L.	30	0.2 $\pm$ 0.1
Poaceae (Barnhart)	28	2.4 $\pm$ 1.5
<i>Impatiens capensis</i> Meerb.	22	1.7 $\pm$ 1.0
<i>Osmunda cinnamomea</i>	21	8.5 $\pm$ 3.0
<i>Coptis trifolia</i> (L.) Salisb.	17	0.1 $\pm$ <0.1
<i>Maianthemum canadense</i> Desf.	16	0.1 $\pm$ 0.1
<i>Mitella nuda</i> L.	16	0.2 $\pm$ 0.1
<i>Trientalis borealis</i> Raf.	15	0.1 $\pm$ 0.1
<i>Oxalis acetosella</i> Raf.	12	0.1 $\pm$ <0.1
<i>Equisetum pratense</i> Ehrh.	11	0.2 $\pm$ 0.1
<i>Lycopus uniflorus</i>	10	0.6 $\pm$ 0.4
<i>Athyrium filix-femina</i>	9	2.3 $\pm$ 1.3
<i>Bidens</i> spp. L.	9	0.4 $\pm$ 0.3
<i>Equisetum sylvaticum</i> L.	9	0.3 $\pm$ 0.1

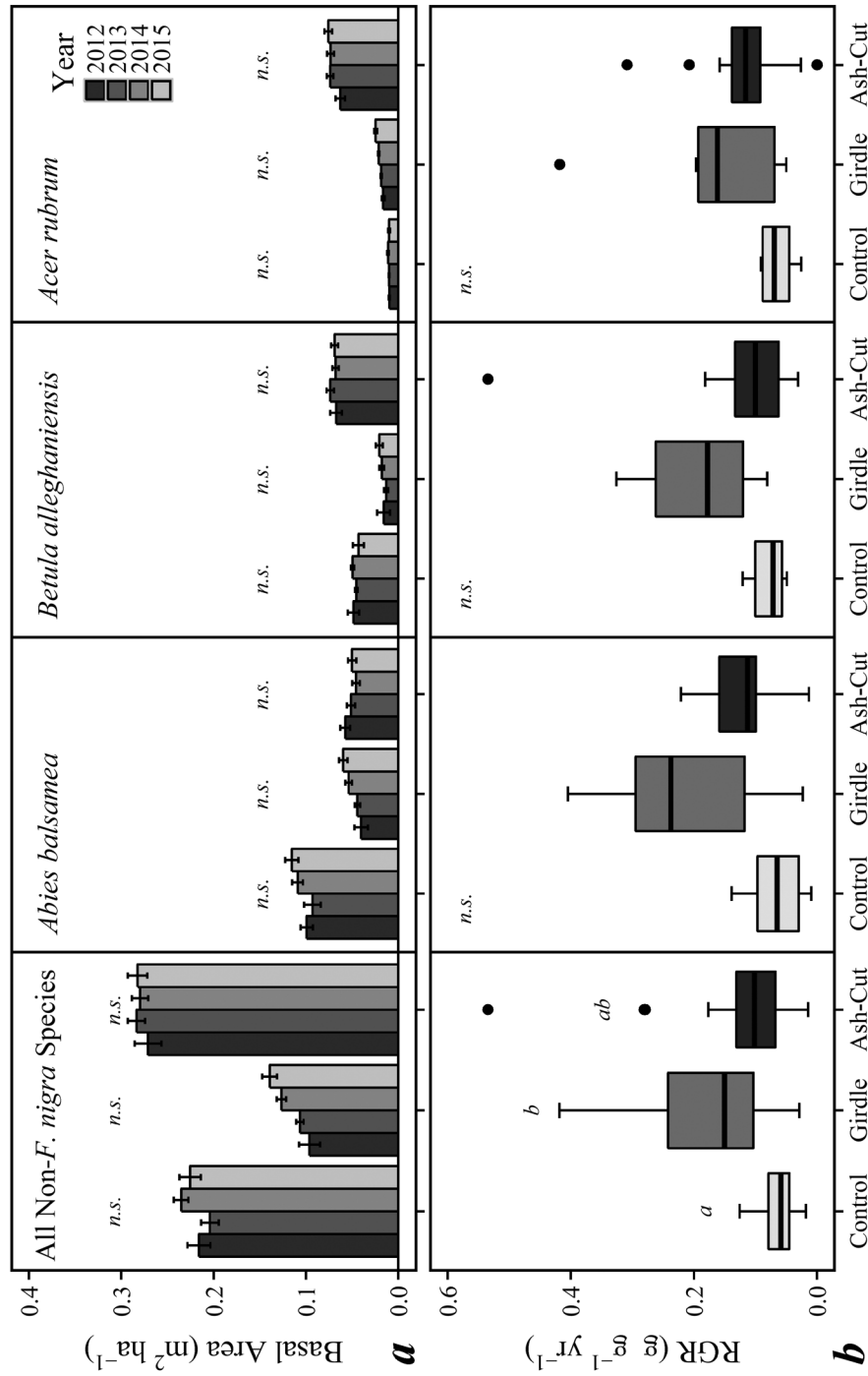
## 2.8 Figures



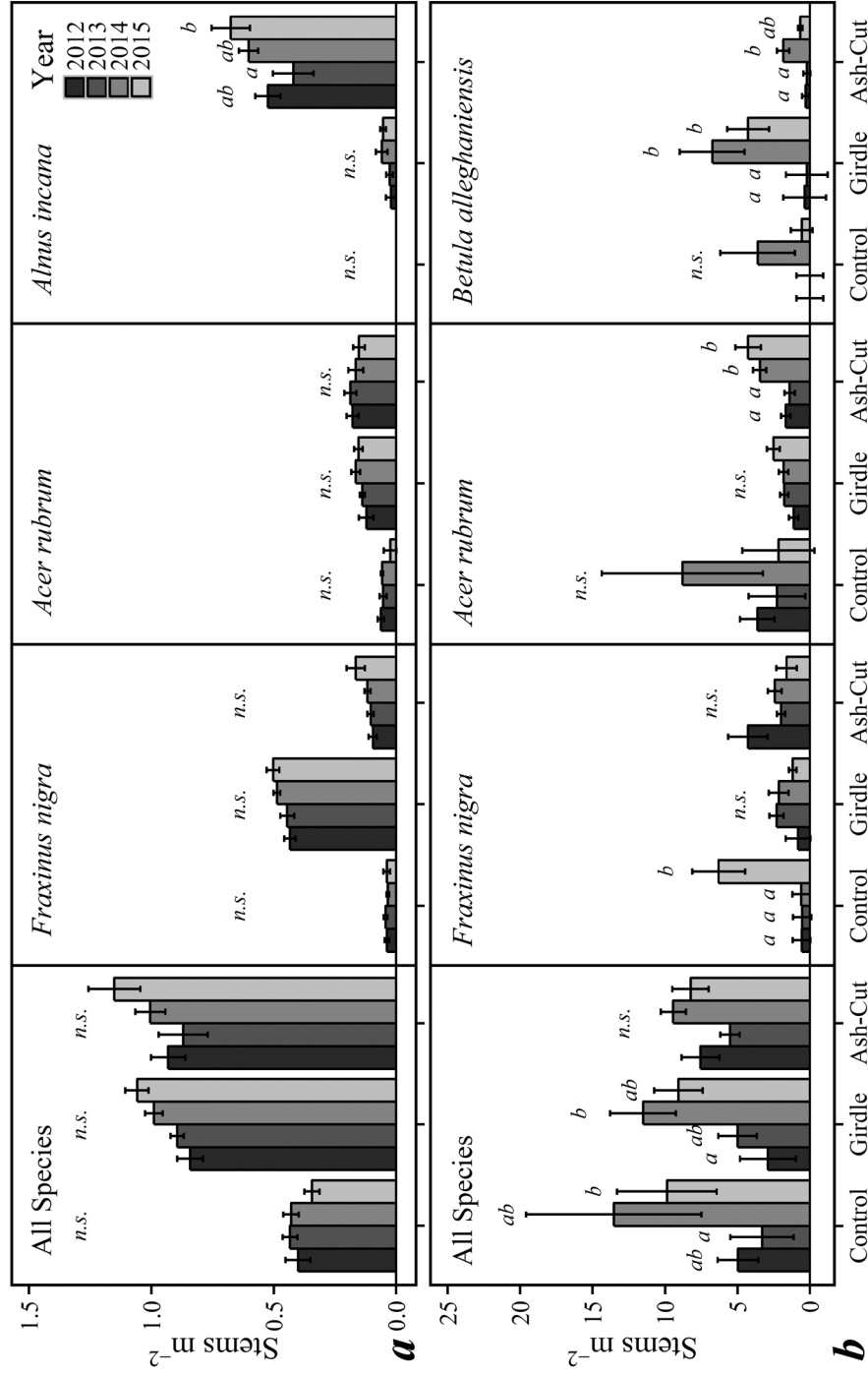
**Figure 2.1.** Regional map (left) with Forest Inventory and Analysis (FIA) importance value (Prasad and Iverson 2003) and detail map of the Ottawa National Forest in western Upper Michigan (right) with study site locations (diamonds). Each block of three sites contained one replicate of each treatment.



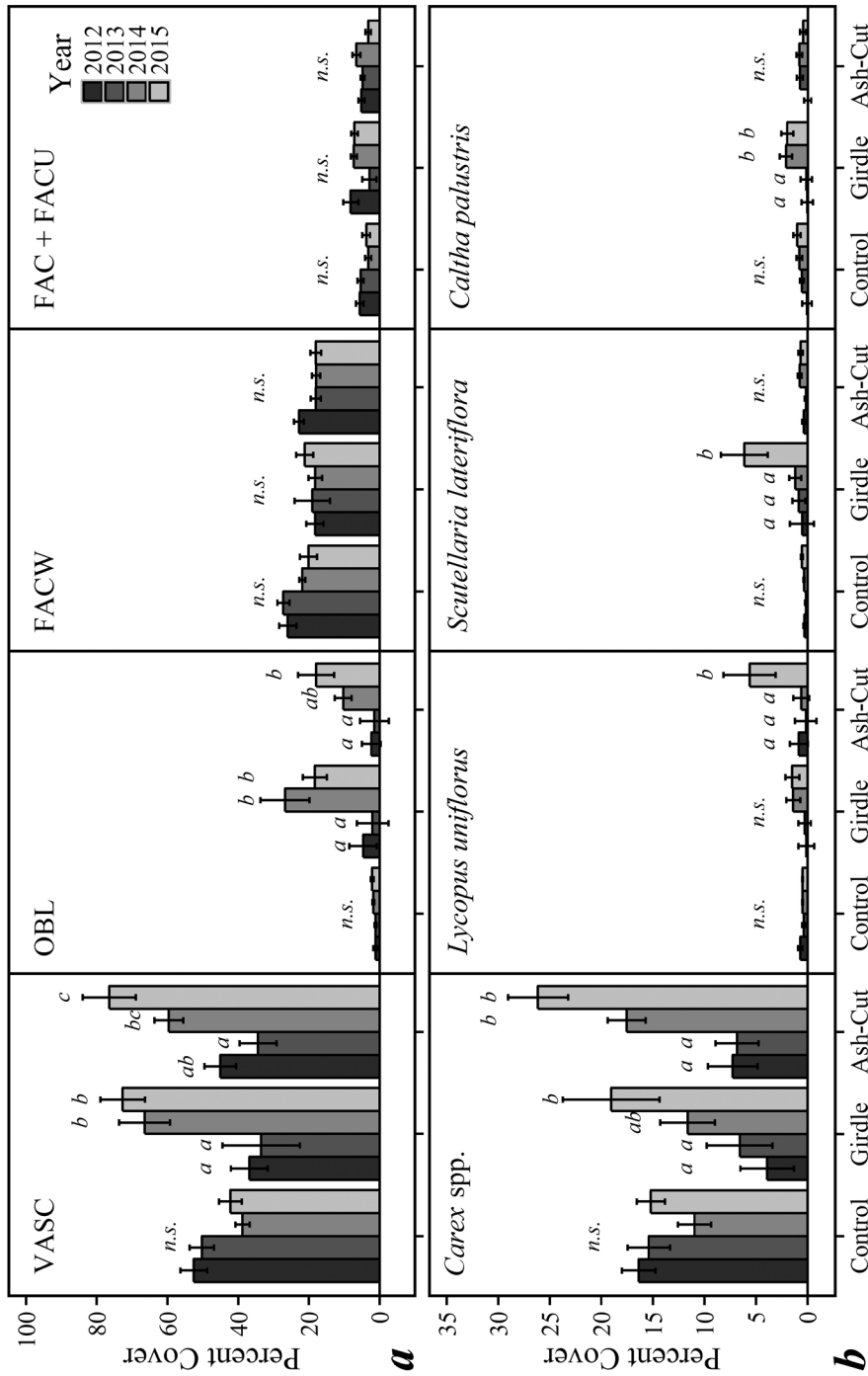
**Figure 2.2.** Vegetation monitoring plot design: *a*) 11.3 m, radius of overstory plot; *b*) 5.5 m, distance from overstory plot center to subplot center; *c*) 4 m, radius of sapling subplot; *d*) 1.5 m, radius of large woody stem subplot, *e*) 0.56 m, radius of small woody stem/herbaceous subplot



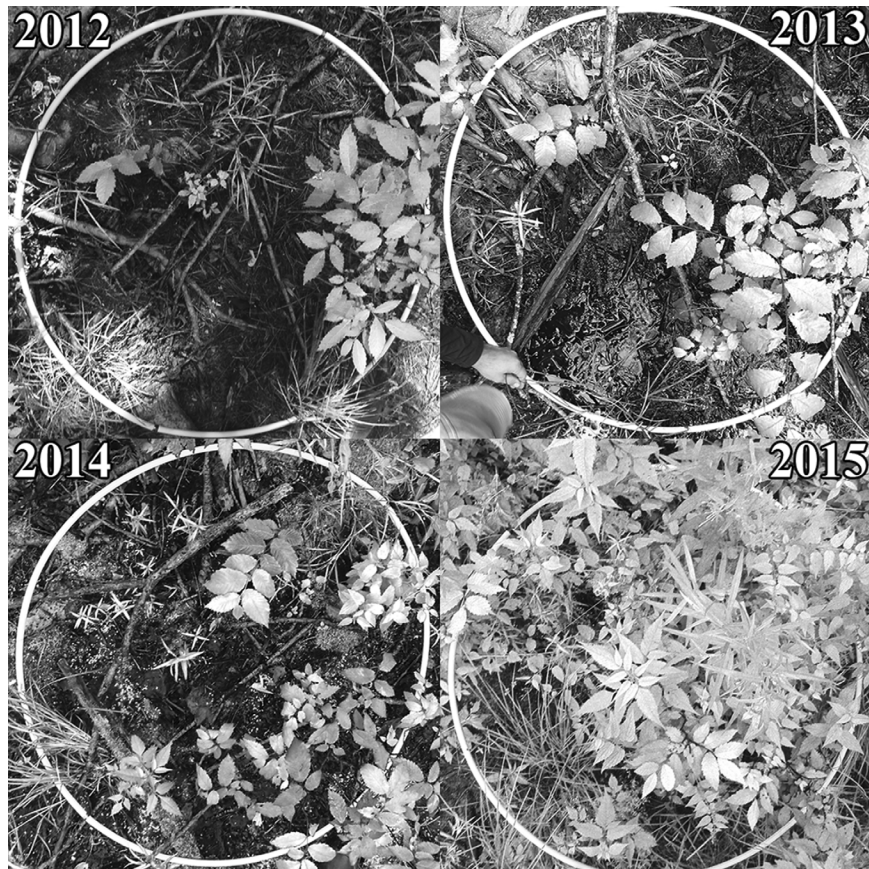
**Figure 2.3.** Sapling (stems 2.5 cm < DBH < 10 cm) basal area by year (**a**) and relative growth rate across three growing seasons (**b**) for all non-*Fraxinus* species combined and the non-*Fraxinus* sapling species with the greatest basal area. Basal area data are mean  $\pm$  standard error. Relative growth rate boxplots represent the 25th (lower edge) and 75th percentiles (upper edge) and medians (middle line). Fences extend to 1.5 times the interquartile range, with outliers beyond these values indicated by discrete points. Labels that do not share a letter indicate a statistically significant difference ( $p \leq 0.05$ ) within a given treatment.



**Figure 2.4.** Woody stem density (mean  $\pm$  standard error) for all species combined and the most abundant large (**a**, stems  $\leq 2.5$  cm DBH and  $> 50$  cm in height) and small (**b**, stems  $\leq 2.5$  cm DBH and  $\leq 50$  cm in height) woody species. Labels that do not share a letter indicate a statistically significant difference ( $p \leq 0.05$ ) within a given treatment.



**Figure 2.5.** Areal cover (mean  $\pm$  standard error) of vascular plants by functional group (**a**) and select common taxa (**b**). Functional groups in **a** represent all vascular herbaceous species (VASC), non-graminoid obligate wetland (OBL), facultative wetland (FACW), and facultative plus facultative upland species (FAC + FACU). Labels that do not share a letter indicate a statistically significant difference ( $p \leq 0.05$ ) within a given treatment.



**Figure 2.6.** Serial photographs of a single representative subplot (radius = 0.56 m) within a 'Girdle' treatment. Photos were taken yearly at the time of survey (late July). Green hues were enhanced during the conversion to a black and white image to improve contrast.



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### 3.0 Simulated EAB infestation effects on biogeochemical processes and foliar characteristics<sup>2</sup>

#### 3.1 Abstract

Mortality of ash trees (*Fraxinus* spp.) resulting from the invasive emerald ash borer (EAB, *Agilus planipennis* Fairmaire (Coleoptera: Buprestidae)) has the potential to significantly alter biogeochemical processes within infested stands throughout North America. The effects on *Fraxinus nigra* Marshall (black ash)-dominated swamps are of particular interest, due to the high proportion of *F. nigra* in the canopy, and the unique nature of the biogeochemical cycles within these forested wetlands. A multiyear manipulative study of nine *F. nigra*-dominated wetlands in the Upper Peninsula of Michigan, USA was undertaken to investigate the potential response of above- and belowground biogeochemical processes and cycles to EAB infestation. Early- and late-stage infestations were simulated by girdling or felling *F. nigra* saplings and overstory trees. Treatments had only minimal impacts on inorganic soil nitrogen (N), with no change in N availability compared to control sites, though a significant increasing trend in nitrate was observed in the early-stage infestation simulation. This response may be attributable to reduced N-limitation and subsequent immobilization of N by the soil microbial community, as measurements of N return via litterfall indicated a reduction in demand for soil N from the dominant canopy species. The response of foliar morphological and biochemical characteristics of the retained overstory species appeared to be driven primarily by aboveground growing conditions and a change from shade- to sun-acclimated leaves as loss of *Fraxinus* leaf area led to greater light availability for the residual trees. Resultant changes included decreased mass-based N content, increased leaf mass per area, and decreased mass-based chlorophyll content. The litter of both co-dominant species in these stands, *Acer rubrum* L. (red maple) and *Betula alleghaniensis* Britton (yellow birch), had lower N content in unmanipulated stands than did *F. nigra*, which was further reduced by the experimental treatments. The shift from

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<sup>2</sup> Material contained Chapter 3 is in preparation for submission to a peer-reviewed journal.

N-rich *F. nigra* leaf litter to the lower N litter of residual species may have important feedback effects on soil processes, decreasing both forest floor carbon storage and the rate of N cycling through the influence of litter quality on decomposition rate and extent. Additionally, while increased microbial immobilization of N immediately following EAB infestation may reduce long-term site fertility changes, these responses may also limit the potential for short-term growth rate increases of extant woody vegetation.

### 3.2 Introduction

Invasive insects are a serious threat to forests across North America (Lovett et al. 2006). The effects of introduced insects and associated pathogens have resulted in widespread alteration to the structure of these forests and ecosystem processes (Gandhi and Herms 2010). Among the more recently discovered invasive insects in North America, the emerald ash borer (EAB, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)) has received considerable attention for the breadth, depth, and severity of its potential impacts (Herms and McCullough 2014). Concerns over the potentially profound effects of EAB on forest composition and ecosystem function stem from the ubiquity of the genus *Fraxinus* (ash) across eastern North America (Cappaert et al. 2005). Members of the genus are common in a wide variety of forest types throughout the region, though they often comprise relatively minor proportions of the canopy (MacFarlane and Meyer 2005). In contrast, *Fraxinus nigra* Marshall (black ash) is frequently a major component of deciduous and deciduous-conifer forested wetlands (Wright and Rauscher 1990) and occurs in nearly pure stands in portions of the range (Erdmann et al. 1987). In the Upper Peninsula of Michigan, USA, the *F. nigra*-*Ulmus americana* L. (American elm)-*Acer rubrum* L. (red maple) forest type tends to be dominated by *F. nigra*, particularly in areas with very poor drainage and organic soils (Erdmann et al. 1987). As a result, the potential fate *F. nigra* of is of particular concern, owing to the potential severity of impacts that may result from the mortality of a significant portion of the forest canopy (Kashian and Witter 2011).

The loss of a large proportion of the woody vegetation is likely to have profound direct and indirect effects on biogeochemical cycles within these ecosystems (Gandhi and Herms 2010), which may have lasting effects on future forest recovery. Following *F. nigra* mortality, both above- and belowground growing conditions may be altered. Belowground, soil nutrient dynamics are likely to be altered by EAB-induced mortality via a number of mechanisms (Hunter 2001); among these, the role of plant demand for nitrogen (N) and decomposition and nutrient mineralization rates have been suggested to be of the greatest importance to biogeochemical cycling (Vitousek and Melillo 1979). In addition, aboveground changes in the light environment and microclimate may stimulate morphological and biochemical acclimation of leaves to the new growing conditions (Naidu and DeLucia 1997 and 1998, Jones and Thomas 2007).

Given their fundamentally interconnected nature, perturbation in above- or belowground ecosystem properties and processes are likely to induce changes throughout the system, potentially resulting in cascading effects through the forest community (Castello et al. 1995, Ellison et al. 2005). Damage due to phytophagous insects has frequently been associated with increased soil N availability (e.g. Jenkins et al. 1999, Orwig et al. 2008, Keville et al. 2013), which is subsequently either redistributed within the system or potentially subject to export (Lovett et al. 2002). As a result, this change in belowground processes may result in improved aboveground nutrient status of residual vegetation (Levy-Varon et al. 2014, Griffin et al. 2011) or may result in nutrient losses from the system (Swank et al. 1981, Eshleman et al. 1998), potentially with negative consequences for downstream ecosystems and long-term site productivity (Lovett et al. 2002). Changes in aboveground plant properties may, in turn, impact soil processes through the role of the canopy in regulating the soil physical environment and litter production, the characteristics of which exert strong influences on nutrient cycling (Prescott 2002). As such, it is critical to consider the implications of EAB-induced mortality on both above- and below-ground processes (Bardgett et al. 1998). Additionally, potential changes within these systems are likely to occur at different temporal scales (e.g. short-term/fast cycle impacts such as direct effects on litter input quantity or plant demand versus long-

term/slow cycle effects, including changes in litter quality, community composition, and nutrient cycling rates; Hunter 2001, Lovett et al. 2006). It is thereby necessary to consider effects at a range of severities and time scales to more fully understand how EAB infestation may affect forest processes (Stadler et al. 2003).

The foundation upon which our understanding of the effects that forest pathogens and pests may have on ecosystem nutrient dynamics, as well as plant responses to disturbance, is built from the multitude of studies examining the biogeochemical impacts of abiotic or anthropogenically-induced changes in growing conditions (e.g. Likens et al. 1970, Vitousek and Melillo 1979, Goulet and Bellefleur 1986). These investigations may provide a useful framework by which to understand the effects of biotic disturbances on forest processes (Orwig et al. 2008). However, it is important to recognize that the effects of biotic disturbances on nutrient cycling may differ fundamentally from other disturbance types (Castello et al. 1995), and substantial differences frequently exist even among various types of biotic disturbances (Hunter 2001). Additionally, the vast majority of studies into the effects of forest disturbance on biogeochemical cycles have been conducted in upland systems, while much of the limited research into these cycles within forested wetlands has focused on managed systems (Nakagawa et al. 2012). As such, the extent to which patterns suggested by results from upland forests and managed wetlands are applicable to the *F. nigra*-dominated forested wetlands of this region is unknown.

A multiyear manipulative study was initiated on the Ottawa National Forest, Michigan, USA to assess the potential impacts of EAB on ecosystem processes in *F. nigra*-dominated wetlands. Emerald ash borer-induced mortality of *F. nigra* was simulated across a series of stands to emulate the effects of early- and late-stage infestation on above- and belowground nitrogen cycling as well as canopy morphological and biochemical acclimation. The objective of this investigation was to determine the near-term effects of *F. nigra* mortality on nutrient dynamics to assess how changes in these cycles may affect long-term site fertility and future vegetation recovery. Additionally, the inclusion of two fundamentally different disturbance severities may

provide further insight into the drivers of change on these ecosystem processes, as sudden large impacts may induce an ecological response that differs fundamentally from slower more subtle changes (Orwig et al. 2013).

### **3.3 Methods and Materials**

#### *3.3.1 Study site description*

Study sites were located on the Ottawa National Forest in the western Upper Peninsula of Michigan, USA (Figure 3.1). The regional climate is continental, with average monthly temperatures ranging from a minimum of -11.3 °C in January to a maximum of 18.2 °C in July (1981-2010 normals data from the Bergland Dam, MI station, UTM Zone 16N 304840 5162412, Arguez et al. 2010). Over the same period, mean annual precipitation was 1010 mm year<sup>-1</sup>, with the majority occurring from June through November (Arguez et al. 2010). During this study, surface water in these wetlands typically persisted from the onset of snowmelt until mid-summer and remained near the soil surface throughout the growing season (Van Grinsven 2015). Surface soils consisted of woody peat histosols ranging in depth from 40 to greater than 690 cm, typically underlain by a layer of clay or clay loam.

Nine wetlands were selected based upon overstory composition, size, and landscape position. All nine study sites contained *F. nigra* as the most abundant overstory species, ranged in size from 0.20 to 1.25 ha, and were located in isolated depressions within first-order watersheds. Study sites were divided into three blocks based upon geographical location, in which a ‘greedy’ algorithm was used to create blocks with minimized in-block spatial variation, using the blockTools package (Moore 2011) for the R statistical environment (R Core Team 2015). To assign treatments within each block, scaled and weighted values for initial estimates of percent *F. nigra* basal area (BA), total site area, and average depth of organic soil were calculated. These values were then used to assign treatments such that among-treatment variation in these values was minimized. Each block contained one site of each treatment type, which consisted of ‘Control,’ ‘Girdle,’ and ‘Ash-Cut,’ where ‘Girdle’ and ‘Ash-Cut’ treatments were intended to mimic early-

and late-stage EAB infestations, respectively. Within ‘Girdle’ sites, all *F. nigra* stems  $\geq 2.5$  cm in diameter at breast height (DBH, 1.37 m) were hand-girdled with a drawknife to a depth sufficient to sever all phloem and cambium tissue. All *F. nigra* stems  $\geq 2.5$  cm DBH within ‘Ash-Cut’ sites were felled by chainsaw and allowed to remain onsite. Both treatment types were applied in the winter of 2012/2013. The lower diameter limit for treatment of *F. nigra* was based on reports that EAB-induced mortality is typically confined to ash stems  $> 2.5$  cm DBH (McCullough et al. 2015). No additional treatment of epicormic sprouting that occurred following treatment was undertaken.

Within each study site, three instrument clusters were established at spatially distributed random locations throughout the wetland. Each cluster contained infrastructure for the assessment of soil gas fluxes, the isotopic and nutrient composition of precipitation throughfall, and litterfall. Cluster centers were located such that all instrumentation was located  $> 5$  m from the wetland edge and  $> 10$  m from another set of instruments. Raised boardwalks were installed from the upland/wetland ecotone to each instrument cluster to minimize the effects of frequent site visits on soil and site hydrology.

### 3.3.2 Sample collection

Ion exchange resin (IER) bags (Binkley and Hart 1983) were used to evaluate inorganic soil nitrogen availability, using a modification of the protocol described in Giblin et al. (1994). Bags were constructed of undyed nylon/Lycra® fabric, each containing 5 g of mixed bed IER (Dowex Marathon MR-3, Sigma-Aldrich Corp., St. Louis, MO, USA), closed with a nylon cable tie. After construction, bags were soaked for 1 hr in a 1.2M HCl solution, rinsed thoroughly in multiple changes of deionized water, and frozen in polyethylene bags until deployment. At each study site, a cluster of three IER bags was buried at each of ten randomly generated locations (ArcGIS, Esri, Redlands, CA, USA) such that clusters were located a minimum of 5 m from either the study site edge or another cluster, and at least 1 m from any other infrastructure. To minimize disruption of upper soil layers, an angled incision was made into the soil approximately 30 cm from the center flag at each location. One bag was then placed in the incision so that the

lowermost point was at a depth of 10 cm below the soil surface, after which the soil was pressed back into place. The remaining two IER bags were placed in this manner at 120° intervals around the center point. Nylon monofilament was attached to each bag and tied to a marking flag to facilitate recovery.

Three deployments of IER bags occurred seasonally, with each deployment keyed to phenological events. Initial deployments occurred in mid-November of 2012, prior to treatment application. These bags were allowed to overwinter and removed after the leaves of all deciduous tree species were fully expanded in the spring (late June/early July). At the time of removal, a new IER bag was placed in the same soil opening. The second set of bags was removed and replaced at the onset of senescence (late August/early September); this third set of IER bags remained in place until mid-November. Deployments were repeated on the same schedule in 2013/14, though the bags placed from mid-November 2013 forward were offset from the initial locations by 60°. After removing the IER bags from the soil, they were thoroughly rinsed with deionized water, placed in polyethylene bags, and stored on ice while onsite. Bags were immediately frozen at -20 °C upon return to the laboratory and remained frozen until extraction.

Twenty 0.5 m<sup>2</sup> litter traps constructed of polyvinyl chloride pipe frames and fiberglass window screen material were installed at each site. A pair of litter traps was located opposite each other at 3 m from the center point of each instrument cluster, while the remaining 14 were placed at randomly generated points along installed boardwalks, with a minimum of 3 m between traps. Traps were elevated to prevent contamination from surface water or soil particles. Leaf litter was collected monthly at each site beginning in mid-June until mid-October from 2012 to 2014. Collected litter was returned to the laboratory where it was dried at 65 °C until no further weight change was observed, after which it was stored at room temperature. All litter samples were subsequently sorted into *F. nigra* and non-*F. nigra* components and weighed, while a subset of traps from each site ( $n = 5$ ) were further sorted to separate *Betula alleghaniensis* Britton (yellow birch)



and *A. rubrum* litter. Litter from all months for a given trap and species were pooled for nutrient analyses (see *Sample processing and analysis*, below). Litter trap locations and the subset of traps that received supplemental sorting remained fixed between study years, though if a sample in a given month was unusable due to damage to the trap (e.g. animal damage or flooding), it was replaced by another trap from the site, so that each composite annual sample for nutrient analysis contained litter from each month of the growing season.

Live foliage samples were obtained by shotgun at each site in late August from 2012 to 2015, prior to the onset of senescence. For each of the three major overstory species (*F. nigra*, *A. rubrum*, and *B. alleghaniensis*), five dominant or co-dominant overstory stems nearest the vegetation study plot centers (see Chapter 2) were selected and used throughout the course of the study. Samples were taken from at least three different east- or west-facing locations (Law et al. 2008) of the upper third of the crown, yielding a composite sample for each tree comprised of at least 25 leaves. Within the ‘Girdle’ sites, foliage samples from epicormic branching that occurred following treatment were obtained using the same criteria. Foliage samples were immediately placed in a zip-top polyethylene bag and stored in a cooler with ice while onsite, and subsequently stored at 4 °C until further processing.

### 3.3.3 *Sample processing and analysis*

Prior to extraction, IER bags were thawed at 4 °C overnight, after which they were again rinsed with deionized water to remove any remaining soil particles. Any bags that exhibited evidence of ruptures in the fabric or other forms of resin loss were discarded. Each cluster of bags was extracted as a group in 50 ml of 2M KCl solution (Kjønaas 1999) per bag for 1 hr at 150 strokes per minute on an orbital shaker table (New Brunswick Innova 2100, Eppendorf, Hamburg Germany). The resulting eluent was passed through a 1.6 µm-pore size borosilicate glass fiber filter (Grade A, Sterlitech Corp., Kent, WA, USA) and frozen at -20 °C in a polypropylene centrifuge tube until analysis. Colorimetric analysis of the inorganic nitrogen content of each sample was

performed on a microplate reader (SpectraMax M2, Molecular Devices LLC, Sunnyvale, CA, USA) using the techniques described for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in Sinsabaugh et al. (2000) and Doane and Horwath (2003) respectively. Each polystyrene well plate contained triplicate aliquots of each sample along with a set of known standards prepared in 2M KCl. The concentration of each sample was calculated by means of a linear regression equation fit to the set of standards on each plate, using the mean absorption of each set of triplicates.

Live foliage samples obtained in 2012 and 2013 were dried at 65 °C until a stable weight was reached. In 2014 and 2015, the area of 25 fresh leaves from each sample tree was measured with a leaf area meter set to a resolution of 1 mm<sup>2</sup> (LI-3100, LI-COR Inc., Lincoln, NB, USA), after which the leaves were dried as above. Foliage samples obtained in 2015 received additional processing prior to drying to determine chlorophyll content (Warren 2008). A stainless steel cork borer was used to cut a 0.58 cm<sup>2</sup> disk from a randomly selected subsample of 10 leaves. Leaf disks were subsequently freeze-dried and weighed. In a 2 ml polypropylene microcentrifuge tube, a 0.22 cm stainless steel ball bearing was combined with a pair of leaf disks from each sample tree, which was then ground for 60 s at 36 Hz in a vibratory bead mill (Mini-Beadbeater-96, BioSpec Products Inc., Bartlesville, OK, USA). To each microcentrifuge tube, 1 ml of  $\text{MgCO}_3$  neutralized ethanol (Ritchie 2006) was pipetted and the samples were returned to the vibratory mill for 120 s. Samples were then centrifuged, after which the supernatant was decanted to a new microcentrifuge tube, and the retained pellet was resuspended in an additional 1 ml of neutralized ethanol and again milled for 120 s. Following centrifugation of this second extraction, the two supernatants were combined and centrifuged a final time. The chlorophyll *a* and *b* content of the pooled supernatants was determined following the microplate reader technique with pathlength correction for a neutralized ethanol matrix described in Warren (2008). Chlorophyll content was calculated as the average concentration of three replicates per sample using published equations for ethanol (Ritchie 2006). At all times during the processing of samples for chlorophyll analysis, leaf disks, extractant, and extracts were kept at 4 °C during routine handling and for

longer periods at -20 °C. All handling and processing took place in a darkened lab or under aluminum foil cover to limit photodegradation of chlorophylls.

Dried litter and live foliage samples were ground to a fine powder (8000M Mixer/Mill, Spex SamplePrep LLC, Metuchen, NJ, USA), from which the mass-based carbon and nitrogen ( $N_{\text{MASS}}$ ) concentration was determined via elemental combustion analysis (ECS 4010 CHNSO Analyzer, Costech Analytical Technologies Inc., Valencia, CA, USA).

### 3.3.4 Analytical methods

The mass of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  eluted from the IER was determined from the measured concentration and volume of eluent, from which the mass of the nitrogen component of each molecule was then calculated. Due to the loss of individual IER bags and the variable length of deployment between seasons, these values were standardized by resin mass and time, yielding soil N availability as the mass of N per mass of resin per day ( $\mu\text{g g}^{-1} \text{d}^{-1}$ ). A large early snowfall event in November of 2014 prevented access to six of the nine sites to remove the final set of IER bags. These bags were not retrieved until May of 2015, and as a result were excluded from statistical analyses. Soil N data were square root transformed to improve normality and non-constant variance. Mixed effects models fit to these data used season and treatment as fixed effects, and cluster nested within site as random effects.

Leaf mass per area (LMA) for live foliage was calculated as oven-dry mass divided by leaf area ( $\text{g m}^{-2}$ ). Measured live foliage  $N_{\text{MASS}}$  and LMA were then used to determine leaf nitrogen on an area basis ( $N_{\text{AREA}}$ ,  $\text{g m}^{-2}$ ). Concentrations of chlorophyll *a* and *b* for each pair of leaf disks was averaged to yield a single value per sample tree, which was then used in concert with leaf disk area and mass to calculate chlorophyll content as a sum of both measured forms on an area and mass basis ( $\text{Chl}_{\text{MASS}}$ ,  $\mu\text{g g}^{-1}$ ;  $\text{Chl}_{\text{AREA}}$ ,  $\mu\text{g m}^{-2}$ ). Mixed models fit to live foliage N and LMA data used treatment and year as fixed effects, and sample tree nested within site as random effects. Models were fit to chlorophyll data using identical random effects, with treatment as the sole fixed effect.

Data were log transformed prior to fitting models with the exception of LMA, for which a square root transformation was superior at improving non-normality and heteroskedasticity.

Mass-based nitrogen concentration ( $N_{\text{MASS}}$ ,  $\text{mg g}^{-1}$ ) of litter samples was multiplied by the mass of litterfall for a given species to calculate N return via litterfall for each year ( $\text{kg ha}^{-1} \text{yr}^{-1}$ ). Because lost samples from damaged traps were replaced by litter from other traps within the site, litter N return to a specific point could not be utilized in subsequent analyses. As a result, the litter N return to each site was calculated as the mean of the five composite samples, and models were fit using year and treatment as fixed effects, with study site as the sole random effect. Data were log transformed prior to fitting models.

All analyses were performed in the R statistical environment. Comparisons of pre-treatment litter and fresh foliage  $N_{\text{MASS}}$  site means were made using the Kruskal-Wallis rank sum test provided in the stats R library (R Core team 2015) followed by pairwise comparisons using Nemenyi tests (package PMCMR, Pohlert 2014). Mixed effects models were fit using the R package lme4 (Bates et al. 2015). Prior to model fitting, data were transformed as described above to improve normality and non-constant variance. Visual examination of residual plots from all fitted models did not reveal significant deviations from the assumptions of normality or heteroskedasticity. Post-hoc pairwise comparisons and comparisons of trends were made using the lsmeans package (Lenth 2016) using the Tukey-HSD method to adjust  $p$ -values. A significance level of  $p \leq 0.05$  was used for all comparisons. Standard errors for presentation in figures were calculated using a method of accounting for within-subjects designs presented in Morey (2008).

### **3.4 Results**

#### *3.4.1 Soil N availability*

No significant differences among treatments in ion exchange resin capture of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  for a given season were observed over the two years post-treatment (Figure 3.2).

However, a significant increasing trend within ‘Girdle’ treatments was observed compared to the ‘Ash-Cut’ treatment ( $p = 0.043$ ), though this trend was not significantly different from the ‘Control.’ It should be noted that the  $y$ -axis scale of the lower panel was reduced for ease of interpretability, resulting in the omission of 4 outliers of  $\text{NO}_3^-$  accumulation from the figure, all of which were measured in the ‘Girdle’ treatment. These values were as follows:  $17.25 \mu\text{g NO}_3\text{-N g}^{-1} \text{ day}^{-1}$  in Autumn 2013; 12.71, 23.85, and  $44.28 \mu\text{g NO}_3\text{-N g}^{-1} \text{ day}^{-1}$  in Summer 2014. Significant seasonal effects were also observed within treatments, indicating that soil  $\text{NH}_4^+$  availability was typically greater in summer and autumn compared to spring (Table 3.1).

### 3.4.2 Foliar nitrogen, LMA, and chlorophyll

Prior to treatment, foliar  $\text{N}_{\text{MASS}}$  was significantly greater in *F. nigra* and *B. alleghaniensis* compared to *A. rubrum* ( $p = 0.006$  and  $p < 0.001$ , respectively; Figure 3.3). Foliar  $\text{N}_{\text{MASS}}$  responses to simulated EAB infestation varied by species and treatment (Figure 3.4). No significant change in *A. rubrum*  $\text{N}_{\text{MASS}}$  was observed in any year within the ‘Girdle’ treatment, however *A. rubrum*  $\text{N}_{\text{MASS}}$  declined in ‘Ash-Cut’ sites in 2013 and 2014 compared to pre-treatment ( $p = 0.002$  and  $p = 0.036$ , respectively), but returned to pre-treatment levels by 2015 (Figure 3.4). *Betula alleghaniensis*  $\text{N}_{\text{MASS}}$  responded consistently to treatment, with significant declines compared to pre-treatment across all years in both the ‘Girdle’ and ‘Ash-Cut’ ( $p < 0.001$  for all years and treatments, Figure 3.4). Canopy leaf-out from treated *F. nigra* trees in ‘Girdle’ sites occurred for a single year post-treatment, and this foliage was significantly elevated in N concentration compared to pre-treatment ( $p < 0.001$ , Figure 3.4). Foliar  $\text{N}_{\text{MASS}}$  of samples taken from *F. nigra* epicormic branching in the ‘Girdle’ sites in 2013 was significantly higher than canopy  $\text{N}_{\text{MASS}}$  ( $p < 0.001$ ), and N concentrations of epicormic foliage were greater than 2012 canopy foliage across all post-treatment years ( $p < 0.001$  for all years). No significant differences in  $\text{N}_{\text{AREA}}$  between treatments were observed in either 2014 or 2015 (Figure 3.5).

*Acer rubrum* LMA (Figure 3.6) was significantly higher in the ‘Ash-Cut’ sites compared to ‘Control’ ( $p = 0.036$ ) in 2014, but declined slightly in 2015 such that the differences were no longer significant ( $p = 0.112$ ). Significantly greater LMA values compared to ‘Control’ were observed in *B. alleghaniensis* foliage from both ‘Girdle’ and ‘Ash-Cut’ sites in 2014 ( $p = 0.010$  and  $p = 0.006$ , respectively), but were not significant in 2015 ( $p = 0.167$  and  $p = 0.111$ ). This does not appear to be related to changes in 2015 LMA within treated sites, as no significant differences compared to 2014 were observed. Rather, *B. alleghaniensis* LMA increased within the ‘Control’ sites in 2015 ( $p < 0.001$ ). In contrast to the increased LMA observed for other species, *F. nigra* epicormic foliage LMA from ‘Girdle’ sites was lower in 2014 compared to ‘Control’ canopy foliage ( $p = 0.004$ ), but was no longer significantly different in 2015 ( $p = 0.152$ ).

Mass-based chlorophyll content ( $\text{Chl}_{\text{MASS}}$ , Figure 3.7 upper panel) of *A. rubrum* foliage was significantly lower in the ‘Ash-Cut’ compared to ‘Control’ ( $p = 0.044$ ), while  $\text{Chl}_{\text{MASS}}$  of foliage from *F. nigra* epicormic branches in the ‘Girdle’ treatment was higher than canopy foliage in ‘Control’ sites ( $p = 0.011$ ). No significant differences in *B. alleghaniensis*  $\text{Chl}_{\text{MASS}}$  were observed, though concentrations were lower in both ‘Girdle’ and ‘Ash-Cut,’ and approached significance in the ‘Ash-Cut’ treatment ( $p = 0.097$ ). While patterns of  $\text{Chl}_{\text{AREA}}$  (Figure 3.7 lower panel) were similar to  $\text{Chl}_{\text{MASS}}$  across treatments and species, no significant differences were observed for any species, though the decreased  $\text{Chl}_{\text{AREA}}$  observed in *A. rubrum* foliage in the ‘Ash-Cut’ treatment approached significance, compared to ‘Control’ ( $p = 0.087$ ), as did the increased  $\text{Chl}_{\text{AREA}}$  observed in *F. nigra* foliage from ‘Girdle’ sites ( $p = 0.065$ ).

### 3.4.3 Litter mass and nitrogen content

Prior to treatment, litter  $\text{N}_{\text{MASS}}$  varied by species; N concentrations in *F. nigra* litter were significantly greater than *B. alleghaniensis* ( $p = 0.050$ ), which in turn were significantly greater than *A. rubrum* ( $p = 0.040$ , Figure 3.3). Litter  $\text{N}_{\text{MASS}}$  (Figure 3.8) of *F. nigra* was significantly higher in both 2013 and 2014 in ‘Girdle’ sites ( $p = 0.008$  and  $0.003$ ), and in 2014 in ‘Ash-Cut’ sites ( $p = 0.002$ ) compared to pre-treatment  $\text{N}_{\text{MASS}}$ , while measured

values in the 2013 ‘Ash-Cut’ treatments approached significance ( $p = 0.057$ ). No significant change in  $N_{\text{MASS}}$  of *A. rubrum* or *B. alleghaniensis* was observed. Total *A. rubrum* litter deposition (Figure 3.9) increased in both treatment types in 2013 compared to 2012, but was significant only in the ‘Girdle’ treatment (‘Girdle’  $p = 0.043$ , ‘Ash-Cut’  $p = 0.072$ ), and returned to approximately pre-treatment levels by 2014. No significant changes in litterfall from *B. alleghaniensis* or all remaining species combined was observed. Significant declines in *F. nigra* litterfall were observed in both treatment types in the first year following treatment (‘Girdle’  $p = 0.003$ , ‘Ash-Cut’  $p < 0.001$ ), and remained highly significant in 2014 ( $p < 0.001$  for both treatments). Nitrogen return via *F. nigra* litter (Figure 3.10) followed a similar pattern, though decreases in the ‘Girdle’ treatment were not significant until the second year post-treatment ( $p = 0.004$ ), while they were significant immediately following treatment in the ‘Ash-Cut’ sites ( $p = 0.003$  in both 2013 and 2014). No other differences in litterfall N-return were observed.

### 3.5 Discussion

#### 3.5.1 Soil nitrogen availability

A change in N availability is perhaps the most commonly reported biogeochemical change following biotic disturbance of forest ecosystems (Lovett et al. 2006). Increased  $\text{NH}_4^+$  availability has been observed in forests affected by the invasive hemlock woolly adelgid (HWA, *Adelges tsugae* Annand; Jenkins et al. 1999, Orwig et al. 2008, but see Knoepp et al. 2011) and the native mountain pine beetle (MPB, *Dendroctonus ponderosae* Hopkins; Griffin et al. 2011, Clow et al. 2011, Keville et al. 2013). As no increase in  $\text{NH}_4^+$  availability was observed in this study (Figure 3.2), it is perhaps tempting to attribute the lack of response to a mechanism of disturbance (i.e. manual girdling) that poorly simulated infestation by phytophagous insects. However, girdling has been used to successfully simulate HWA infestation, and yielded positive soil N availability responses similar to actual infestation (Yorks et al. 2003, Orwig et al. 2013, Trahan et al. 2015). Large increases in  $\text{NH}_4^+$  have also been reported following salvage logging of *Tsuga canadensis* (L.) Carriere (eastern hemlock; Kizlinski et al. 2002, Orwig et al. 2013). Additionally, numerous studies have reported increased  $\text{NO}_3^-$  availability in

soil following HWA (Jenkins et al. 1999, Orwig et al. 2008, Cessna and Nielsen 2012) and MPB (Clow et al. 2011) infestation, though this response is not consistent across all studies (Keville et al. 2013), potentially due to differences in atmospheric N deposition (Griffin et al. 2011).

As previously noted, much of the research into biogeochemical responses to disturbance has been conducted in upland systems, and thus the applicability of these previously observed responses to *F. nigra*-dominated wetlands is unknown. Increased soil water N content has been observed in forested wetlands following harvest (Trettin et al. 1997), harvest followed by site preparation (McLaughlin et al. 2000) and fire (Belova et al. 2014). Similarly, increased export of N in streams draining disturbed forested wetlands has also been observed (Bayley et al. 1992, Nieminen 2004). However, no change in N availability following forest harvest has also been observed following large-scale vegetation removals in forested wetlands (Nakagawa et al. 2012, Westbrook and Devito 2004).

Though exceptions have been observed, given the results of similar studies across both upland and wetland forests, the lack of soil  $\text{NH}_4^+$  increase in either treatment type would seem contrary to expectation. Increased  $\text{NH}_4^+$  availability following disturbances is frequently attributed to increased mineralization rates and decreased plant demand following biotic disturbances (Jenkins et al. 1999, Cobb et al. 2006 Orwig et al. 2008, Clow et al. 2011, Keville et al. 2013). However, no response in mean growing season water table height or soil temperature to treatment was observed, though water table heights in the first year post-treatment were significantly higher within all sites and treatments (Van Grinsven 2015). While the increased water table height had the potential to reduce gross mineralization rates, these responses should be similar across all sites, and as a result, no change in gross mineralization rates due to differences in environmental conditions among treatments was likely. As such, no treatment effect on soil N availability resulting from a change in environmental conditions was expected, though an increase in  $\text{NH}_4^+$  availability due to N mineralization from pre-treatment litter



inputs and reduced post-treatment plant demand remained a hypothesized effect of these simulated infestations.

The lack of soil N response may be attributable, in part, to changes in the microbial community, a major competitor for soil N (Kuzyakov and Xu 2013). Decomposition rates in northern peatlands are predominantly controlled by the elevated water table, resulting in the accumulation of the partially decomposed organic matter characteristic of surface soils in these ecosystems (Gorham 1991). This pool of organic matter is assumed to be relatively labile in the presence of oxygen, as evidenced by increases in decomposition rates when anoxic conditions are reduced (Kasimir-Klmedtsson 1997, Laiho 2006). If microbial activity in these saturated soils is secondarily limited by N availability (Kaye and Hart 1997, Hart and Stark 1997), then the reduction in competition for N by plant roots (Kuzyakov and Xu 2013) may allow for increased uptake and immobilization by microbial communities. Soil heterotrophs are known to respond rapidly to changes in N availability (Zak et al. 1990, Brooks et al. 1998, Zogg et al. 2000, Micks et al. 2004), which may subsequently be immobilized in soil organic matter (Koopsman et al. 1996, Nadelhoffer et al. 1999a and 1999b, Magill et al. 2000). As a result, total consumption by plants and microorganisms of  $\text{NH}_4^+$  may have remained relatively unchanged following treatment (Norton and Firestone 1996), resulting in the lack of response in  $\text{NH}_4^+$  availability observed in this study. In addition to the large, relatively labile pool of C present in the accumulated peat, fine root mortality following *F. nigra* decline and continued inputs of leaf litter may serve as additional N sinks. Both types of plant residues typically result in net N immobilization during the initial stages of decomposition (Aber and Melillo 1980, McClaugherty et al. 1984, Attiwill and Adams 1993), though the strength of the sink and point at which net N mineralization begins to occur varies considerably by tissue type and species (Berg and Ekbohm 1983, Chen et al. 2001, Hobbie et al. 2010).

Soil  $\text{CO}_2$  efflux following disturbances is commonly observed to decline, following both high-severity disturbances (e.g. forest harvest, Mattson and Swank 1989, Striegl and

Wickland 1998, Kurth et al. 2014) and low-severity disturbances (e.g. tree girdling, Nave et al. 2011, Levy-Varon et al. 2014). The results of girdling studies have been shown to mimic patterns of declining soil respiration following insect infestation (Nuckolls et al. 2009, Moore et al. 2013), which are attributed to decreased root activity. Efflux of CO<sub>2</sub> from the soil surface has also been observed to remain constant or increase following disturbance; such positive effects are frequently ascribed to increased microbial activity (Hendrickson et al. 1989, Toland and Zak 1994, Lytle and Cronan 1998). Van Grinsven (2015) reported increased fluxes of CO<sub>2</sub> in both ‘Girdle’ and ‘Ash-Cut’ treatments in this study, lending support for the hypothesis that the similar levels of NH<sub>4</sub><sup>+</sup> availability between treatments resulted from increased activity and biomass of soil heterotrophs in the manipulated sites, resulting in increased demand for NH<sub>4</sub><sup>+</sup>. Though rapid recovery of soil respiration following disturbance has also been hypothesized to result from the effects of vegetation regrowth or uptake from existing vegetation (Nave et al. 2011, Kurth et al. 2013, Levy-Varon et al. 2014), it seems unlikely that the epicormic sprouts from the stems of girdled or felled *F. nigra* require belowground infrastructure and activity (Vitousek and Melillo 1979) sufficient to account for the observed increases in soil CO<sub>2</sub> fluxes.

Among the potential mechanisms proposed by Vitousek and Melillo (1979) for retention of N within an ecosystem following disturbance was the uptake of NH<sub>4</sub><sup>+</sup> by decomposers, particularly in situations where disturbance results in large quantities of woody debris onsite. The role of organic matter in N retention following disturbance was shown experimentally soon thereafter, where microbial immobilization in woody residues reduced NO<sub>3</sub><sup>-</sup> export following harvest (Vitousek and Matson 1984 and 1985). Since that time, the importance of N-poor woody debris as a sink for N (Laiho and Prescott 2004), reducing soil N availability (Zimmerman et al. 1995) and mitigating potential N losses (Carlyle et al. 1998, Brais et al. 2006) has been well-demonstrated. Within the ‘Ash-Cut’ sites, *F. nigra* stems were allowed to remain onsite after felling, and so represent a potentially large sink for NH<sub>4</sub><sup>+</sup>. As a result, the availability of NH<sub>4</sub><sup>+</sup> to nitrifiers, which tend to compete poorly in the presence of high demand from heterotrophic

microorganisms (Hart et al. 1994), may be reduced. Though nitrification is an aerobic process that may be limited in anoxic wetland soils (Reddy et al. 1984),  $\text{NH}_4^+$  oxidation can occur in flooded soils by a number of processes (e.g. Reddy et al. 1989). The accumulation of  $\text{NO}_3^-$  on IER in all treatment types suggests the potential for  $\text{NO}_3^-$  production within these wetlands. Thus the role of woody debris as a sink for  $\text{NH}_4^+$  provides a possible explanation for the lack of  $\text{NO}_3^-$  production in the ‘Ash-Cut’ sites compared to the ‘Girdle’ treatment (Figure 3.2), despite similarities in other biotic and abiotic factors that influence the rate of nitrification. Analogous effects have been predicted to result in the latter stages of MPB infestation when snags begin to fell to the forest floor (Edburg et al. 2012).

As  $\text{NO}_3^-$  is highly mobile in the soil, excess production frequently results in export via hydrologic pathways, as observed in many studies following mechanical disturbance of vegetation (Likens et al. 1970, Martin et al. 1986). Export losses of  $\text{NO}_3^-$  in streamwater have also been observed following infestation by the invasive *Lymantria dispar* L. (gypsy moth; Webb et al. 1995, Eshleman et al. 1998) as well as native insect outbreaks (e.g. *Alosophila pometaria* Harris (fall cankerworm, Swank et al. 1981), *Ennomos subsignarius* Hübner (elm spanworm, Lewis and Likens 2007)). Leaching losses of  $\text{NO}_3^-$  below the rooting zone have likewise been reported following HWA infestation (Cessna and Nielson 2012). Though increased soil  $\text{NO}_3^-$  following HWA damage has been predicted to result in N leaching (Jenkins et al. 1999), such losses are not always observed (Block et al. 2012). A number of hypotheses to explain the lack of N export following HWA and MPB infestation (Block et al. 2012, Clow et al. 2011, Mikkelsen et al. 2013) have been suggested, and emphasize that factors beyond the impacted species and scale of damage or mortality must be considered when predicting impacts of phytophagous insects on ecosystem N losses (Lovett et al. 2002). Additionally, denitrification often plays an important role in the wetland N cycle (Saunders and Kalff 2001), and small depressional wetlands may be capable of retaining much of the increased  $\text{NO}_3^-$  available following harvest (Spoelstra et al. 2010). Thus, while soil  $\text{NO}_3^-$  availability was increasing within the ‘Girdle’ sites (Figure 3.2) during time periods in

which water was leaving the wetlands via ephemeral streams (Van Grinsven 2015), further research will be required to determine if the increase in available  $\text{NO}_3^-$  leads to N losses from the ecosystem.

Reported N mineralization rates in other *F. nigra*-dominated wetlands of the region are lower than surrounding upland forests, as would be expected given the frequently anoxic conditions (Zak and Grigal 1991). In forested wetland systems where gross mineralization rates are low, competition for N is strong and thus gross immobilization rates may be similar, and as a result little change in net mineralization or N availability following disturbance may be observed (Westbrook and Devito 2004, Nakagawa et al. 2012). These results lend further support for the hypothesis that microbial activity may control the short-term response of N availability to disturbance in these forests.

In addition to the microbial response, changes in the plant community may provide further explanation for the lack of change in soil N availability. By the second year post-treatment (2014) herbaceous vegetation cover had increased significantly (see Chapter 2), likely resulting in increased uptake which also provided a new sink for  $\text{NH}_4^+$  from the soil. This expansion in herbaceous biomass likely also contributed to the observed increase in soil  $\text{CO}_2$  flux during the post-treatment period (Van Grinsven 2015). Finally, though pre-treatment litter inputs were anticipated to result in a pulse of N availability immediately post-treatment, the reduction in litter inputs following treatment application were expected to be equivalent to the reduction in uptake soon thereafter. As decomposition rates in these wetlands are low and likely to be little changed by environmental conditions following treatment, N availability should be expected to approach equilibrium rapidly after the initial disturbance.

### 3.5.2 Foliar nitrogen, LMA, and chlorophyll

The link between belowground conditions and canopy characteristics in modified landscapes is well-established, with increased soil N availability associated with increased foliar  $\text{N}_{\text{MASS}}$  as a result of experimental additions of N (Aber et al. 1998,

Gunderson et al. 1998, Magill et al. 2004), across stands of varying disturbance histories (Ollinger et al. 2002), following disturbance- or succession-simulating girdling (Nave et al. 2011, Levy-Varon et al. 2014), and after insect infestation (Griffin et al. 2011). However, disturbances that affect the aboveground growing environment, such as increased light availability in canopy gaps, may complicate predictions, as the covariation of these conditions may tend to obscure how each will impact the physiological response of remaining vegetation (Fownes and Harrington 2004, Kranabetter et al. 2010). Additionally, drawing comparisons to other studies can be problematic, as the response of plants to changing light conditions can depend on species-specific traits, such as their tolerance of shade (Goulet and Bellefleur 1986).

Foliar morphological and biochemical characteristics of retained overstory species in both ‘Girdle’ and ‘Ash-Cut’ sites generally reflected expected changes resulting from the exchange of shade- for sun-acclimated leaves in higher light conditions (Walters 2005), including decreased  $N_{MASS}$  and increased LMA (Figures 3.4 and 3.6). However,  $N_{AREA}$  (Figure 3.5) remained unchanged, and  $Chl_{MASS}$  (Figure 3.7) showed little response, contrary to expectations. Among North American hardwood species, the response of *Acer saccharum* Marsh. (sugar maple) – a more shade tolerant species than either *A. rubrum* or *B. alleghaniensis* (Godman et al. 1990, Walters and Yawney 1990, Erdmann 1990) – to changing light conditions is among the more well-studied. Jones and Thomas (2007) found an increase in both LMA and  $N_{AREA}$  of retained *A. saccharum* following harvest, though the increase was not significant until the second year post-harvest and was restricted to lower canopy layers. Similarly, decreased LMA,  $N_{AREA}$ , and increased  $N_{MASS}$  were observed following experimental shading of branches in *A. saccharum* canopies (Coble and Cavaleri 2015). Fewer studies have considered the effect of light conditions on both the suite of leaf characteristics and species considered here. A positive relationship between LMA and light has been reported for both *A. rubrum* (Jurik 1986, Abrams and Kubiske 1990, Kloeppel et al. 1993, Gottschalk 1994, Lei and Lechowicz 1998) and *B. alleghaniensis* (Abrams and Kubiske 1990, Beaudet and Messier 1998). Decreased  $N_{MASS}$  and  $Chl_{MASS}$ , and increased  $N_{AREA}$  with increasing light has also been

shown in *A. rubrum* saplings (Lei and Lechowicz 1998), and similar patterns between  $N_{\text{AREA}}$  and light for *B. alleghaniensis* have also been observed (Delagrangue et al. 2004).

From these studies, it is possible to conclude that, while specific predictions of the scale of plant response to a change in growing conditions may not be feasible, the species under consideration here should at least be expected to follow typical response patterns, absent additional confounding factors. Among these potential factors are the interrelated nature of the measured foliar morphological and biochemical characteristics. Ellsworth and Reich (1993) showed that *A. saccharum*  $N_{\text{MASS}}$  was not related to canopy position, and thereby light availability, and thus the increase in  $N_{\text{AREA}}$  along a vertical gradient was wholly attributable to the positive correlation between height and LMA. Similarly, no change in *A. saccharum*  $N_{\text{MASS}}$  was observed at any canopy position by Jones and Thomas (2007). The severity of the disturbance resulting from the simulated EAB-infestation in this study may have resulted in a sufficiently dramatic  $N_{\text{MASS}}$  response to obscure any response in  $N_{\text{AREA}}$ . However, it has also been suggested that  $N_{\text{MASS}}$  in shade tolerant species, such as *A. saccharum*, may not be sensitive to light levels (Kranabetter et al. 2010), and so making comparisons to the less shade-tolerant species considered here is difficult.

Increased competition with soil microorganisms may also play a role. Both  $N_{\text{AREA}}$  (Dejong et al. 1989) and  $N_{\text{MASS}}$  (Fownes and Harrington 2004) may increase with increasing N availability in the soil, potentially to such an extent that this factor may offset the negative correlation between  $N_{\text{MASS}}$  and irradiance (Fownes and Harrington 2004). Given the decreased demand from *F. nigra* for soil N expected in the treated sites, a positive effect of increased soil N on foliar N of retained species was expected. However, no increase in N availability was observed, and this lack of soil N response cannot be attributed to demand from retained woody species (see *Litter mass and nitrogen content*, below). As such, it seems likely that from the perspective of these overstory species, soil N availability was unchanged, and thus the foliar response observed is dominated by the plants' response to changing light conditions. Finally,

among the described stress responses of plants to a sudden change in light environment is chlorotic foliage (Sharma et al. 2006). Reduced  $\text{Chl}_{\text{MASS}}$  was observed in *A. rubrum*, and, though not significant, a similar pattern was observed in *B. alleghaniensis* (Figure 3.7). While decreased  $\text{Chl}_{\text{MASS}}$  may be expected in high-light environments (Walters 2005), these responses may also be an indicator of stress in retained overstory stems (Sharma et al. 2006), and should be considered as a potential explanation for the lack of observed overstory growth increase (Chapter 2).

### 3.5.3 Litter mass and nitrogen content

The forest canopy plays a critical role in nutrient cycling, particularly through the production of litter and the influence of litter on rates and quantities of nutrients cycled through the ecosystem (Prescott 2002). Investigations into the quantity and quality of litter inputs can provide considerable insight into the current biogeochemical processes occurring within an ecosystem, as well as future trajectories. Frequently, the return of nutrients via litterfall is used as a proxy for plant demand for soil nutrients, as the growth requirement must be met either by soil nutrient supply, or retranslocation from within plant storage (Attiwill and Adams 1993, Aerts and Chapin 1999). The decrease in total N return via litterfall from the three dominant species in these sites (Figure 3.10), which together comprise approximately 88% of the overstory basal area (see Chapter 2), can be interpreted as a significant reduction in N demand by these species. This reduction in demand is driven primarily by *F. nigra*, as litterfall from *A. rubrum* and *B. alleghaniensis* slightly increased (Figure 3.9), while litter  $\text{N}_{\text{MASS}}$  decreased (Figure 3.8), such that N return via litterfall in these two species remained essentially unchanged (Figure 3.10). That litter  $\text{N}_{\text{MASS}}$  of *A. rubrum* and *B. alleghaniensis* was not significantly affected by treatment, despite an increase in fresh foliage  $\text{N}_{\text{MASS}}$ , is potentially due to inputs of lower canopy and sapling litter, from which fresh foliage was not sampled. The observed pattern, however, is similar to declines in  $\text{N}_{\text{MASS}}$  of fresh foliage (Figure 3.4), suggesting that techniques of statistical analysis may also play a role. Because fresh foliage was collected from the same crown each season, it was possible to include the individual tree as a random effect within each model. However, sampling and analytical techniques

dictated that litter  $N_{\text{MASS}}$  be analyzed as an average value for each site and growing year, potentially obscuring subtle treatment effects.

Prior to treatment, significant differences in litter  $N_{\text{MASS}}$  were observed between species, wherein both *A. rubrum* and *B. alleghaniensis* had significantly lower mass-based N content than *F. nigra* (Figure 3.3). Should regenerative capacity of these species overcome increased herbaceous competition to become the dominant overstory species in a future forested wetland (see Chapter 2), the change in litter quality is likely to significantly affect the rate and quantity of nutrient cycling in these stands (Prescott 2002). A number of controls on litter decomposition rates have been investigated, with initial lignin and N content of freshly shed litter frequently cited as being among the most important controls (e.g. Melillo et al. 1982). In a mixed species forest, the presence of nutrient-rich litter may also positively influence combined litter decomposition rates of all species (Rothe and Binkley 2001). In general, there appears to be a positive effect of N content on initial rates of decomposition. As decomposition proceeds, however, the resulting increase in N concentration has a rate-retarding effect on decomposition through the inhibition of lignin degradation (Berg and Matzner 1997, Berg 2000), though other nutrients likely also play a role (Berg et al. 1996).

This dual role of initial N concentrations appears to have two major consequences for N cycling in forests. Canopy and litter N concentrations tend to be positively correlated with soil N availability (Vitousek 1982, Reich et al. 1997, Ollinger et al. 2002), suggesting a positive feedback mechanism between above- and belowground processes that may enhance site fertility. Thus the loss of N-rich *F. nigra* litter may reduce N availability and thereby growth rates of the future forest, although species-specific rates of nutrient use efficiency may mitigate the magnitude of these effects (Aerts and Chapin 1999). Secondarily, as the latter stages of decomposition are reached, N-rich litter is expected to reach a higher limit value compared to N-poor litter, beyond which little further mass loss will occur (Berg et al. 1996). Consequently, increased soil organic matter accumulation may occur in sites where composition shifts towards species with



higher initial concentrations of litter N (Berg and Meentemeyer 2002), and is similar in effect to that which has been reported following experimental N addition (Nave et al. 2009). In *F. nigra*-dominated wetlands, the opposite effect may be the case, as the loss of N-rich litter following EAB infestation could result in the slowing of organic matter accumulation (Berg 2000). However, given the importance of the water table in controlling decomposition in peatlands, additional research is required to determine how significant a role litter and litter N content might play in peat formation in these forests.

### **3.6 Conclusion**

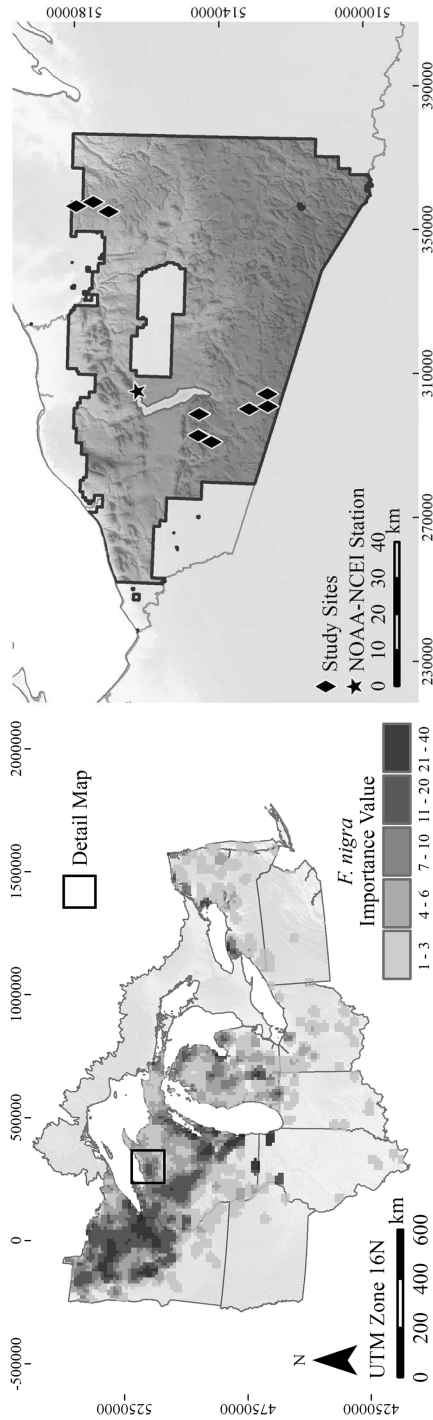
The biogeochemical responses to *F. nigra* mortality are likely to have both positive and negative effects on ecosystem function, due to the unique nature of ecological processes in these forests and the characteristics of the woody species they contain. The abundant organic matter that has accumulated on the forest floor due to the extended periods of inundation during the growing season, as well as woody debris from the ‘Ash-Cut’ treatment, appears to be serving as a sink for soil N, mitigating potential changes in availability resulting from reduced plant demand. While this response may reduce long-term changes in site fertility due to N export, the increased competition may also be limiting current vegetation, slowing short-term responses in woody species. Additionally, the replacement of *F. nigra* by *A. rubrum* or *B. alleghaniensis* will likely result in a reduction of the positive feedback between litter quality and site fertility, reducing carbon accumulation rates in living biomass, while also potentially reducing soil carbon storage.

### 3.7 Tables

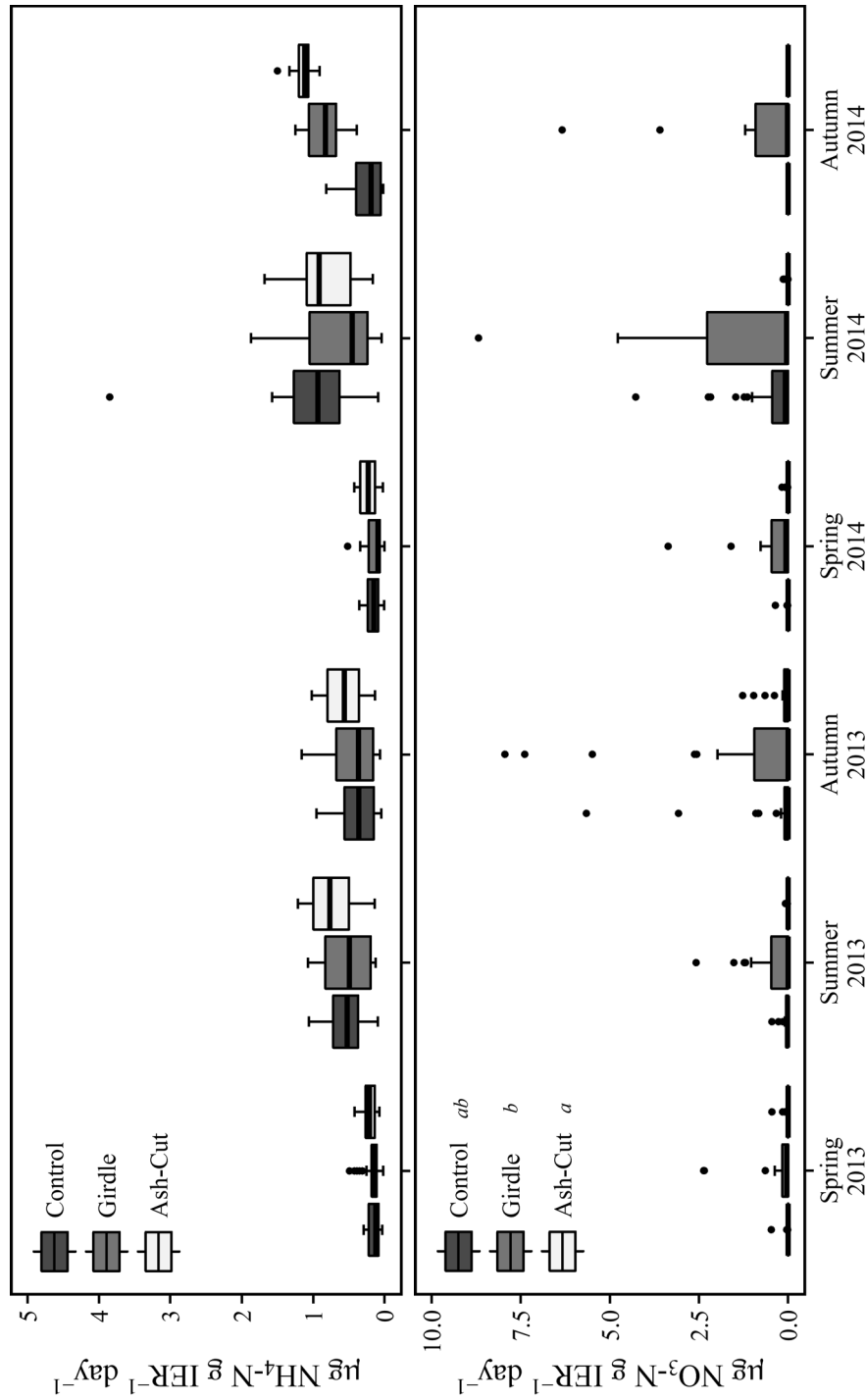
**Table 3.1.** Results of pairwise comparisons of modeled inorganic soil nitrogen availability by treatment and season. Columns that do not share a letter indicate a statistically significant difference ( $p \leq 0.05$ ) between seasons within a given year, treatment, and inorganic-N species.

	2013			2014		
	Spring	Summer	Autumn	Spring	Summer	Autumn
<b>NH<sub>4</sub><sup>+</sup></b>						
Control	<i>a</i>	<i>b</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>
Girdle	<i>a</i>	<i>b</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>b</i>
Ash-Cut	<i>a</i>	<i>b</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>b</i>
<b>NO<sub>3</sub><sup>-</sup></b>						
Control	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>
Girdle	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>a</i>
Ash-Cut	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>

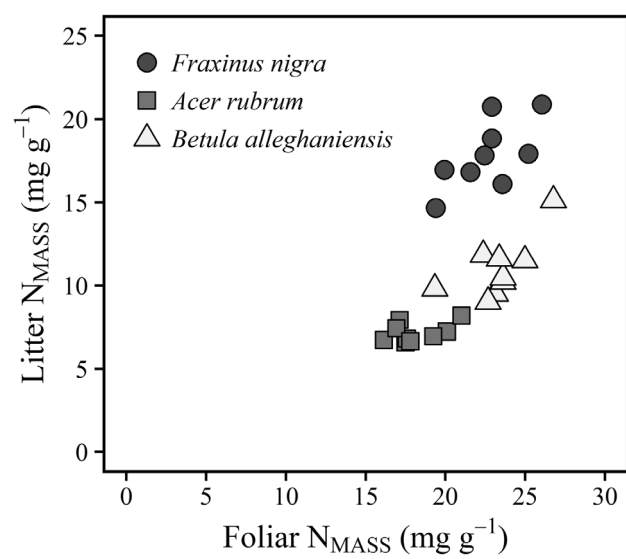
3.8 Figures



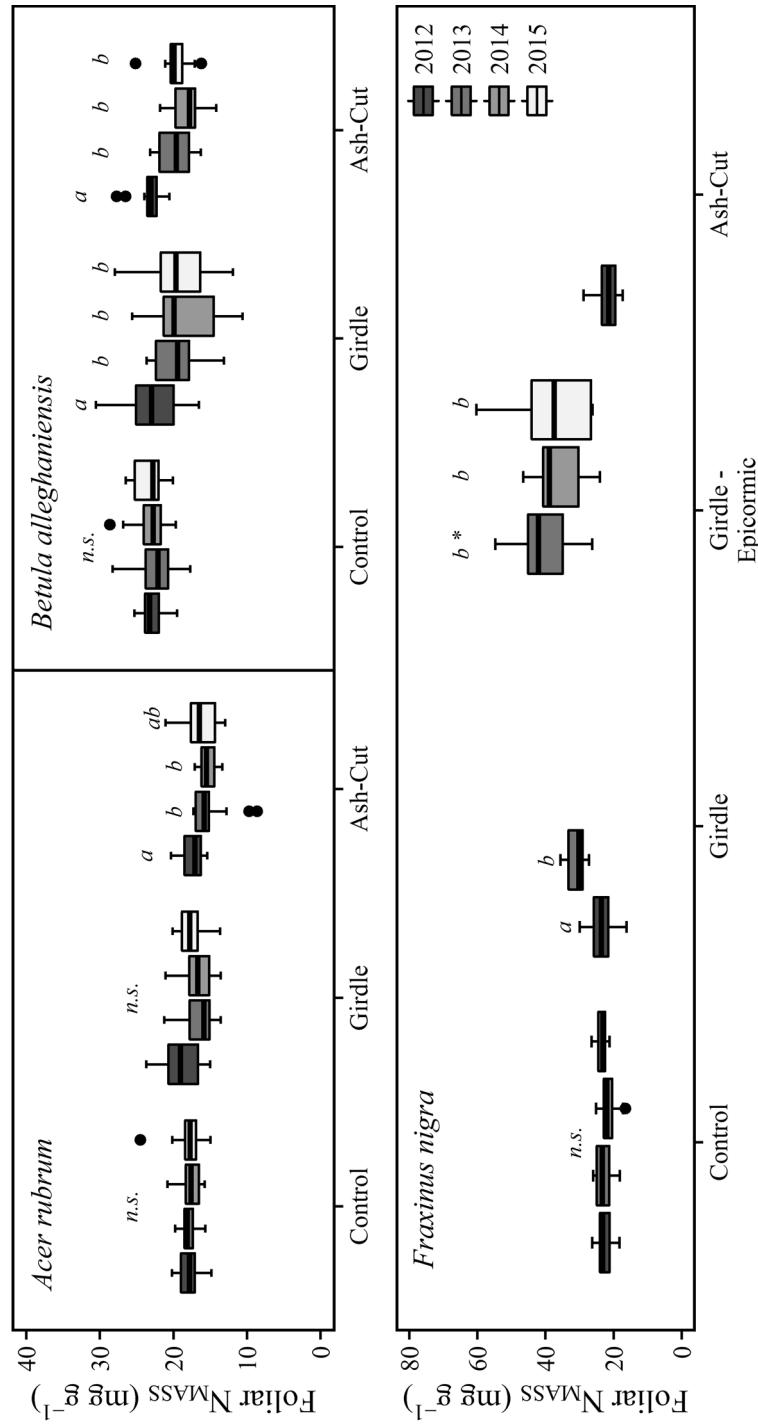
**Figure 3.1.** Regional map (left) with Forest Inventory and Analysis (FIA) importance value (Prasad and Iverson 2003) and detail map of the Ottawa National Forest in western Upper Michigan (right) with study site locations (diamonds). Each block of three sites contained one replicate of each treatment.



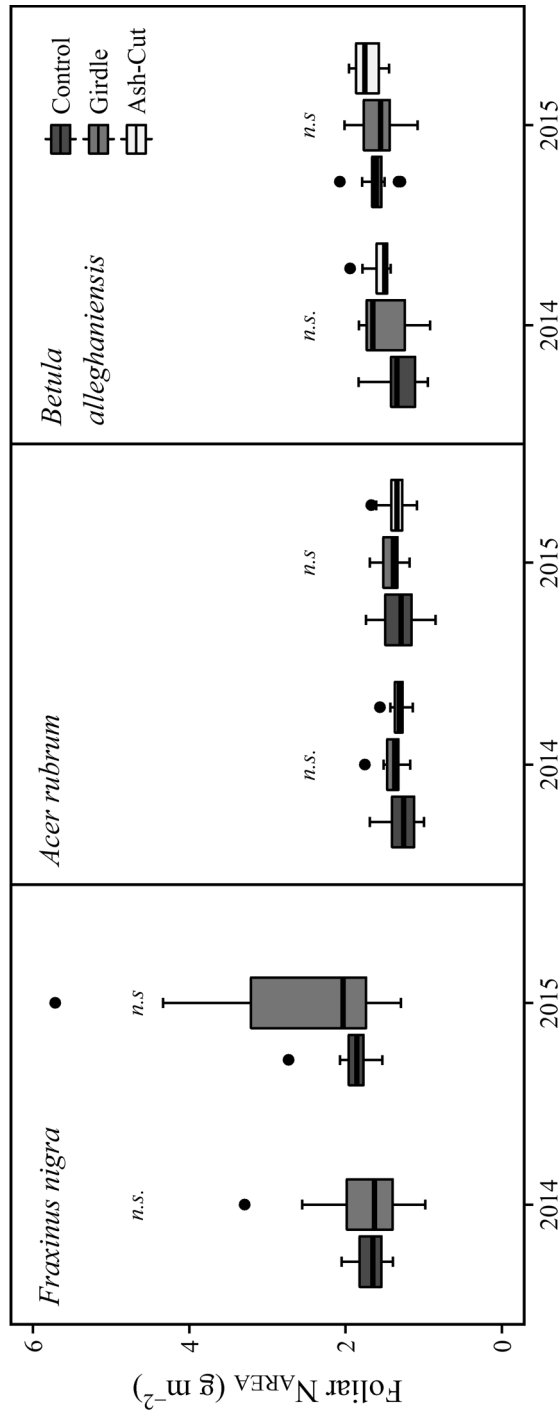
**Figure 3.2.** Soil  $\text{NH}_4^+$  (upper) and  $\text{NO}_3^-$  (lower) capture on ion exchange resins by season. Boxplots represent the 25th (lower edge) and 75th percentiles (upper edge) and medians (middle line); fences extend to 1.5 times the interquartile range. Note that the scale in the lower panel has been reduced for ease of interpretability, resulting in the omission of 4 outliers (see text for values). Legend labels that do not share a letter indicate a statistically significant difference in trend across seasons.



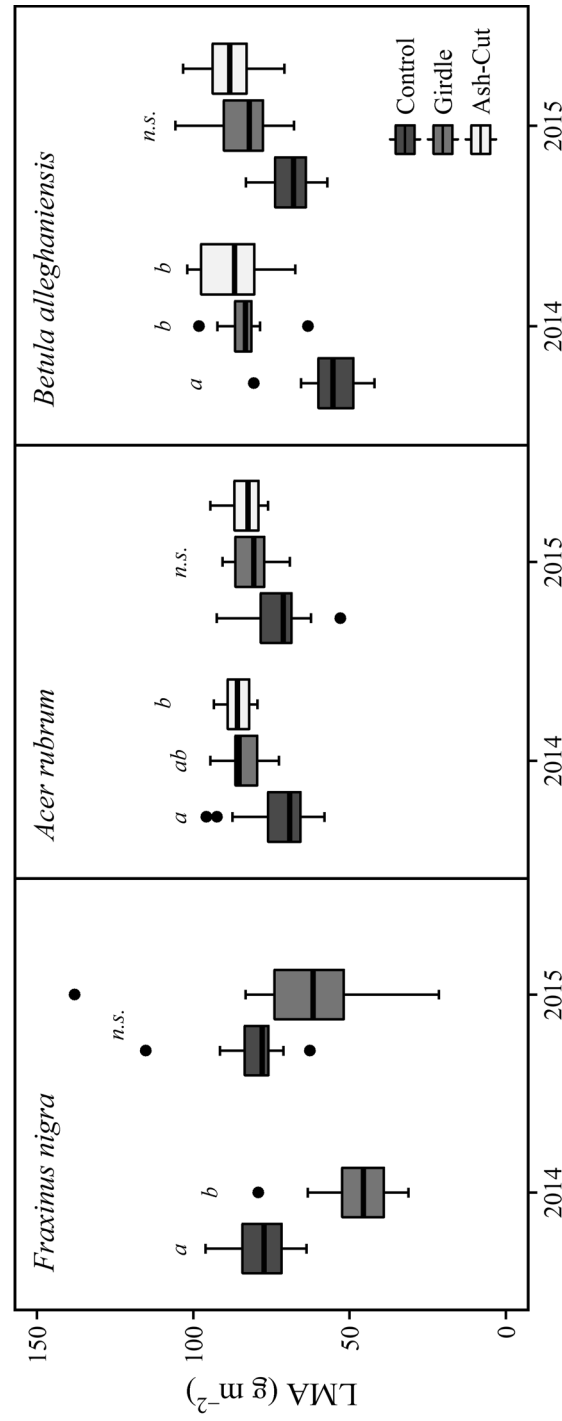
**Figure 3.3.** Pre-treatment nitrogen concentrations by mass ( $N_{MASS}$ ) for fresh foliage and litter across all sites.



**Figure 3.4.** Mass-based foliar concentration ( $N_{MASS}$ ). Boxplots represent the 25th (lower edge) and 75th percentiles (upper edge) and medians (middle line). Fences extend to 1.5 times the interquartile range, with outliers beyond these values indicated by discrete points. Labels that do not share a letter indicate a statistically significant difference within a treatment across years. Significance values in the ‘Girdle – Epicormic’ foliage are compared to the pre-treatment (2012) canopy foliage within the ‘Girdle’ treatment. An asterisk indicates significant differences between canopy and epicormic foliage within a given year. Note that upper and lower panels differ in scale.

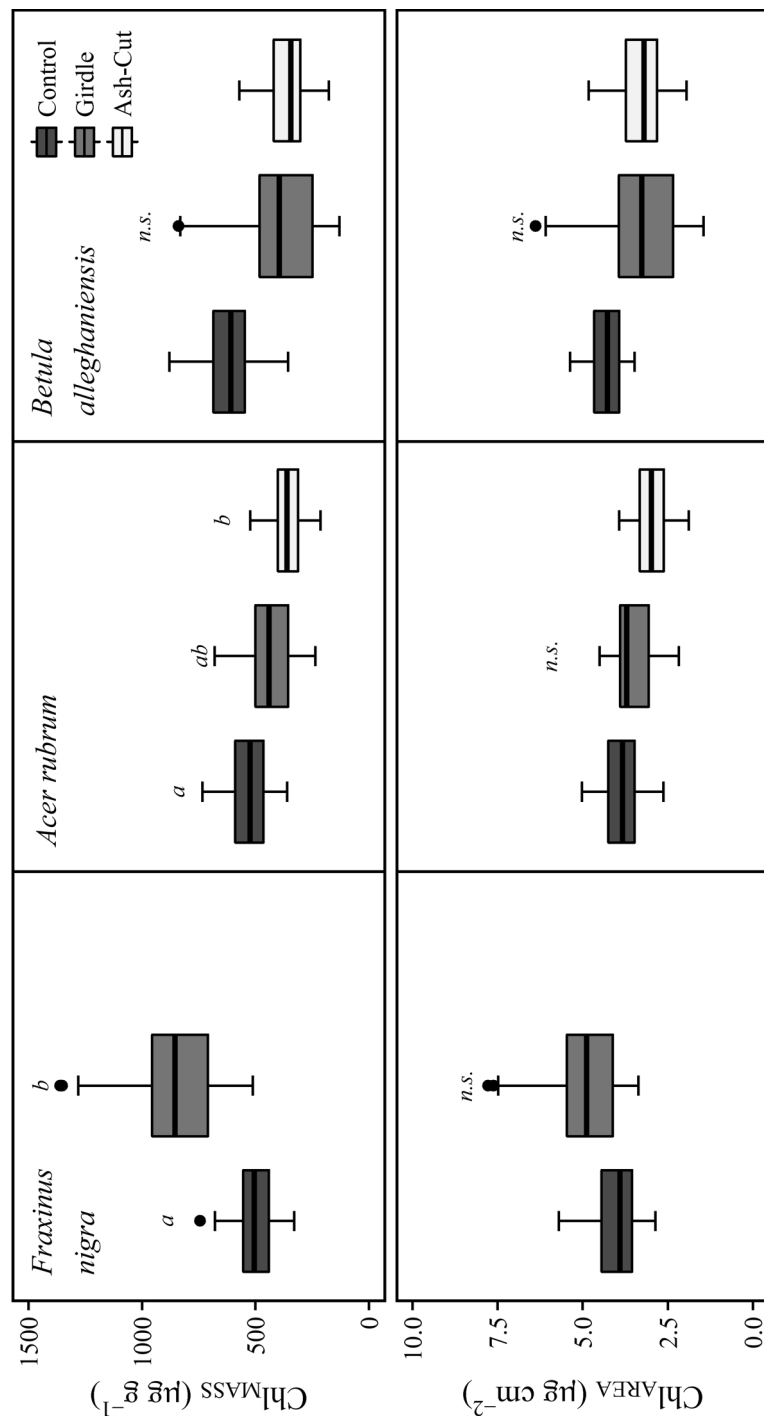


**Figure 3.5.** Area-based foliar concentration ( $N_{AREA}$ ) by species. Boxplots represent the 25th (lower edge) and 75th percentiles (upper edge) and medians (middle line). Fences extend to 1.5 times the interquartile range, with outliers beyond these values indicated by discrete points. Note that *F. nigra* samples in the 'Girdle' treatment were taken from epicormic branching.

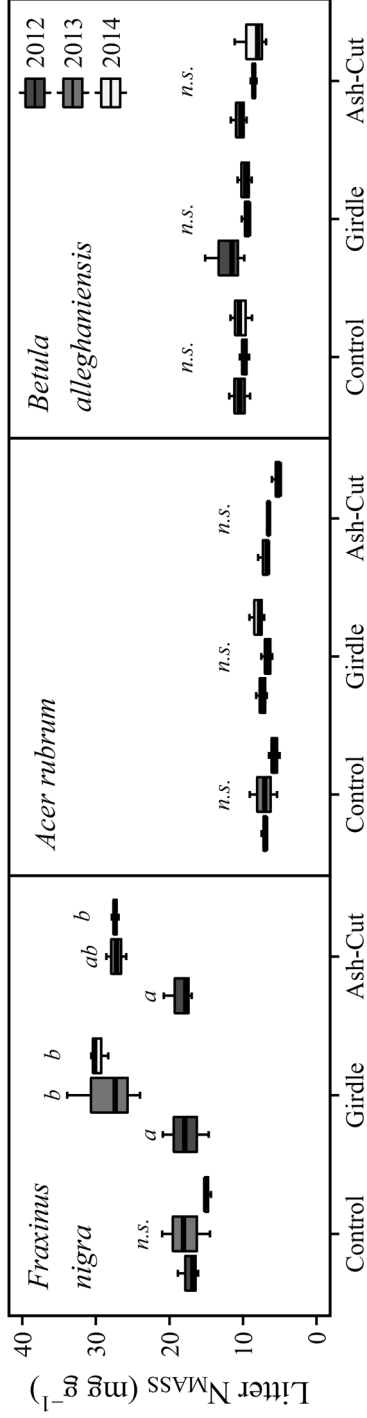


**Figure 3.6.** Leaf mass per area (LMA) by species. Boxplots represent the 25th (lower edge) and 75th percentiles (upper edge) and medians (middle line). Fences extend to 1.5 times the interquartile range, with outliers beyond these values indicated by discrete points. . Labels that do not share a letter indicate a statistically significant difference within a treatment across years. Note that *F. nigra* samples in the ‘Girdle’ treatment were taken from epicormic branching.

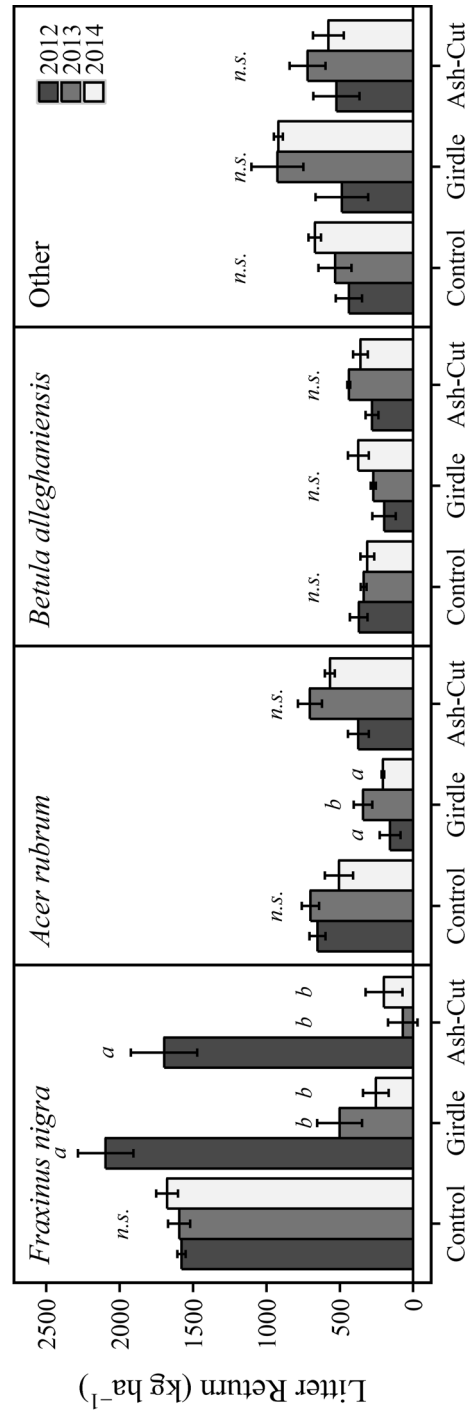




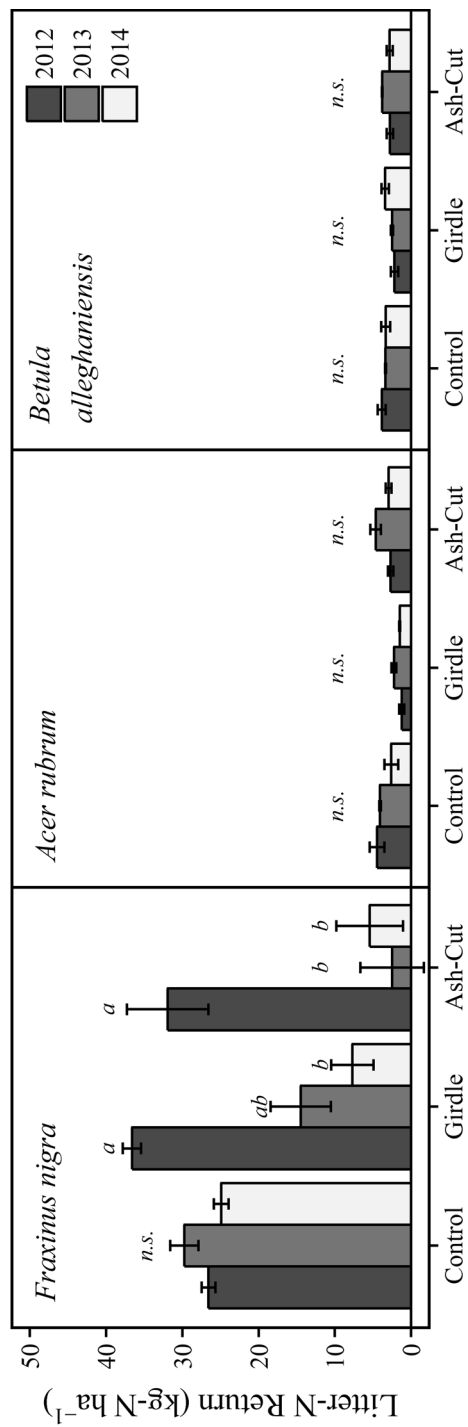
**Figure 3.7.** Chlorophyll concentrations by mass (Chl<sub>MASS</sub>, upper) and area (Chl<sub>AREA</sub>, lower) in 2015. Boxplots represent the 25th (lower edge) and 75th percentiles (upper edge) and medians (middle line). Fences extend to 1.5 times the interquartile range, with outliers beyond these values indicated by discrete points. . Labels that do not share a letter indicate a statistically significant difference within a treatment across years. Note that *F. nigra* samples in the ‘Girdle’ treatment were taken from epicormic branching.



**Figure 3.8.** Litter nitrogen concentrations by mass ( $N_{\text{MASS}}$ ) and species. Boxplots represent the 25th (lower edge) and 75th percentiles (upper edge) and medians (middle line). Fences extend to 1.5 times the interquartile range, with outliers beyond these values indicated by discrete points. Labels that do not share a letter indicate a statistically significant difference within a treatment across years.



**Figure 3.9.** Litterfall mass (mean  $\pm$  standard error) by species, with 'Other' representing all litter that was not *F. nigra*, *A. rubrum*, or *B. alleghaniensis*. Labels that do not share a letter indicate a statistically significant difference within a treatment across years.



**Figure 3.10.** Litterfall N return (mean  $\pm$  standard error) by species. Labels that do not share a letter indicate a statistically significant difference within a treatment across years

### 3.9 References

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## 4.0 Simulated EAB infestation effects on inorganic-N deposition via throughfall<sup>3</sup>

### 4.1 Abstract

The quantity and nutrient content of precipitation inputs via throughfall are strongly influenced by forest density and species composition. As a result, disturbances that result in canopy mortality or removal, or changes in species assemblages, are likely to result in changes in throughfall nutrient deposition. These changes may impact soil nutrient availability, and in areas with increased levels of atmospheric nutrient inputs – such as the high deposition of anthropogenically-derived nitrogen (N) deposition observed in many areas of eastern North America – may increase the potential for negative effects from this form of pollution. Native ash trees (genus: *Fraxinus*) across North America are currently threatened by the invasive emerald ash borer (EAB, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)). The mortality of members of this genus may significantly affect the delivery of inorganic N via throughfall to the forest floor. These changes are likely to be most dramatic in forests where *Fraxinus* species comprise large proportions of the forest overstory, such as in the *Fraxinus nigra* Marshall (black ash)-dominated wetlands of the upper Great Lakes region. To investigate the role of this species in throughfall deposition of N and the potential for future changes, early- and late-stage infestations were simulated in nine *F. nigra* dominated wetlands in the Upper Peninsula of Michigan, USA. Infestation stages were simulated by girdling or felling *F. nigra* saplings and overstory trees. Following treatment, all forest canopies remained a net sink for both ammonium and nitrate, though the sink strength for inorganic-N species was reduced by both the early- and late-stage simulations. Deposition of N via throughfall was unaffected in the early-stage infestation, while nitrate deposition increased in the late-stage infestation. Though the effects of this disturbance were relatively minor, the region also receives relatively low levels of atmospheric N

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<sup>3</sup> Material contained Chapter 4 is in preparation for submission to a peer-reviewed journal.

deposition. The results of this study may have important implications for regions in which both *Fraxinus* species are abundant and levels of N deposition are greater.

## 4.2 Introduction

Invasive insects are a serious threat to forests across North America (Lovett et al. 2006). The effects of introduced insects and associated pathogens have resulted in widespread alteration to the structure of these forests and ecosystem processes (Gandhi and Herms 2010). Among the more recently discovered invasive insects in North America, the emerald ash borer (EAB, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)) has received considerable attention for the breadth, depth, and severity of its potential impacts (Herms and McCullough 2014). Concerns over the potentially profound effects of EAB on forest composition and ecosystem function stem from the ubiquity of the genus *Fraxinus* (ash) across eastern North America (Cappaert et al. 2005). Members of the genus are common in a wide variety of forest types throughout the region, though they often comprise relatively minor proportions of the canopy (MacFarlane and Meyer 2005). In contrast, *Fraxinus nigra* Marshall (black ash) is frequently a major component of deciduous and deciduous-conifer forested wetlands (Wright and Rauscher 1990) and occurs in nearly pure stands in portions of the range (Erdmann et al. 1987). In the Upper Peninsula of Michigan, USA, the *F. nigra*-*Ulmus americana* L. (American elm)-*Acer rubrum* L. (red maple) forest type tends to be dominated by *F. nigra*, particularly in areas with very poor drainage and organic soils (Erdmann et al. 1987). As a result, the potential fate *F. nigra* of is of particular concern, owing to the potential severity of impacts that may result from the mortality of a significant portion of the forest canopy (Kashian and Witter 2011).

Throughout North America, human perturbations of biogeochemical cycles and species assemblages are examples of drivers of ecosystem change that may act in concert, resulting in greater impacts than any single change agent (Turner 2010, Peters et al. 2011). Over the past several centuries, anthropogenic modifications of the global nitrogen (N) cycle have doubled the annual rate of N-fixation, which in turn has increased

emissions to the atmosphere and subsequent deposition that may be 10- to 100-fold greater than background deposition (Vitousek et al. 1997, Galloway et al. 2004). As a result, much research attention has focused on the potential for forests to become “nitrogen saturated,” with subsequent negative impacts on ecosystem function (Ågren and Bosatta 1988, Aber et al. 1989 and 1998).

However, many forests across North America remain N-limited (Fenn et al. 1998), and as a result have a high retention capacity for N inputs (Aber et al. 1995, Magill et al. 1997), frequently with positive effects on plant growth rate and carbon sequestration (Vitousek et al. 1997, Magnani et al. 2007, Thomas et al. 2010). This retention may be due, at least in part, to the ability of the forest canopy to scavenge N from both wet and dry atmospheric deposition (Lovett 1994, Sparks 2009, Nair et al. 2016). Though the forest canopy is frequently a source of many nutrients, wherein the deposition of these nutrients via throughfall exceeds that of gross precipitation, both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are frequently retained within forest canopies (Parker 1983, Lovett and Lindberg 1993). This retained N may have important ecological significance, providing mineral nutrition to canopy epiphytes (Van Stan and Pypker 2015) or incorporated into tissues of woody species (Garten et al. 1998). This direct incorporation into plant tissues has the potential to positively affect plant performance (Sparks 2009) to a greater degree than that observed when N amendments are applied directly to the soil to simulate chronic N deposition (Nair et al. 2016).

Anthropogenic disturbances that affect the presence or condition of the forest canopy are as a result expected to modify the quantity and nutrient content of throughfall. For ions such as  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , for which the canopy is frequently a sink, changes in the forest canopy that reduce interception losses may similarly be expected to reduce nutrient scavenging. When canopy trees are removed, for example during harvest or salvage logging following insect infestation, canopy water storage is expected to decrease (Gash et al. 1995). However, while increased spacing between trees may reduce interception per unit land area, the interception per tree is likely to increase, mitigating some of the direct

effects of canopy removal alone (Teklehaimanot et al. 1991). Similar effects, at least insofar as direct changes resulting from differences in canopy storage, may increase N deposition via throughfall within canopy gaps (e.g. Klopatek et al. 2006). By opening gaps in the canopy, enhanced dry deposition to forest edges and solitary trees (Tulloss and Cadenasso 2015) may also occur. This dry deposition may be subsequently rinsed during rainfall events (Weathers et al. 2001), resulting in enhanced nutrient deposition along forest edges (De Schrijver et al. 2007). Dry deposition is especially important for  $\text{NO}_3^-$  (Lovett 1994), and high levels of dry  $\text{NO}_3^-$  deposition to the forest canopy may result in inputs of  $\text{NO}_3^-$  via throughfall that exceed gross precipitation (Parker 1983, Lovett and Lindberg 1993). Thus the potential for increased losses of N from disturbed ecosystems frequently ascribed to a reduction in plant demand (Vitousek and Melillo 1979) may include both a reduction in belowground demand (i.e. root uptake) and increased N inputs (Orwig et al. 2008). However, exceptions to this potential pattern have been reported (e.g. Scharenboch and Bockheim 2008), which emphasizes the need for caution when predicting the result of a disturbance within complex systems.

Insect infestations add still further complication to the interactions between the forest canopy, precipitation, and nitrogen deposition. Leaf-feeding insects, such as the invasive hemlock wooly adelgid (*Adelges tsugae* Annand), have been reported to increase the inorganic-N content of throughfall (Stadler et al. 2005, Orwig et al. 2008, but see Knoepp et al. 2011). However, it has been hypothesized that this change may result from insect exudates or damaged foliage (Orwig et al. 2008), though enhanced dry deposition may also be important (Templer et al. 2010). As defoliation due to EAB is negligible (Hermes and McCullough 2014), it seems unlikely that enhanced N inputs via leaching from damaged foliage should be expected to play a significant role in throughfall nutrient dynamics following EAB infestation. Additionally, unlike removal of trees during harvest, forest canopies following insect infestation are likely to contain dead stems and branches for considerable lengths of time, creating conditions similar to the leafless period in a deciduous forest. Precipitation throughfall below a leafless canopy is typically greater than when foliated (Staelens et al. 2008), and dry nutrient deposition can also be

substantial (Staelens et al. 2007), while foliar retention is reduced (Houle et al. 1999). This suggests that the retained canopy structures following insect infestation may result in a different throughfall response than that which may occur following forest harvest.

To examine canopy interactions and potential changes in throughfall quantity and nutrient content following EAB infestation in *F. nigra*-dominated wetlands, a multiyear manipulative study was undertaken on the Ottawa National Forest, MI, USA. Emerald ash borer-induced mortality of *F. nigra* was simulated across a series of stands to emulate the effects of early- and late-stage infestation on throughfall deposition and canopy retention. The objectives were to characterize this wetland forest canopy as a source or sink for inorganic-N and determine the potential effects of the loss of the dominant overstory species on interception and the scale of canopy interaction with these N species. The retention of standing dead trees, mimicking an early-stage infestation, may provide further insight into the importance of leafless canopy structures on precipitation and nutrient inputs to forests.

#### **4.3 Methods and Materials**

##### *4.3.1 Study site description*

Study sites were located on the Ottawa National Forest in the western Upper Peninsula of Michigan, USA (Figure 4.1). The regional climate is continental, with average monthly temperatures ranging from a minimum of -11.3 °C in January to a maximum of 18.2 °C in July (1981-2010 normals data from the Bergland Dam, MI station, UTM Zone 16N 304840 5162412, Arguez et al. 2010). Over the same period, mean annual precipitation was 1010 mm year<sup>-1</sup>, with the majority occurring from June through November (Arguez et al. 2010). During this study, surface water in these wetlands typically persisted from the onset of snowmelt until mid-summer and remained near the soil surface throughout the growing season (Van Grinsven 2015). Surface soils consisted of woody peat histosols ranging in depth from 40 to greater than 690 cm, typically underlain by a layer of clay or clay loam.



Nine wetlands were selected based upon overstory composition, size, and landscape position. All nine study sites contained *F. nigra* as the most abundant overstory species, ranged in size from 0.20 to 1.25 ha, and were located in isolated depressions within first-order watersheds. Study sites were divided into three blocks based upon geographical location, in which a ‘greedy’ algorithm was used to create blocks with minimized in-block spatial variation, using the blockTools package (Moore 2011) for the R statistical environment (R Core Team 2015). To assign treatments within each block, scaled and weighted values for initial estimates of percent *F. nigra* basal area (BA), total site area, and average depth of organic soil were calculated. These values were then used to assign treatments such that among-treatment variation in these values was minimized. Each block contained one site of each treatment type, which consisted of ‘Control,’ ‘Girdle,’ and ‘Ash-Cut,’ where ‘Girdle’ and ‘Ash-Cut’ treatments were intended to mimic early- and late-stage EAB infestations, respectively. Within ‘Girdle’ sites, all *F. nigra* stems  $\geq 2.5$  cm in diameter at breast height (DBH, 1.37 m) were hand-girdled with a drawknife to a depth sufficient to sever all phloem and cambium tissue. All *F. nigra* stems  $\geq 2.5$  cm DBH within ‘Ash-Cut’ sites were felled by chainsaw and allowed to remain onsite. Both treatment types were applied in the winter of 2012/2013. The lower diameter limit for treatment of *F. nigra* was based on reports that EAB-induced mortality is typically confined to ash stems  $> 2.5$  cm DBH (McCullough et al. 2015). No additional treatment of epicormic sprouting that occurred following treatment was undertaken.

Within each study site, three instrument clusters were established at spatially distributed random locations throughout the wetland. Each cluster contained infrastructure for the assessment of soil gas fluxes, the isotopic and nutrient composition of precipitation throughfall, and litterfall. Cluster centers were located such that all instrumentation was located  $> 5$  m from the wetland edge and  $> 10$  m from another set of instruments. Raised boardwalks were installed from the upland/wetland ecotone to each instrument cluster to minimize the effects of frequent site visits on soil and site hydrology.

#### 4.3.2 *Data collection*

Construction of throughfall collectors and ion exchange resin (IER) columns was modified from Simkin et al. (2004) and Fenn and Poth (2004). Collectors consisted of an HDPE funnel (upper diameter 10 cm, SKS Bottle & Packaging, Watervliet, NY, USA) placed inside a PVC coupler, and sealed in place with aquarium-grade silicone adhesive (American Sealants Inc., Fort Wayne, IN, USA), creating a total collection area of 103.5 cm<sup>2</sup>. The funnel/coupler assembly was then attached to a 76.2 cm length of PVC pipe (nominal i.d. 10.16cm) with a sealed lower opening, attached to a length of galvanized conduit driven into the soil such that the upper opening of the collector was 1 m above the wetland surface (Figure 4.2).

Ion exchange resin columns were constructed from 15.2 cm lengths of Schedule 40 PVC pipe (nominal i.d. 1.27cm). A PVC end cap with a 1.6 mm drain hole drilled in the center was fit to the bottom portion of each column, with a 1.58 cm diameter Porex® porous polyethylene filter disc (POR-4712, 0.32 cm thickness, 35 µm pore size, Porex Corporation, Fairburn, GA, USA) placed between the cap and PVC column. Assembled columns were acid-washed prior to packing with 15 g of mixed bed exchange resins (Dowex® Marathon™ MR-3 IER, Dow Chemical Company, Midland, MI, USA). After packing, IER columns were rinsed with three bed volumes of deionized water to ensure elimination of air pockets and hydration of resin material. The open upper portion of the filled column was then covered in polypropylene film, and completed IER columns were subsequently stored in sealed polyethylene zip-top bags in the dark at 4 °C until deployment.

In 2012 and 2013, columns were deployed monthly from mid-May until mid-October. At the beginning of each sampling period, IER columns were added to the throughfall collector by inserting the lower portion of the funnel inside the open top of the IER column. The attachment was made watertight by adding a short length of Tygon® tubing (1.27cm i.d., Saint-Gobain North America, Valley Forge, PA, USA) between the funnel and resin column. Acid-washed polyester fiberfill material was placed inside the neck of

the funnel to act as a filter. At the end of each sampling period, each throughfall collector was emptied and the volume of precipitation collected inside was measured. Field tests of assembled collectors with IER columns in place showed no measurable losses of collected precipitation to evaporation, obviating the need for additional measures to protect against evaporation. Following measurement of collected throughfall, the IER column attached to each funnel was removed, covered with polyethylene film, and stored in a cooler on ice while onsite. Upper collector assemblies were examined for signs of potential contamination (e.g. bird droppings or missing filter material); any potentially contaminated columns were flagged for closer examination during analysis. Fiberfill material in upper collector was replaced, and a new IER column fit to each funnel, after which the fully assembled collector was leveled. Used IER columns were stored in the dark at -20 °C until further processing.

A total of 16 collectors were installed at each of the nine study sites in 2012 and 2013. Pairs of throughfall collectors were co-located at each of three instrument clusters established to monitor soil gas and plant litter fluxes. These collector pairs were installed opposite each other, 3 m from each cluster center at a random bearing. The remaining ten collectors were randomly located (ArcGIS, Esri, Redlands, CA, USA) along installed boardwalks with a minimum of 3 m separating collectors. In 2014, 32 additional throughfall columns were added to the ‘Control’ and ‘Girdle’ sites within a single study block. A set of IER columns was installed in all collectors in mid-May, and allowed to remain throughout the growing season. Monthly measurements of throughfall volume at the original 16 collector locations continued as described previously, as did monthly contamination inspections and replacement of fiberfill material at all 48 collectors. Gross precipitation collectors and IER columns ( $n = 1$  in 2012 and 2013,  $n = 3$  in 2014), constructed and sampled identically to throughfall collectors, were located in a clearing as near as feasible to each study site. Gross precipitation collectors were first installed at all sites during the mid-August 2012 deployment of throughfall resin columns, thereby gross deposition data from mid-May until that time were not available.

#### 4.3.3 *Sample processing and analysis*

Extraction of adsorbed ions on the IER material was conducted using three successive elutions (Simkin et al. 2004) in a 2M KCl solution (Fenn and Poth 2004). Resin was removed from each column and placed in a disposable polypropylene extraction cup with 50 mL of 2M KCl, after which the samples were shaken on an orbital shaker table (New Brunswick Innova 2100, Eppendorf, Hamburg Germany) at 120 strokes  $\text{min}^{-1}$  for 30 min. The supernatant from each sample was then decanted through the original IER column into a second polypropylene container, retaining the IER material in the extraction cup. This extraction procedure was repeated twice more, resulting in a composite 150 mL sample from which a subsample was placed in a polypropylene centrifuge tube and frozen at  $-20\text{ }^{\circ}\text{C}$  until analysis. Blanks columns of resin unexposed to throughfall were identically processed for quality assurance and to monitor for potential interference from molecules produced under field conditions (Fenn and Poth 2004). No measurable quantity of  $\text{NH}_4^+$  or  $\text{NO}_3^-$  was eluted from resins subjected to either complete lab processing procedures or field conditions.

Colorimetric analysis of the inorganic nitrogen content of each sample was performed on a microplate reader (SpectraMax M2, Molecular Devices LLC, Sunnyvale, CA, USA) using the techniques described for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in Sinsabaugh et al. (2000) and Doane and Horwath (2003), respectively. Each polystyrene well plate contained triplicate aliquots of a given sample along with a set of known standards prepared in 2M KCl. The concentration of each sample was calculated by means of a linear regression equation fit to the set of standards on each plate, using the mean absorption of each set of triplicates.

#### 4.3.4 *Analytical methods*

The mass of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  eluted from the IER was determined from the measured concentration and volume of eluent, from which the mass of the nitrogen component of each molecule was then calculated. The area of each throughfall collector was used to yield N deposition via throughfall on an area basis ( $\text{kg ha}^{-1}$ ). Measured throughfall volumes were similarly used to determine precipitation depth (cm). Gross N deposition

and precipitation at each site was calculated as the inverse distance weighted mean of the N mass deposited or precipitation received at the three gross precipitation collectors for each block using the following formula:

$$\hat{N} = \frac{\sum_{i=1}^n \frac{1}{d_i} N_i}{\sum_{i=1}^n \frac{1}{d_i}}$$

where  $\hat{N}$  is the estimated nitrogen deposition or precipitation to the study site canopy,  $N_i$  is the measured gross N deposition or precipitation, and  $d_i$  is the distance from the gross precipitation collector to the study site center. Canopy retention of gross N deposition was then calculated by subtracting the gross N deposition for each study site from the throughfall N deposition for each constituent. Interception loss was calculated as gross precipitation depth minus throughfall depth, expressed as a percent of gross precipitation.

Mixed effects models were fit to 2012 and 2013 throughfall deposition data using the time period of interest (year or month and year) and treatment as fixed effects, and collector nested within site as random effects. Models fit to the 2014 data used treatment and collector as the sole fixed and random effects, respectively. Deposition data (both N deposition and precipitation) were square root transformed prior to model fitting to resolve violations of the assumptions of normality and non-constant variance, while canopy N retention values, which were frequently negative, were transformed as  $\ln(x + 1)$ . Precipitation interception loss values were approximately normally distributed and were not transformed. Visual examination of residual plots from all fitted models did not reveal deviations from the assumptions of normality or homoskedasticity.

All analyses were performed in the R statistical environment. Mixed effects models were initially fit using the R package lme4 (Bates et al. 2015); comparisons of gross N deposition and throughfall depth to throughfall N deposition were subsequently modeled using the nlme package (Pinheiro et al. 2016) to obtain  $p$ -values for each predictor. Model coefficients calculated using both packages were identical. The R package MuMIn (Bartoń 2016) was used to calculate marginal ( $r_m^2$ ) and conditional ( $r_c^2$ ) coefficients of

determination to assess model fit. Post-hoc pairwise comparisons and comparisons of trends were made using the lsmeans package (Lenth 2016), using the Tukey-HSD method to adjust  $p$ -values. A significance level of  $p \leq 0.05$  was used for all comparisons. Modeled responses and associated confidence intervals were generated using the effects package (Fox 2003). Standard errors for presentation in figures were calculated using a method of accounting for within-subjects designs presented in Morey (2008).

#### 4.4 Results

Gross inorganic-N deposition during the 2013 growing season, the period during which data were available across the longest time frame and broadest range of sites, was estimated to be  $1.41 \text{ kg ha}^{-1}$  ( $9.21 \text{ g ha}^{-1} \text{ day}^{-1}$ ). This compares favorably with calculated mean daily gross wet deposition ( $11.79 \text{ g ha}^{-1} \text{ day}^{-1}$ ) at a nearby National Atmospheric Deposition Program (NADP) over a similar time frame (Site MI99, Houghton MI, UTM Zone 16N 382270 5217956, NADP 2016). Though collectors were continuously open, and thus are not capable of discriminating between wet and dry gross deposition, the efficiency of this collector type at capturing dry deposition is unknown (Klopatek et al. 2006), and as a result the calculated gross deposition reported here may underestimate total (wet + dry) N deposition.

Percent of incoming precipitation lost to canopy interception during the first post-treatment growing season (2013) was  $28.60 \pm 3.03$ ,  $27.83 \pm 9.69$ , and  $15.88 \pm 7.28$  in the ‘Control,’ ‘Girdle,’ and ‘Ash-Cut’ sites, respectively, though these differences were not statistically significant. No significant differences were observed when interception losses were calculated on a monthly basis.

Within a given time period, both throughfall deposition and canopy retention differed significantly in the first year post-treatment (Figure 4.3,  $p$ -values vary by constituent and time period, see caption for details). Throughfall deposition of  $\text{NH}_4^+$  was typically lower in 2013 compared to 2012, with the exception of the Aug/Sept time period, whereas deposition of  $\text{NO}_3^-$  was consistently higher. Despite these observed changes, differences

in deposition or canopy retention between treatments within a given month were uncommon. Throughfall deposition of  $\text{NO}_3^-$  was higher during May/Jun 2013 in the ‘Ash-Cut’ sites, compared to both the ‘Control’ ( $p = 0.007$ ) and ‘Girdle’ sites ( $p = 0.049$ ). Canopy retention of  $\text{NO}_3^-$  was lower in the ‘Ash-Cut’ treatment during this time period ( $p < 0.001$  and  $p = 0.026$  compared to the ‘Control’ and ‘Girdle,’ respectively), and was also lower than the ‘Control’ treatment during Jun/Jul ( $p = 0.034$ ). Time periods during which the 95% confidence intervals around the modeled mean retention value included zero were infrequent, but were observed for both inorganic-N species during the post-treatment year. No significant differences between treatments in either deposition or canopy retention were observed in the pre-treatment periods, and all canopies were found to be significant sinks for both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  throughout the 2012 growing season.

Changes in monthly deposition between years can in part be attributed to differences in gross deposition during those time periods. A significant positive relationship ( $p < 0.001$ ) between throughfall deposition of both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  was observed (Figure 4.4, left panel), though considerable variability in the response was seen, leading to moderate  $r_m^2$  values for both constituents ( $\text{NH}_4^+ r_m^2 = 0.406$ ,  $\text{NO}_3^- r_m^2 = 0.370$ ). In both cases, inclusion of the random effects increased the amount of variance explained by the model ( $\text{NH}_4^+ r_c^2 = 0.407$ ,  $\text{NO}_3^- r_c^2 = 0.465$ ). Though a significant positive effect of throughfall depth on deposition (Figure 4.4, right panel) was indicated for both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  ( $p = 0.005$  and  $p = 0.027$ , respectively), both the  $r_m^2$  and  $r_c^2$  were very small ( $\text{NH}_4^+ r_m^2 = 0.053$ ,  $r_c^2 = 0.122$ ;  $\text{NO}_3^- r_m^2 = 0.031$ ,  $r_c^2 = 0.162$ ), indicating that throughfall depth was a weak predictor of throughfall deposition. Models that used both predictors simultaneously were deemed inappropriate due to multicollinearity and tests on these models revealed high variance inflation factors.

Treatment effects become more evident when examined at the scale of the entire growing season for  $\text{NO}_3^-$  deposition, while no effects on  $\text{NH}_4^+$  deposition were observed at this timescale (Figure 4.5). Deposition of  $\text{NO}_3^-$  was significantly greater in the ‘Ash-Cut’ treatment compared to the ‘Control’ ( $p = 0.036$ ), and approached significance compared

to the ‘Girdle’ treatment ( $p = 0.056$ , Figure 4.6). The effect of treatment on canopy retention of individual inorganic-N constituents was not significant, but total inorganic-N retention was reduced in the ‘Ash-Cut’ treatment compared to the ‘Control’ ( $p = 0.022$ , Figure 4.6), and comparisons between these sites approached significance when  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were considered separately ( $p = 0.082$  and  $p = 0.053$ ). Modeled total growing season retention showed that canopies within all treatments were significant sinks for both inorganic-N species.

Patterns in canopy N retention during the first year post-treatment were suggestive of a small but seemingly consistent effect of the ‘Girdle’ treatment on the apparent sink strength of the canopy (Figure 4.6). Intensive sampling during the 2014 growing season indicated that, by the second year post-treatment, canopy retention of  $\text{NH}_4^+$  was reduced by the ‘Girdle’ treatment ( $p < 0.001$ , Figure 4.7), though no effect on either  $\text{NO}_3^-$  retention or throughfall N deposition was observed.

#### **4.5 Discussion**

The simulated EAB-induced mortality of overstory and understory *F. nigra* had a subtle but significant effect on N deposition and retention in these wetlands, the examination of which highlights the complex nature of canopy interactions with atmospheric deposition.

Monthly throughfall deposition of  $\text{NH}_4^+$  varied considerably between pre- and post-treatment study years (Figure 4.3). However, these changes cannot be directly ascribed to experimental treatments, as differences between pre- and post-treatment within a given month typically followed a similar pattern across all treatment types, including ‘Control.’ Throughfall  $\text{NH}_4^+$  deposition decreased in 2013 within most time periods, resulting in total growing season throughfall deposition of  $\text{NH}_4^+$  that was lower in all treatment types in 2013 compared to 2012 (Figure 4.5), despite increased throughfall deposition in all sites in Aug/Sept. Though gross deposition data were not available for all pre-treatment periods, the decline in throughfall deposition in Jul/Aug and Sept/Oct can be attributed, at least in part, to a decline in gross deposition, which was significantly lower in 2013



compared to 2012 ( $p = 0.029$  and  $p < 0.001$ ). However, no difference in gross deposition between study years was found for Aug/Sept; as a result the increased deposition observed in the ‘Girdle’ and ‘Ash-Cut’ sites appeared to result from a decline in canopy retention, though this decline was significant in the ‘Ash-Cut’ treatment only (Figure 4.3).

Similar mechanisms appeared to drive the observed patterns in  $\text{NO}_3^-$  throughfall deposition, though the trend between study years was reversed. The greater mass of  $\text{NO}_3^-$  deposited in July – October (Figure 4.5) was correlated with significantly increased gross  $\text{NO}_3^-$  deposition (Figure 4.3) in Aug/Sept ( $p < 0.001$ ), which also approached a significant increase in Jul/Aug ( $p = 0.084$ ). Gross deposition in Sept/Oct 2013 declined significantly compared to 2012; however, canopy retention was lower, resulting in the increased  $\text{NO}_3^-$  deposition observed in this time period. The mechanism behind the decreased canopy retention of  $\text{NO}_3^-$  in this time period, or the decline in  $\text{NH}_4^+$  retention during Aug/Sept, is not clear.

From these results, it is evident that the increase in  $\text{NO}_3^-$  and decrease in  $\text{NH}_4^+$  deposition in 2013 (Figure 4.5) was driven predominantly by changes in gross N deposition. Further evidence for the link between gross N deposition and throughfall deposition can be seen in the positive correlation between these variables in the unmanipulated stands (Figure 4.4). These results make clear the important role that the quantity of inorganic nitrogen deposited to the top of the forest canopy plays in controlling the mass of N deposited to the forest floor (Lovett and Lindberg 1993, Lovett et al. 1996, Knoepp et al. 2008). However, the relatively small coefficients of determination of the model fit to gross and throughfall N deposition, in concert with patterns observed in the monthly data and the lack of correlation between throughfall depth and N deposition emphasize the critical effect that the forest canopy has on the process of N deposition. In the case of these forests, the canopy represents a significant sink for inorganic N (Figures 4.6 and 4.7), which was reduced by the simulated infestation. While this change in sink strength did not impact  $\text{NH}_4^+$  deposition,  $\text{NO}_3^-$  deposition was greater in the post-treatment ‘Ash-Cut’

sites. That combined inorganic-N deposition was not significantly greater in these same sites may be attributable to the decrease in gross  $\text{NH}_4^+$  deposition during the 2013 growing season.

While the canopy sink for total inorganic-N deposition was reduced by the first year post-treatment in the ‘Ash-Cut’ site (Figure 4.6), no significant effects resulting from the ‘Girdle’ treatment were initially observed. However, as the  $p$ -values of many of these pairwise comparisons were quite small, and a clear pattern across both constituents and treatments emerged, the lack of significance may have resulted from a relatively small sample size. To examine this potential limitation of the original study design, the number of throughfall collectors at a single pair of sites was tripled for the 2014 growing season. This increased sample size revealed that the pattern observed in  $\text{NH}_4^+$  deposition in 2013 may be the result of a treatment effect and that the ‘Girdle’ treatment reduced the sink strength of the forest canopy by the second growing season following the simulated infestation. These results, along with the lack of significant differences observed in interception losses, also emphasize the potential importance of sample size when monitoring highly heterogeneous parameters such as throughfall, even in stands dominated by a single species and over relatively long time frames (Zimmermann and Zimmermann 2012). It should also be noted, however, that girdled *F. nigra* stems produced a small quantity of canopy foliage in 2013, but did not do so in 2014. This foliage, though minor compared to a full canopy (see Chapter 3, Figure 3.9), may have contributed to the apparent canopy sink for N in 2013. The absence of any *F. nigra* canopy foliage in 2014 may have reduced the sink strength sufficiently that a significant effect was observed during this growing season.

Forest canopies are frequently sinks for atmospheric N deposition (Parker 1983, Lovett and Lindberg 1993, Johnson and Lindberg 1992, but see Eaton et al. 1973, Fenn and Poth 2004 among others), particularly in areas where gross N deposition is relatively low (Lovett and Lindberg 1993), and thus the retention mechanisms involved are not saturated (Lovett et al. 1996). Net canopy retention of N within intact canopies has been

observed in deciduous (Potter et al. 1991, Lovett et al. 1996, Knoepp et al. 2008), coniferous (Carleton and Kavanagh 1990, Klopatek et al. 2006), and mixed species canopies (Lovett et al. 1996, Knoepp et al. 2008). A significant effect of species has frequently been reported, though canopies remained sinks for N despite differences in composition (Houle et al. 1999, Lovett et al. 2006, Knoepp et al. 2008). As the ‘Ash-Cut’ treatment – within which the remaining canopy is now dominated by non-*Fraxinus* species – remained a net sink for N indicates that in the long-term, these canopies will continue to retain much of the N contained in gross deposition, though changes in throughfall deposition of N are possible.

The responses observed in ‘Girdle’ versus ‘Ash-Cut’ and between inorganic-N species likely resulted from differences in canopy structure following treatment. For nutrients that interact significantly with the forest canopy, the removal of a significant portion of that canopy is expected to result in an immediate reduction of that direct interaction – in the case of these forests, a reduction in the sink strength of the canopy. The amount of reduction may increase with increasing scale of the removal (Schliemann and Bockheim 2014). Additionally, the removal of trees increases space between retained canopy stems, which enhances interactions between individual trees and the atmosphere (Teklehaimanot et al. 1991). As a result, the forest edges surrounding gaps are frequently observed to have higher rates of nutrient deposition via throughfall than either the interior forest or the gap itself (Draaijers et al. 1988, Weathers et al. 2001). This edge effect on ions that are frequently delivered to the forest canopy as dry deposition, such as  $\text{NO}_3^-$  (Lovett and Lindberg 1993) is especially pronounced (Lindberg and Owens 1993, De Ridder 2004), though increased rates of  $\text{NH}_4^+$  deposition along forest edges have also been observed (Draaijers et al. 1988 and 1994, Weathers et al. 2001, De Schrijver et al. 2007, Wuyts et al. 2008). Canopy openings need not be large to induce nutrient deposition enhancement along forest edges (De Ridder et al. 2004), and as a result internal edges, such as along forest roads or around canopy gaps (Lindberg and Owens 1993, De Schrijver et al. 2007), or single trees within an opening (Tulloss and Cadenasso 2015) can enhance nutrient deposition via throughfall. The reduced canopy retention of inorganic-N and the

increased deposition of  $\text{NO}_3^-$  (Figures 4.6 and 4.7) in the ‘Ash-Cut’ treatment may thus have resulted from the combined effects of reduced canopy interaction with incoming precipitation on the canopy sink strength, as well as enhanced deposition below retained trees.

In contrast, *F. nigra* canopies with little to no foliage in the ‘Girdle’ sites continued to interact with incoming precipitation, functioning, at least physically, much like that of a deciduous forest canopy during the dormant season. Precipitation interactions with a leafless canopy can be greatly reduced, frequently resulting in increased throughfall (Staelens et al. 2008, Herbst et al. 2008). However, only a very small and non-significant difference in interception losses from the ‘Girdle’ canopy was observed compared to ‘Control’ sites, which indicates canopy interactions with incoming precipitation remained important. Additionally, though the absence of foliage removes an important potential pathway for the uptake of N (Houle et al. 1999), ion deposition may still be significantly affected by leafless branches (Staelens et al. 2006). While the deposition of N via throughfall below a leafless deciduous canopy can approach or even exceed gross deposition (Hamburg and Lin 1998, Houle et al. 1999), leafless canopies may also retain significant proportions of N deposition (Staelens et al. 2007). Canopy characteristics that enhance nutrient deposition, such as facilitation of dry deposition or leaching from foliage, may also be reduced (Parker 1983, Staelens et al. 2007, Van Stan et al. 2012), further increasing the apparent sink strength of the forest canopy. As a result, retention of N within the ‘Girdle’ stands was much more similar to that observed in the ‘Control’ treatment. The observed differences in retention during the 2014 growing season (Figure 4.7) may be due to the characteristics of the N species under study. The relative sink strength of the leafless canopy may be preserved by the interaction of the retained branches with dry deposition of  $\text{NO}_3^-$  (Staelens et al. 2007), which would maintain retention of this ion within the ‘Girdle’ canopy. In contrast, a number of observers have reported that  $\text{NH}_4^+$  is preferentially retained by foliage (Bowden et al. 1989, Brumme et al. 1992, Lumme 1994, but see Garten et al. 1998, Gaige et al. 2007), and as a result the

leafless canopy was insufficient to preserve a sink comparable in strength to the ‘Control’ treatment.

Changes in N deposition to the forest floor may result in increased soil N availability (Aber et al 1998, Fenn et al. 1998), an effect which has been noted in other studies of invasive phytophagous insects (Orwig et al. 2008). However, despite increased  $\text{NO}_3^-$  inputs during the 2013 growing season in the ‘Ash-Cut’ treatment, no increase in soil  $\text{NO}_3^-$  was observed (see Chapter 3). Soil  $\text{NO}_3^-$  availability was increasing, however, in the ‘Girdle’ treatment during the 2013 and 2014 growing season, though no change in throughfall deposition was observed. These contrasting results indicate that soil processes dominated over throughfall deposition effects in controlling N availability in these forests, buffering the potential effects of minor changes in aboveground nutrient inputs on belowground conditions (Stadler et al. 2001). The modest ecological importance of N deposition to the ecosystem N budget in these forests is further underscored by comparing gross deposition with annual N requirements from woody species. Incoming gross N deposition was calculated to be  $1.41 \text{ kg ha}^{-1}$  during the 2013 growing season, of which approximately 53% was deposited to the forest floor via throughfall. By comparison, the annual N requirement for foliage alone in the three dominant species in these forests (*F. nigra*, *A. rubrum*, and *Betula alleghaniensis* Britton) was estimated to be  $37.17 \pm 7.17 \text{ kg ha}^{-1}$  (mean  $\pm$  standard error, calculated as the N return via litterfall, see Chapter 3) over the course of the growing season, with additional N required for woody growth increment and root production. Gross annual wet deposition of inorganic N measured at a nearby NADP station was  $4.41 \text{ kg ha}^{-1}$  (NADP 2016), still less than 12% of the N return via litterfall.

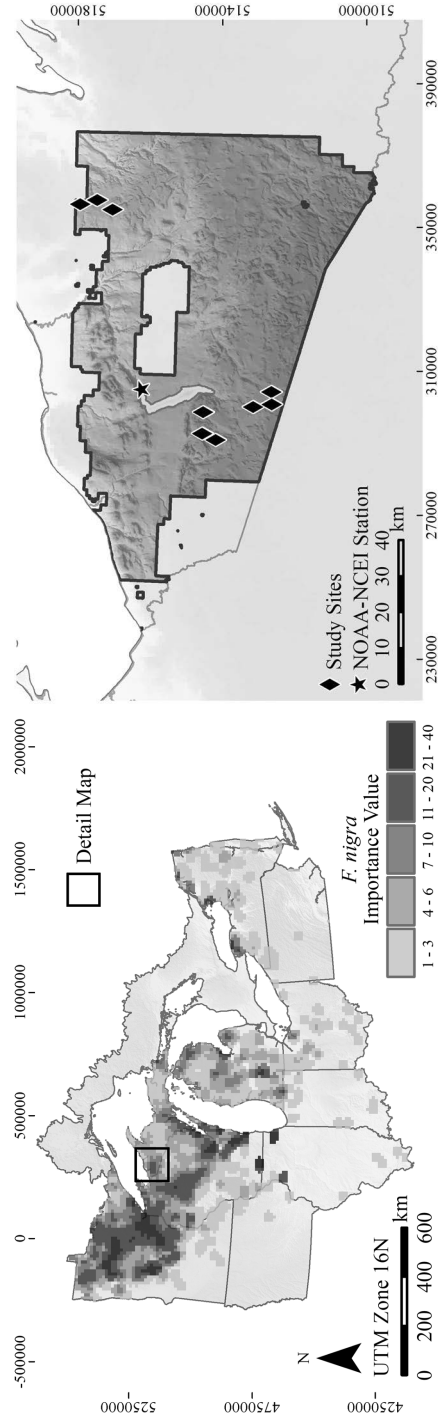
Levels of gross N deposition in this region are relatively low, totaling less than half that which may be currently received elsewhere in the eastern United States (NADP 2016). As a result, atmospheric N deposition is a minor component of the ecosystem N budget, and the relatively small change in throughfall N deposition observed following simulated infestation may also be the result of these low levels of deposition. While concern over

the effects of EAB on the forests in this study stems in part from the very high proportion of *F. nigra* in the canopy, other members of the genus may also be important components of North American forests. *Fraxinus americana* L. (white ash), for example, is common in a number of forest types throughout the eastern half of the continent but is rarely the dominant species (Schlesinger 1990). However, *F. americana* is abundant in several regions that currently receive high levels of N deposition, such as western Ohio and eastern Pennsylvania (Prasad and Iverson 2003, NADP 2016), where atmospheric N deposition may be of greater importance to the forest N budget. Large quantities of gross N deposition can reduce the apparent importance of canopy retention on throughfall deposition (Hamburg and Lin 1998), overwhelming the retention mechanisms (Lovett et al. 1996) and potentially resulting in a positive net flux of N through the canopy (Lovett 1994). This effect of increased gross deposition is clearly observed in the results of this study, as changes in monthly gross deposition significantly affected throughfall N deposition. Consequently, in areas where both the abundance of *F. americana* (or other *Fraxinus* species) and levels of N deposition are high, the reduction in canopy sink strength resulting from EAB infestation may exacerbate the potential negative effects of N pollution (Aber et al 1998, Fenn et al. 1998).

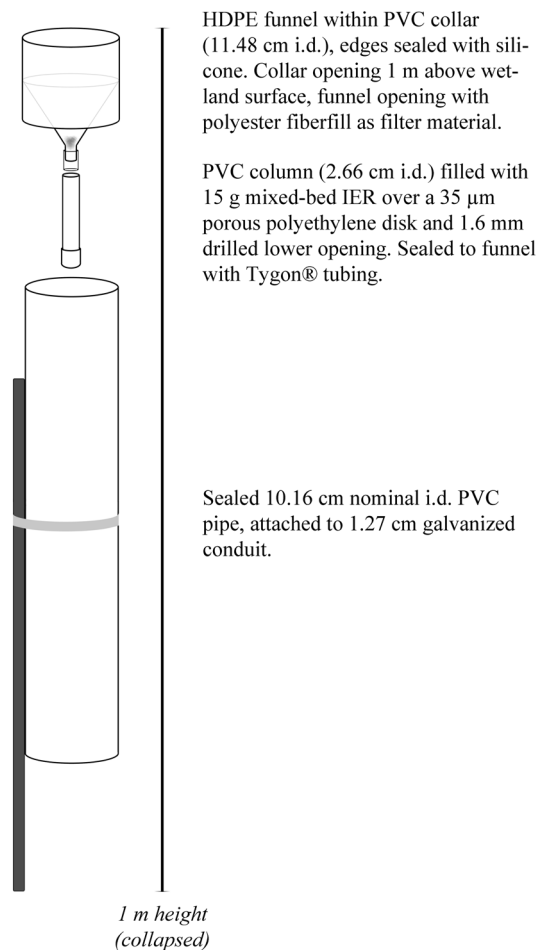
#### **4.6 Conclusion**

Simulated EAB infestation significantly affected both the canopy retention and deposition of inorganic-N species in these forested wetlands. Though the size of the effect in this system was relatively small, these results have important implications for regions where anthropogenic N deposition is greater, which may have significant negative consequences for both forests and the aquatic ecosystems to which they are linked. The potential for positive interactions between these drivers of ecosystem change emphasizes the importance of research that considers how synergistic effects between change agents may affect the scale of ecosystem impacts.

## 4.7 Figures

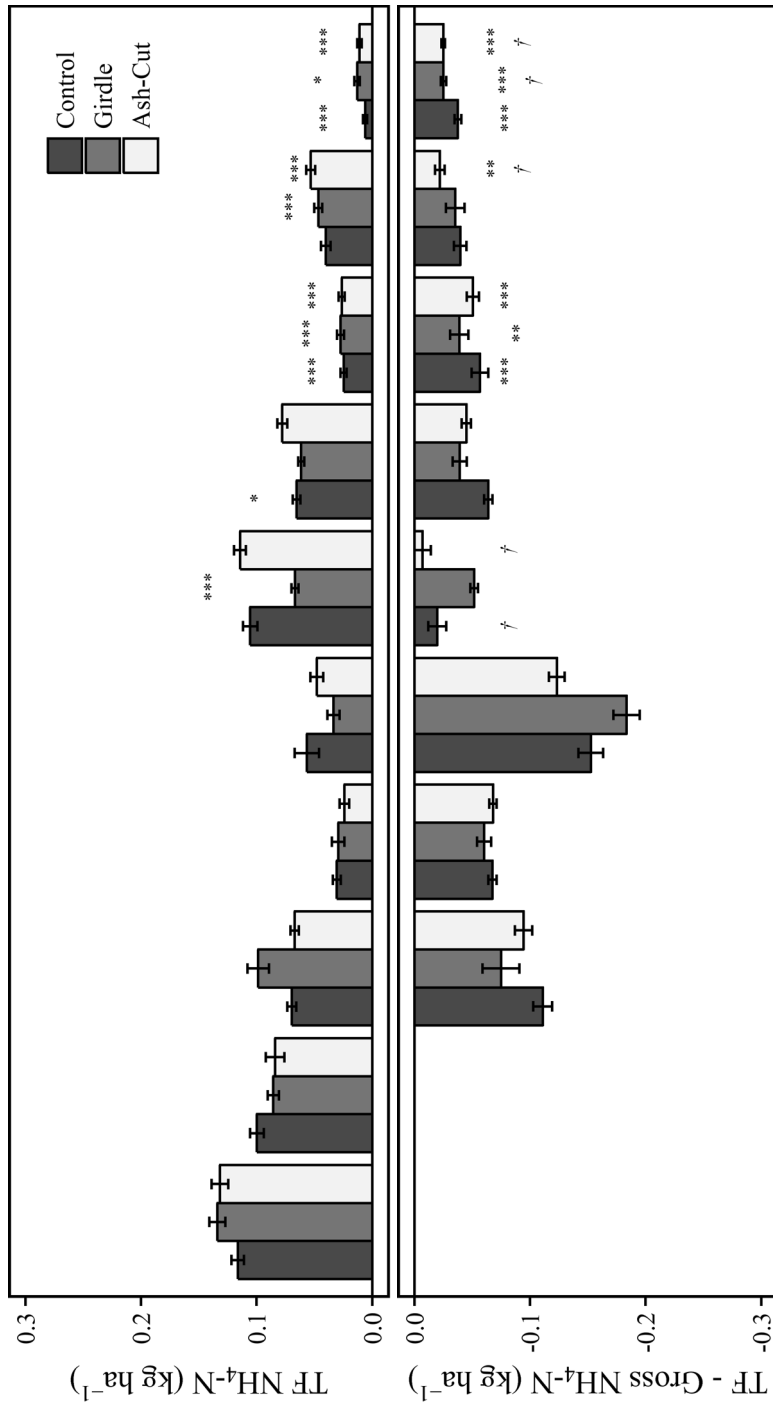


**Figure 4.1.** Regional map (left) with Forest Inventory and Analysis (FIA) importance value (Prasad and Iverson 2003) and detail map of the Ottawa National Forest in western Upper Michigan (right) with study site locations (diamonds). Each block of three sites contained one replicate of each treatment.

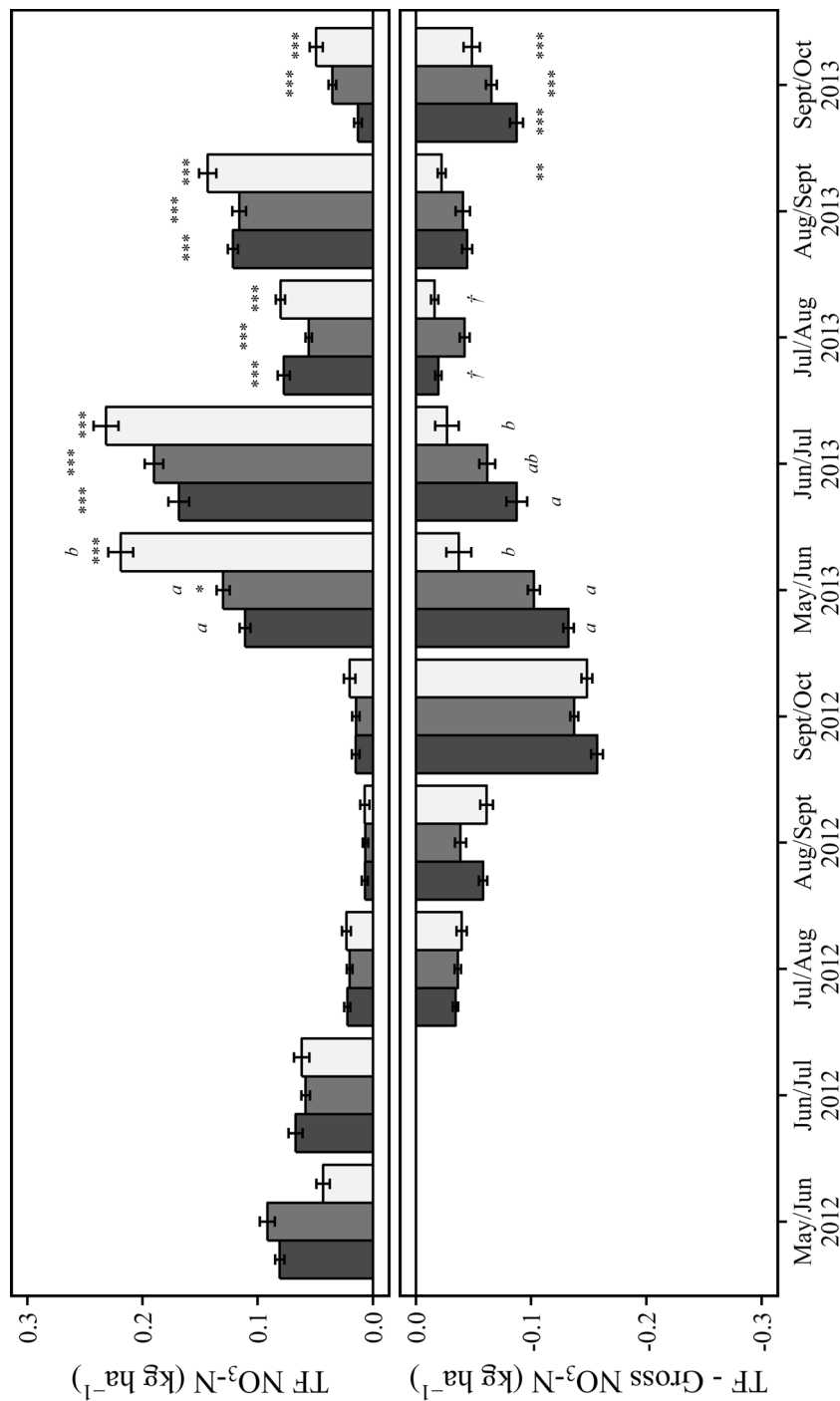


**Figure 4.2.** Throughfall collector and ion exchange resin column design.

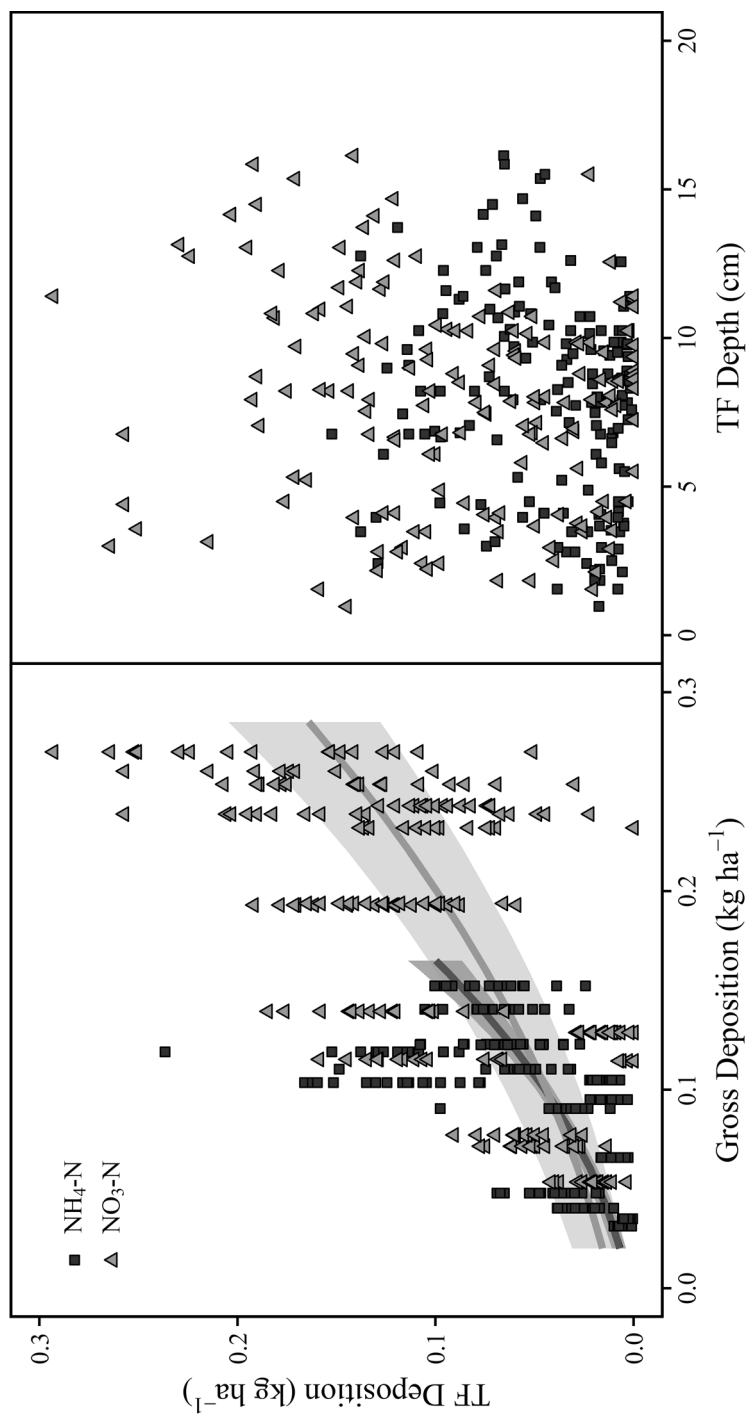




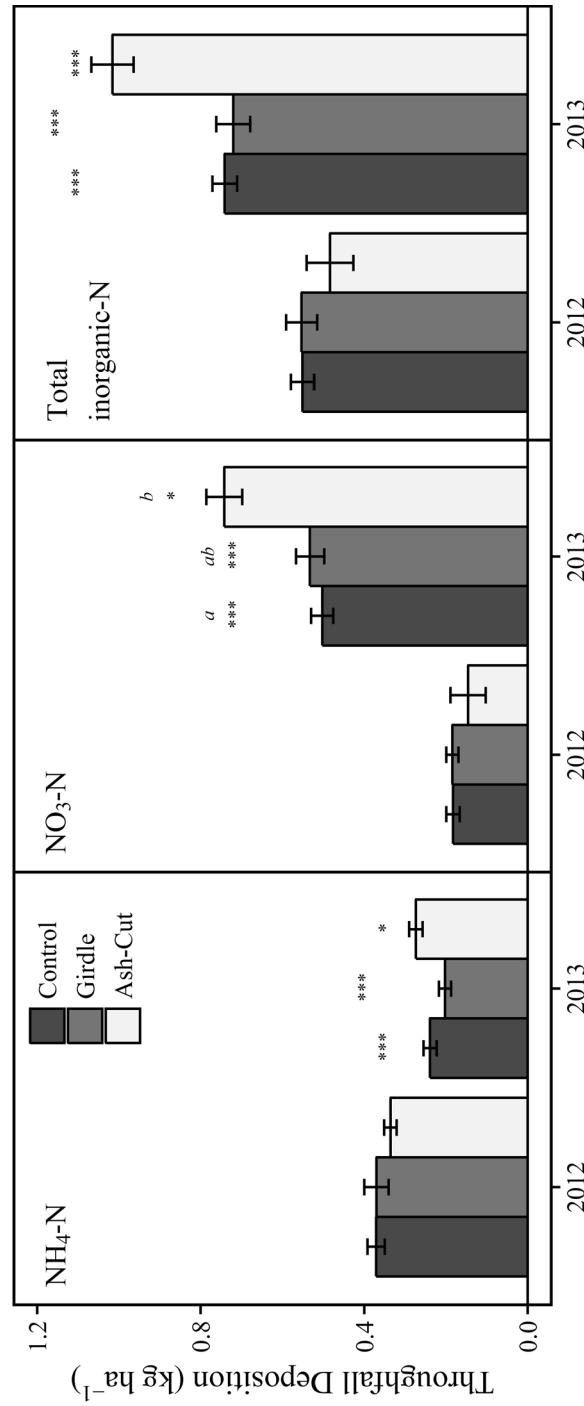
**Figure 4.3.** Throughfall deposition (first and third panels) and canopy retention (second and fourth panels) of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (mean  $\pm$  se). More negative retention values represent greater canopy retention. Letters indicate statistical differences between treatments; labels that do not share a letter indicate a statistically significant difference ( $p \leq 0.05$ ) within the sample period. Columns marked with asterisks indicate a statistically significant difference compared to the pre-treatment period (\*  $p \leq 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ). Columns marked with a  $\dagger$  indicate modeled 95% confidence intervals around the mean include zero **ADD Axis here!**.



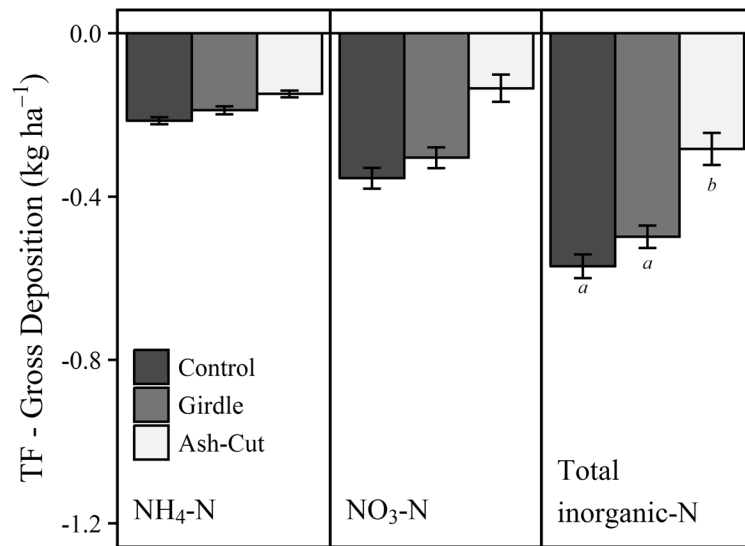
**Figure 4.3 (continued).** Throughfall deposition (first and third panels) and canopy retention (second and fourth panels) of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (mean  $\pm$  se). More negative retention values represent greater canopy retention. Letters indicate statistical differences between treatments; labels that do not share a letter indicate a statistically significant difference ( $p \leq 0.05$ ) within the sample period. Columns marked with asterisks indicate a statistically significant difference compared to the pre-treatment period (\*  $p \leq 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ). Columns marked with a  $\dagger$  indicate 95% confidence intervals around the mean include zero.



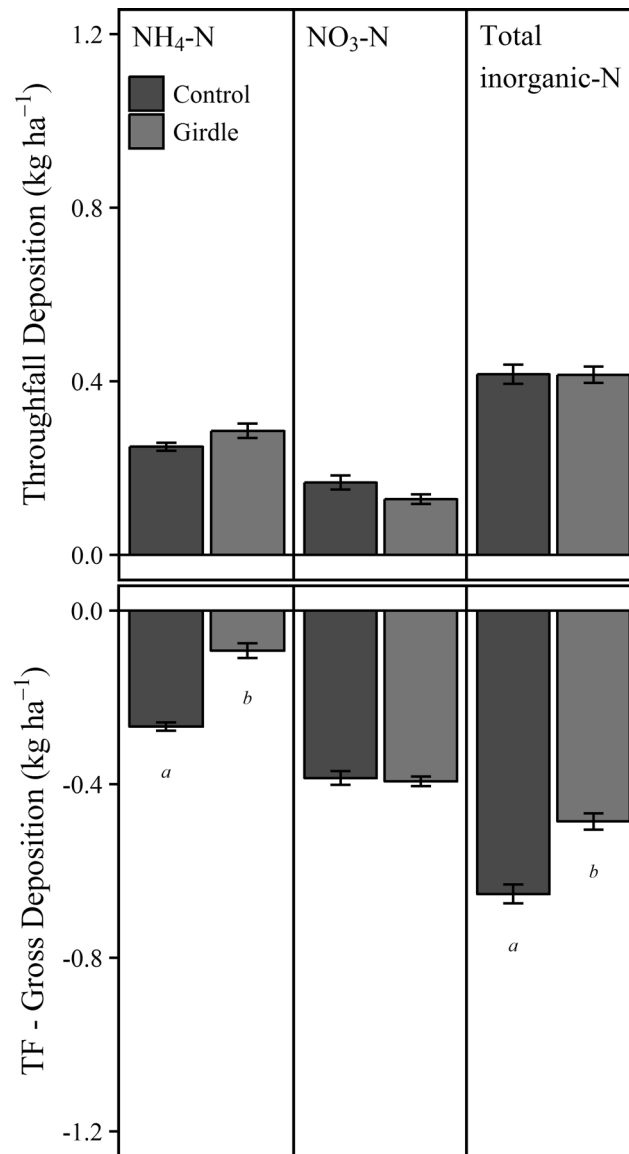
**Figure 4.4.** Gross deposition and throughfall depth versus throughfall deposition of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  within 'Control' sites during the 2013 growing season. Lines and shaded areas in the left panel represent modeled response surfaces bounded by 95% confidence intervals.



**Figure 4.5.** Sum of monthly deposition values by growing season for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  and total inorganic-N (mean  $\pm$  se). Letters indicate statistical differences between treatments; labels that do not share a letter indicate a statistically significant difference ( $p \leq 0.05$ ) within the growing season. Columns marked with asterisks indicate a statistically significant difference compared to the pre-treatment period (\*  $p \leq 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ).



**Figure 4.6.** Sum of monthly retention values for the first post-treatment growing season (2013). More negative retention values represent greater canopy retention. Letters indicate statistical differences between treatments; labels that do not share a letter indicate a statistically significant difference ( $p \leq 0.05$ ) within the sample period.



**Figure 4.7.** Growing season deposition and retention values for the second year post-treatment (2014). More negative retention values represent greater canopy retention. Letters indicate statistical differences between treatments; labels that do not share a letter indicate a statistically significant difference ( $p < 0.05$ ) within the sample period.

## 4.8 References

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## 5.0 Conclusions

The loss of *Fraxinus nigra* Marshall (black ash) as a dominant overstory component in deciduous forested wetlands due to the invasive emerald ash borer (EAB, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)) will have profound short- and long-term consequences for structure and function in these ecosystems. While many of these consequences may in part be offset by the recovery of the forest canopy, the composition of that canopy, and indeed the recovery itself is uncertain. As a result, it is very difficult to predict how the loss of this species will affect forest processes over the next several decades.

Three years following the simulated invasion, the response of woody vegetation within these forests can perhaps best be described as limited. Growth rates of overstory stems have not increased, and a number of individuals of co-occurring species have died following treatment. This mortality cannot be attributed to physical damage relating to the treatment application, and as such the lack of response in growth rate, in concert with mortality of retained stems, may indicate stressful growing conditions following treatment. While sapling growth rate did increase, this occurred only in the early-stage simulation. Seedling density of *Acer rubrum* L. (red maple) and *Betula alleghaniensis* Britton (yellow birch) also increased, suggesting that mortality of *F. nigra* saplings and overstory trees favored regeneration of these co-occurring species. These seedlings will need to compete with the large expansion of herbaceous species, which nearly doubled in areal cover in the treated sites by the end of the study. Though this increase has not appeared to result in a decline in regeneration currently, competition-related reductions in seedling establishment remain a possibility in the future, emphasizing the need for continued monitoring of these sites. Additionally, while no increase in cover or number of invasive species occurred in these relatively remote wetlands, the herbaceous response suggests that EAB mortality may facilitate invasion in locations where invasive plant pressure is greater.



Following the simulated disturbance, soil nitrogen (N) availability was not significantly impacted, though an increasing trend in  $\text{NO}_3^-$  was observed in the early-stage infestation simulation. This response is likely due to changes in soil microbial activity and biomass, as litterfall measurements indicated that demand for soil N from the dominant co-occurring species was reduced. Additionally, the presence of woody debris on the forest floor in the late-stage simulation may have provided an additional sink that reduced N availability to nitrifying bacteria, and thereby production of  $\text{NO}_3^-$ . Aboveground, the acclimation response of foliage in *A. rubrum* and *B. alleghaniensis* appeared to be dominated by the replacement of shade- with sun-acclimated leaves including a reduction in foliar N content and leaf mass per area. Though litter N content was unaffected by either treatment, the inherent differences in litter quality of *A. rubrum* and *B. alleghaniensis* compared to *F. nigra* will affect N return via litter to the forest floor, should regeneration of these woody species overcome potential increases in competition with herbaceous species. This reduction in litter quality will likely influence the rate and extent of decomposition, which may reduce forest floor carbon storage and nutrient availability to the recovering forest in the long-term. Finally, while microbial immobilization may reduce nutrient losses and subsequent changes in site fertility, the competition for N may also reduce the capacity of the current forest to respond to gap creation following *F. nigra* mortality.

Though forest canopies in both the early- and late-stage simulations remained net sinks for N deposition, the strength of that sink for both species of inorganic-N was reduced by the loss of *F. nigra*, and the deposition of  $\text{NO}_3^-$  to the forest floor increased in the late-stage simulation. These changes did not appear to have affected soil N status, as the increasing trend in  $\text{NO}_3^-$  availability was observed in the early-stage simulation only, while soil  $\text{NO}_3^-$  remained unchanged in the late-stage treatment, despite the increase in N deposition. Although the change in canopy retention and throughfall deposition in these sites was relatively small, these results have important implications for forests elsewhere in North America. In regions that receive greater N deposition than the Upper Great Lakes, the retention mechanisms for N in the canopy may approach saturation, as may

whole-ecosystem retention capacity. As a result, the loss of *Fraxinus* species in these areas may result in a greater change in N deposition via throughfall, which may exacerbate the negative effects of N pollution in these regions.

The results of these studies highlight the interconnected nature of ecosystem processes. Both the short- and long-term vegetation response may be affected by changes in soil processes and the feedback effects between canopy foliage and soil N cycling. Additionally, while relatively unimportant in these sites, the loss of forest canopy may affect soil nutrient status through its effects on throughfall deposition.

These results also have implications for sustainable management strategies designed to reduce the negative effects of EAB on forest function, yet unfortunately do not yield a single recommendation that positively influences all of the processes examined here. Seedling establishment increased in both the early- and late-stage simulations, which suggests that salvage logging of *F. nigra* would not negatively affect long-term woody vegetation recovery potential, and competition with herbaceous species should be no greater in a logged site. However, overstory mortality was observed in the late-stage simulation, while increased sapling growth occurred only in the early-stage simulation, indicating that removal of *F. nigra* stems may increase stress on middle and upper canopy trees, reducing the rate of near-term recovery. Similarly, the felling of *F. nigra* in the late-stage simulation resulted in no change in soil  $\text{NO}_3^-$ , which exhibited an increasing trend in the early-stage infestation. In this case, salvage logging may help to retain nutrients onsite, and reduce negative effects on downstream ecosystems, provided some woody debris is left on the forest floor. Conversely, removal of *F. nigra* trees from the canopy increased deposition of  $\text{NO}_3^-$  via throughfall, which in areas where N deposition is high may overwhelm the retention capacity of the ecosystem for N, and thus logging may increase leaching or export losses.

Finally, while these manipulative experiments have provided insight into the potential changes that will result from EAB infestation in forested wetlands, it is important to note

that as simulations, they may not adequately represent conditions following an actual infestation. It should also be recognized that the response in other ecosystems will be shaped by a number of factors not considered here, and may differ in scale or fundamental nature to the responses observed in these studies. Continued research into the effects of EAB and other invasive insects is critical to understanding both how these species drive ecosystem change and what measures can be undertaken to reduce their impacts on ecosystem function and the provisioning of ecosystem services.