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THE INTERACTIVE EFFECTS OF MICROTOPOGRAPHY AND HYDROLOGY ON GROUND LAYER VEGETATION AND SOIL GAS FLUX RESPONSES TO A SIMULATED EMERALD ASH BORER INFESTATION IN BLACK ASH WETLANDS

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By

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Preface

This thesis consists of two chapters which are intended for future journal publication. I, Elisabeth Stimmel, carried out the primary data collection, analysis, and writing. Joseph Shannon, Kathryn Hofmeister, and Joshua Davis assisted with study design and data analysis for Chapter 1. Joseph Shannon, Kathryn Hofmeister, and Matthew Van Grinsven also aided in study design and data analysis for Chapter 2. Original article revisions were made by Fengjing Liu, Randall Kolka and Kathryn Hofmeister for Chapter 1 and Fengjing Liu for chapter 2.

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Lastly, I am very lucky to have an immediate family who has been so supportive during my time as a graduate student an entire 500 miles away. My parents Celeste and Bernie have heard about all of the highs and lows, and my siblings Anya and Karl always offered advice from their own schooling or a funny video to cheer me up when needed.

Abstract

Black ash (*Fraxinus nigra* Marshall) wetlands are at risk of significant ecological and functional changes due to the invasive emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)), which kills trees in the *Fraxinus* (ash) genus. Simulated EAB infestations consisting of girdle treatments and ash cut treatments have been implemented in black ash wetlands to study the impacts of black ash canopy dieoff in these systems. Initial findings include ground layer vegetation shifts and impacts to carbon dioxide (CO_2) and methane (CH_4) release from soils, but these factors and their interactions with microtopography in these systems are not well understood. The objectives of this study were to explore how vegetation and greenhouse gas fluxes (GHGs) are currently responding to simulated EAB treatments in the Ottawa National Forest in the Great Lakes Region of North America six years after initial treatment implementation, and to determine how microtopography affects them as well.

Tree seedling counts and diversity were not found to be affected by treatments alone. Microtopography, however, had a larger impact and showed more seedlings and a higher diversity of seedlings growing on hummocks. Seedlings over one year of age were also found in greater numbers and with more diversity on top of hummocks. Herbaceous species have continued their trend of increased cover in treated sites, with higher cover of obligate wetland species and graminoids in treated sites as well. Increased herbaceous cover was found on top of hummocks rather than in hollows. These findings suggest that herbaceous cover is influenced by both treatments and microtopography, while tree regeneration is more influenced by microtopography. The implications of the lack of response between treatments for seedlings is that the increase in water levels at our wetland sites may be buffered by microtopography and our sites could continue to stay forested following an EAB infestation.

CO_2 and CH_4 fluxes showed different responses to treatment and microtopography. CO_2 flux was highest in control sites and on top of hummocks, while CH_4 was not found to be different between treatments or microtopography. The postulated reasons for these findings are depth to water table and root respiration. For CO_2, the lower water levels allowed for more soil to be aerated and decomposed, and there is more root respiration in control sites because of a higher volume of living trees. For CH_4, the low water levels created an environment where little CH4 was produced at all. When landscape-scale estimates of GHG fluxes were created using weighted fluxes from hummocks and hollows, CO_2 fluxes were overestimated and CH_4 fluxed were underestimated when the elevation-based flux differences due to microtopography were not factored in. These findings suggest that microtopography should be included when scaling up gas flux measurements to the landscape scale in order to get the most accurate estimates.

1 Evaluation of Ground Layer Vegetation in Relation to Microtopography Six Years After a Simulated Emerald Ash Borer Infestation in Black Ash Wetlands

1.1 Abstract

The vegetative structure and successional trajectory of *Fraxinus nigra* Marshall (black ash) wetlands is currently at risk for significant change due to the invasive emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)), which causes mortality of trees in the genus *Fraxinus*. Increased water levels, canopy openness, and herbaceous cover caused by the loss of black ash canopy and its associated evapotranspiration can ultimately lead to decreased woody regeneration and shifting herbaceous wetland indicator status group composition. Simulated emerald ash borer infestations have been created to study these shifts, but have not shown consistent initial results of expected impacts on the woody regeneration layer. We returned to a simulated emerald ash borer infestation in Michigan's western Upper Peninsula to explore the responses of ground layer vegetation and woody regeneration to the initial treatments applied six years earlier. We also sought to gain a better understanding of how microtopography might impact tree regeneration and herbaceous composition given the context of the treatments. The treatments alone were found to still have little impact on woody regeneration, while microtopography had a much larger impact with more seedlings and a higher diversity of seedlings growing on hummocks. Seedlings older than one year of age were also found in higher numbers with higher diversity on hummocks. Herbaceous species were found to have continued to increase in percent cover in treated sites, with obligate wetland and graminoid groups responding to treatments the most. More total herbaceous cover was also found on hummocks than in hollows. The sustained increased cover of herbaceous plants but lack of response in woody regeneration to the treatments combined with strong responses of both vegetative layers to microtopography suggests that microtopography may be functioning to buffer the hydrologic impacts created by the simulated emerald ash borer infestation.

1.2 Introduction

Ash trees (*Fraxinus* spp.) native to North America have been under significant threat by the invasive emerald ash borer beetle (EAB) (*Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)) for at least two decades. The beetle larvae kill ash trees by consuming their phloem tissue, girdling the tree. EAB can kill an ash tree in as few as 3-4 years following infestation and can infest trees as small as 2.5 cm diameter at breast height (DBH) (Flower et al. 2013). First detected in 2002 in southeastern Michigan, EAB has killed hundreds of millions of ash trees in the 35 eastern U.S. states it has been found in and continues to spread west (http://www.emeraldashborer.info/about-eab.php;

accessed March 2020). This is of particular concern in areas where ash trees are a dominant overstory species, such as in black ash (*Fraxinus nigra* Marshall) wetlands in the Great Lakes region. Loss of dominant overstory species in any ecosystem can cause significant ecological shifts (Ellison et al. 2005), and for black ash ecosystems this is often a transformation from a forested wetland to a shrub-scrub wetland (Erdmann, 1987). Co-occurring dominant tree species in black ash wetlands often include quaking aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marshall), American elm (*Ulmus americana* L.), or red maple (*Acer rubrum* L.). Even if these species do replace lost black ash overstory, trees like quaking aspen and paper birch may decline in the future due to climate change, and American elm is susceptible to Dutch elm disease (Iverson et al. 2015). Black ash trees specifically are of a limited group of trees that can tolerate inundation for extended periods of the year, making their niche replacement challenging and their loss highly impactful.

Several studies have tracked how EAB might influence environmental function changes in black ash wetlands through the creation of simulated EAB infestations using girdling and ash-cut treatments (Slesak et al. 2014; Van Grinsven et al. 2017). Ecological impacts of black ash overstory loss due to EAB include changes in nitrogen and carbon cycling as well as changes in hydrology and plant species composition (Kolka et al. 2018). Nitrogen cycling is expected to be affected by changes in litterfall following EAB, as ash litter has very high concentrations of nitrogen (Davis et al. 2019). Carbon cycling changes include increased carbon dioxide (CO_2) fluxes from soils at sites where ash trees were girdled or felled, and increased methane (CH_4) production at sites where ash trees were felled (Van Grinsven et al. 2018).

Changes in black ash wetland hydrology are very dramatic and are some of the first ecosystem function changes seen following overstory death. Several studies have shown that both the girdling and clearcutting of overstory black ash causes decreased water table drawdown during the growing season as a result of the loss of evapotranspiration (Slesak et al. 2014; Van Grinsven et al. 2017; Diamond et al. 2018). Diamond et al. (2018) noted that even five years after treatment, the hydrologic changes brought about by girdling and clearcutting did not shift back to their original states. When coupled with the sudden increase in sunlight and other resources, these hydrologic changes appear to be the primary cause of the vegetative species shifts found in black ash wetlands following an EAB infestation (Erdmann et al. 1987; Davis et al. 2017).

Vegetative shifts include changes to both woody regeneration and the herbaceous layer. The response of the herbaceous layer often influences the growth of the woody regeneration. Both clearcutting and girdling of overstory black ash causes an increase in herbaceous layer cover and height, which can in turn outcompete woody regeneration (Davis et al. 2017; Looney et al. 2017). Herbaceous species assemblages also shift towards obligate and facultative wetland groupings when water table levels increase, with a strong increase in graminoid cover (Davis et al. 2017; Looney et al. 2017). Furthermore, the loss of overstory black ash decreases the amount of viable black ash seeds present in the seedbank over time (Klooster et al. 2013), which can further push the ecosystem towards a shrub-scrub structure.

To keep black ash wetlands forested following an EAB infestation, it has been suggested that both herbaceous control and understory replanting is needed (Looney et al. 2017; Bolton et al. 2018; D'Amato et al. 2018). Understory replanting may be bolstered by planting replacement tree seedlings on hummocks specifically (Bolton et al. 2018). Despite the supporting evidence that EAB infestations in black ash wetlands decrease seedling densities (Klooster et. al 2014; Bowen et. al 2018), some studies implementing simulated EAB infestations have reported no initial significant differences between treatments (e.g. Davis et al. 2017; Looney et al. 2017). Furthermore, Looney et al. (2017) found that the understory woody vegetation layer was more correlated with differences between sites than canopy treatments implemented to mimic an EAB infestation. This suggests that there could be other site-specific factors influencing whether the seedling layer is affected by loss of overstory black ash.

Microtopography can be loosely defined as topographic variability at the individual plant scale (Moser et al. 2007) and is worth investigating as a site-specific influence on the seedling layer because it has been shown to affect both herbaceous and seedling patterns and assemblages. Specifically, microtopographic heterogeneity creates pockets of differing soil chemical, structural, and hydrological characteristics, which encourages increased overall plant diversity in wetland landscapes (Bruland et al. 2005; Moser et al. 2007; Wolf et al. 2011). Bruland et al. (2005) reported that the highest herbaceous diversity can often be found not on the top of a hummock or in the bottom of a hollow but instead where plants are protected from the extremes, such as on a flat or a hummock shoulder. Mature tree survival in relation to microtopography can be highly dependent on the actual elevation of hummocks in relation to the water table as well as whether the vegetation present is well adapted to frequently inundated or acidic soils (Lampela et al. 2016). In highly inundated areas, hummocks can offer a competitive advantage due to increased oxic conditions and nutrient availability (Lampela et al. 2016). Diefenderfer et al. (2018) described the effect of microtopography on wetland landscapes in their review as a "bet-hedging strategy" which can ensure that different plant species survive despite fluctuating environmental factors such as hydrology.

In this study, we sought to test the effects of microtopography and explore the response of ground layer vegetation to a simulated EAB infestation six years post-treatment. Our objectives were to (1) determine the current effects of the treatments on the diversity and survivorship of the tree seedling layer and herbaceous functional group composition, and (2) to determine if microtopography influences both tree regeneration and herbaceous functional groups given the hydrologic effects produced by the original ash-cut and girdle treatments.

1.3 Methods

1.3.1 Study Sites

Nine study sites were located in the Ottawa National Forest (ONF) of Michigan's Upper Peninsula in the Great Lakes Region of North America (Figure 1.1). Sites were selected to be similar in terms of hydrology (all in first-order watersheds), canopy composition (all with dominant canopy tree species being *F. nigra*), landscape position (all in isolated depressions), and size (between 0.23 and 1.19 ha) as detailed in Davis et al. (2017). Pre-treatment overstory basal area information can be seen in Table 1.1. Soils at our sites consisted of woody peat Histosols of depths between 5 cm and more than 690 cm with a clay lens or poorly sorted clay-loam underneath. All but one site had a high connectivity with the groundwater flow in the surrounding landscape (Van Grinsven et al. 2017). The average annual precipitation at our sites was 1010 mm year^{-1} from 1981 to 2010 and temperatures ranged from a monthly average of -11.3°C in January up to 18.2°C in July (Arguez et al. 2012).

1.3.2 Treatments

Three treatments were used for the study: a control, ash girdle, and an ash-cut treatment. Treatment names from the first vegetation study by Davis et al. (2017) were kept for clarity between data sets. Each treatment was replicated at three location blocks for a total of nine sites (Figure 1.1). The "girdle" treatment was created to simulate an early emerald ash borer infestation, where all ash trees above 2.5 cm DBH were girdled in a 15-30 cm band by hand at breast height with a draw knife. To simulate an emerald ash borer infestation in later years, an "ash-cut" treatment was applied where all ash trees above 2.5 cm DBH were felled and left on site. The "control" treatment consisted of sites with no manipulation of the overstory trees. All treatments were carried out in the winter of 2012-2013. Within each of the nine sites, 3-5 0.04 ha (11.3 m radius) permanent vegetation monitoring plots (number determined by site area) were placed randomly. Each permanent vegetation plot had three nested subplots (subplot radius 0.56 m for a 1m² area each), located at 120° intervals 5.5 m from the center, for 9-15 1m² subplots per site. The focus of this study for vegetation surveys was on the nested subplots only.

1.3.3 Data Collection

The vegetation survey was completed during the last week of July and the first week of August in 2018 to capture the peak cover of herbaceous plant species at our sites (Davis et al. 2017). Plot centers established in 2012 were relocated earlier in the season to ensure exact permanent plot location. If the original flagging at subplots could not be found, a bearing, measuring tape and reference photo were used to most closely match the plot location. Seedlings (woody stems below 50 cm in height), and herbaceous plants were measured at each of the three 1m² (0.56 m radius) subplots associated with the permanent vegetation plot. Seedlings were counted and identified by species, as well as classified by age. Age classification was described as whether the individual was in its first season of growth or whether it was older than one year (a “surviving” seedling) which was determined by the presence or absence of cotyledons (Klooster et al. 2013). Vascular herbaceous plants were also identified to species, except for graminoids, which overlapped within subplots often enough that cover estimates by species would have been unacceptably erroneous. Each species was assigned one of ten cover classes based on cover within each 1 m^2 subplot (trace, 0%–1%, 1%–2%, 2%–5%, 5%–10%, 10%–25%, 25%–50%, 50%–75%, 75%–95%, and > 95%).

To quantify microtopography at each subplot location, visual cues were used to give each subplot a cover class of “hollow” (cover of hummock at each plot was assumed to be the area left which was not “hollow”). Visual cues included variations in ground height, presence of pooled water, presence of a thick partially decomposed leaf mat, and changes in litter color, which would indicate inundation for part of the growing season (Figure 1.2). The cover classes used for microtopography were identical to the ten classes used for herbaceous cover where the percentage groupings corresponded with the estimated amount of “hollow” present.

1.3.4 Data Analysis

Cover classes of microtopography were clustered into three groups for analysis because each of the 10 cover classes on its own did not have enough observations to use for statistical testing. The groupings were created using the mean of all cover class measurements plus or minus half of a standard deviation. These microtopography groups were either “hummock” (having the lowest cover of hollow; < 5-10% cover), ”mixed” (indicating a plot with intermediate cover of hollow; > 5-10% but < 50-75% cover) or “hollow” (having the highest cover of hollow; > 50-75% cover). Each subplot was then given one of these new groups. With the new groupings, each microtopography group had between 48 and 74 subplots to use for analysis compared with the range of 2 – 30 subplots for a given hollow cover class when they were not grouped.

For herbaceous cover data, plants were grouped based on wetland indicator status (WIS) for analysis. Wetland indicator status groups are as follows: Facultative Upland (FACU) where plants usually occur in non-wetlands, but may be found in wetlands, Facultative (FAC) where plants occur equally in wetlands and non-wetlands, Facultative Wetland (FACW), where plants mostly occur in wetlands but may occur outside of wetlands, and Obligate Wetland (OBL), where plants almost always occur in wetlands (https://plants.usda.gov/core/wetlandSearch; accessed February 2020). If a species did not have a WIS and did not cover a major amount of any plots (< 10% cover) it was included in a separate group of “others” for analysis. Only 7 species in total were placed into the “others” group for analysis. The mean value of each cover class range was used in place of the cover class value for analysis (e.g. for cover class 6 which is 10-25% cover, the average value would be 17.5). *Carex* spp., Moss (all moss including *Sphagnum* spp.), and *Poaceae* spp. were also included as their own groups for analysis along with the WIS groups because they could not be assigned a WIS but still covered a significant amount of the total measured area.

To model vegetation data and its interactions with treatment, microtopography, and site location, mixed-effects models were used with the package lme4 in R (Bates et al. 2015). For all seedling models, treatment, age, and microtopography group were fixed effects with site as a random effect. The seedling count data were zero inflated and had an unequal spread of residuals, as is often seen when dealing with count data. To mitigate this, count data were transformed using ln(x+1) prior to fitting the model following Davis et al. (2017). We also attempted to fit the seedling count data using a negative binomial generalized linear mixed model, but found the original transformed model to better reduce heteroskedasticity, though it did not completely diminish it (O'Hara and Kotze, 2010). For seedling diversity data, untransformed linear mixed models worked well, and did not appear to violate any major assumptions of normality or variance. Seedling diversity measures were calculated using Shannon's Diversity Index (H'). This index takes both the richness of species and their evenness into account, so we found it was a more appropriate measure to use than richness or evenness on their own (Morris et al. 2014). For herbaceous cover data, a general linear mixed model of family distribution gamma was determined to fit and perform well with treatment, WIS, and microtopography group as fixed effects and site as a random effect. Cover response data were transformed (x+1) to ensure only positive values (which do not include zeros) were used to fit the model, which is necessary for a gamma distribution. For all pairwise tests, the Tukey-HSD method was used to adjust *p*-values with a significance level of $p < 0.05$.

Results from mixed models were reported as estimated marginal means (EMM), also known as least-squares means. EMMs give the mean response based on the model used for each factor while accounting for other variables in the model, and are appropriate to use in unbalanced experiments (Lenth 2019). They are reported here to show differences between groups, not direct amounts taken from the observed data.

1.4 Results

1.4.1 Seedling Layer

As a whole, differences in total seedling count between control, girdle, or ash-cut treatments were not significant (Table 1.2). Seedling counts were also not significantly different between treatments when broken into age groups of first year and surviving (> 1 year) (Table 1.3). Furthermore, seedling diversity was not correlated with treatment (Table 1.4). However, the relationship between total seedling count and microtopography group was significant (Figure 1.3), with the hummock group having a significantly higher total seedling count than the wet hollow group (Figure 1.3, $p < 0.001$). When seedling count was split into microtopography groups and given age, total first year seedling count was not significantly different between microtopography groups but count of surviving seedlings was significantly higher in the hummock group than in the hollow group

(Figure 1.4, $p = 0.003$). Seedling diversity was also significantly different between microtopography groups (Figure 1.5), with the hummock group having the highest diversity index overall and the hollow group having the least ($p = 0.01$). Both the hummock and mixed microtopography groups had similar EMM results of diversity (0.62 and 0.61, respectively) and both were significantly different from the hollow group, which had an EMM of 0.37.

When comparing microtopography groups within each treatment, for total count of seedlings there was a significant difference within the control and girdle treatments (Figure 1.6). The hummock microtopography group had the highest EMM in both cases (1.32 for control and 1.61 for girdle) and both hummock microtopography groups were significantly different from their respective hollow microtopography groups ($p = 0.0017$ and $p = 0.0077$, respectively). For seedling diversity, the girdle treatment was the only treatment where microtopography group had significant differences in diversity index (Figure 1.7), with both the hummock and mixed groups being more diverse than the hollow group ($p = 0.0145$ and $p = 0.0296$). When the data were split into first year and surviving seedlings, the first year group showed significant differences in the ash-cut and girdle treatment (Figure 1.8). The highest count of first year seedlings in the ash-cut treatment was in the mixed microtopography group and lowest in the hummock group ($p = 0.03$). In the girdle treatment, in contrast, the highest count of first year seedlings was in the hummock group and the lowest in the mixed group ($p = 0.01$). For the surviving seedlings, the significant differences in total count between microtopography groups were found in the control and girdle treatments (Figure 1.8). In the control, the highest count of surviving seedlings was in the hummock group and the lowest in the hollow group ($p = 0.005$). In the girdle treatment the pattern was similar, with the highest count of surviving seedlings in the hummock group and the least in the hollow group ($p = 0.04$). It is worth noting that the difference between the hummock group and mixed group within the girdle treatment was nearly significant ($p = 0.07$).

1.4.2 Herbaceous Cover

For overall mean cover of herbaceous plants, the ash-cut treatment had the highest cover (EMM = 1.63), then girdle (EMM = 1.55), then control (EMM = 1.21). The ash-cut treatment had significantly higher cover than the control ($p = 0.0067$) and the girdle treatment had marginally significantly higher cover than the control ($p = 0.0677$).

When split into WIS groups and tested between treatments, FACU, FAC, FACW, and Moss group cover did not differ significantly between treatments, while OBL, Carex, and Poaceae cover did (Figure 1.9). For Poaceae, the girdle and ash-cut treatments had significantly higher cover than the control treatment ($p < 0.001$). For Carex, ash-cut had the highest cover and was significantly higher than the girdle treatment ($p = 0.007$), and control treatment had near-significantly higher cover than the girdle treatment as well ($p = 0.0527$). For OBL, the ash-cut treatment had the most cover and was significantly higher than the control treatment ($p = 0.0026$), while girdle was in the middle and not significantly different from either treatment.

For overall mean cover comparisons between microtopography groups, the mixed microtopography group had the highest cover (EMM = 1.62), then hummock (EMM = 1.40), then hollow (EMM = 1.38). The mixed group had significantly higher cover than both the hummock ($p = 0.034$) and hollow group ($p = 0.01$).

When split into WIS groups and tested between microtopography groups, FACU, Carex, and Moss were not significantly correlated with microtopography group, while all other groups were (Figure 1.10). For the FAC group, the hummock and mixed microtopography group had significantly higher mean cover than the hollow microtopography group ($p = 0.003$ and $p = 0.004$, respectively). For FACW the trend was the same ($p = 0.001$ and $p = 0.001$). For the OBL group, the hollow and mixed microtopography groups had significantly higher mean cover than the hummock group ($p < 0.001$ and $p = 0.006$). Finally, for Poaceae, the hollow and mixed microtopography groups had significantly higher mean cover than the hummock group ($p = 0.012$ and $p < 0.001$).

When the data were further split into mean herbaceous cover by microtopography groups given treatment, the overall mean cover was significantly different between microtopography groups in the control and girdle treatments only (Figure 1.11). In the control treatment, mixed and hummock microtopography groups had significantly higher cover than hollow ($p < 0.001$ and $p = 0.003$). In the girdle treatment, the mixed and hollow groups had significantly higher mean cover than the hummock microtopography group ($p = 0.002$ and $p = 0.014$).

When mean cover data were split into WIS cover by microtopography group given treatment, there were too few observations to make a statistical comparison between microtopography groups. However, the data can be visualized in Figure 1.12 where it can be seen that FACW, OBL, Carex and Moss had comparably large variations between microtopography groups within each treatment.

1.5 Discussion

1.5.1 Treatment Effects

The seedling layer as a whole does not seem to be affected by treatments alone. This could be due to the input and output hydrology seen at the majority of our sites. Van Grinsven et al (2017) noted that at our specific wetland sites, the extent to which treatments influenced water level responses is largely impacted by the connection to groundwater flow. Although the water levels were still higher and drawdown slower at the ash-cut and girdled treatment sites (Figure 1.13), it may not be as extreme of a change at our specific sites from pre- to post-treatment compared to black ash systems with shallower water tables (Slesak et al. 2014) or controlled more by evapotranspiration, such as a rainwater or snowmelt only fed systems. At our sites there are also very prominent outflow channels as well, which may have further buffered water level increase. Based on findings from other studies, we would expect the seedling count and diversity in treated sites to be decreased (Klooster et. al 2013 Bowen et. al 2018). The absence of decreased seedling layer counts and diversity in treated sites suggests that water level on the treatment scale is not currently impeding seedling growth.

Additionally, these results could be due to several of the previously cut or girdled trees re-sprouting since the initial treatment application, either drawing down water levels soon enough to avoid a complete shift from seedlings to herbaceous species or providing another seed source that would not otherwise be present in a real EAB infestation. There has not been visual confirmation of re-sprouted treatment trees at our sites producing seeds, but other studies located in real EAB infestation areas have shown that all viable seeds and newly germinated seedlings can disappear from the ground layer and seedbank in only about 4 years following total ash tree mortality (Klooser et al., 2013). Our sites may still have some ash seed sources, given the amount of first-year ash seedlings that were recorded (Figure 1.14). This idea is further supported by Palik et al. (2012), who found that black ash mortality unrelated to EAB is also correlated with decreased ash seedling densities, which suggests that if we still have newly germinated ash seedling at our sites six years after treatment, there could be another seed source. The source would likely not be from trees outside the treatment sites, however, because sites where ash trees were girdled or felled had complete canopy death and were well isolated from other depressional black ash wetlands.

However, even with the continued presence of ash seedlings in the seedling layer, a rise in water table should theoretically still cause other species of seedlings to decrease enough to cause a difference between treatments in count and diversity, such as in Looney et al. (2015) where they found that clearcutting significantly increased water table and therefore decreased planted alternative species seedling survival. This does not appear to be the case at our sites, as water tables are still increased in the girdle and ash-cut treatments, yet no difference in seedling diversity between treatments can be seen.

Along with the decrease in seed-producing trees and increase in water levels, another common explanation for why woody regeneration often decreases following EAB infestations is because of an increase in herbaceous cover (Erdmann et al., 1987; Looney et al., 2017). We did see a difference in overall mean herbaceous cover between treatments, and the higher cover in both the girdle and ash-cut treatments would suggest that if herbaceous cover were to reduce the seedling density, it could do so in those treatments. There is also higher overall graminoid cover in the girdle and ash-cut sites, which partially fits the suggestions in Erdmann et al. (1987) that areas of black ash dying off can shift to a shrub-scrub habitat because of competition with grass species. However, the combined results of the seedling layer with the herbaceous layer indicate that treatment and increased herbaceous cover are not impacting woody regeneration at this point in time. One reason for this could be how low the seedling numbers were (Table 1.4). While the treatments have not appeared to decrease seedling numbers, it is possible that they were not able to increase numbers, either, because of the quick increase in cover by the herbaceous layer. Had seedling numbers been higher, it is possible that we may have seen a larger response to the treatments. Other seedling species which were found in high numbers other than black ash were red maple, yellow birch (*Betula alleghaniensis* Britt.), grey alder (*Alnus incana* L.), and American elm, all of which are not as well adapted to the growing conditions at our sites in comparison to black ash. It is also possible that the types of herbaceous species present at our sites are less likely to impede tree seedlings. If we had for example more invasive and aggressive species present such as Amur honeysuckle (*Lonicera maackii*) then we may have seen a substantial reduction in the tree seedling layer (Hoven at al., 2017). Other studies agree that sites with lower amounts of shrub species and higher established regeneration would be best to focus management efforts to reforest following EAB invasion (Looney et al. 2017).

In regards to wetland functional plant groups, it is unsurprising that the FACW group has the highest cover in all treatments, as the size of our wetlands along with the wetland conditions provide for an ideal habitat for FACW plants, and these plants have been the dominant WIS group surveyed at these sites in the past (Davis et al. 2017). One may wonder why the OBL group is not more dominant in the ash-cut and girdled treated sites with the present higher water levels, and while there is evidence that the OBL group does better in both the ash-cut and girdle treatments, it still has less overall cover than both Carex and the FACW group (both FACW and OBL had 14 species detected each). While the treated sites do have higher water levels which allow for better OBL plant growth, it is possible that the sites have not had a high enough water level increase to allow for total OBL dominance over other groups such as Carex, possibly due again to groundwater connectivity and outflow controls on water levels. Overall, a higher amount of OBL plants and graminoid cover have both been associated with overstory black ash dieoff (Slesak et al. 2014; Davis et al. 2016; Looney et al. 2017), and it appears either can be expected given the sudden increase in water level and higher insolation.

1.5.2 Microtopography Effects

Microtopography appears to affect both seedlings and herbaceous cover similarly and more consistently than treatment. Looney et al. (2017) similarly observed that specific site characteristics had a larger influence on plant community composition, and attributed differences in site hydrology and stand history to the variation in their ground layer community patterns more than treatment. It is consistent that the majority of seedlings at our sites would be found in the hummock microtopography group given that the median height difference between hummocks and hollows at our sites is relatively small (typically < 0.5 m, data not shown) and therefore the best growing sites are likely on top of the highest hummocks where there is still plenty of moisture but complete inundation is less likely. This result is further supported by the higher survival of planted seedlings on top of hummocks in black ash wetlands seen in Bolton et al. (2018). The more moderate growing conditions are a likely explanation for the pattern seen in seedling diversity as well. While seedlings seemed to grow best on top of hummocks, herbaceous cover had a trend similar to Bruland et al. (2005) which found the highest herbaceous biomass and diversity to be in a mixed microtopography zone (called "flats" in their study). This pattern could be due to the FACW's better ability to grow in inundated soils than tree seedlings, making it likely that it would occupy areas such as our very wet hummock shoulders where other plants may not be able to grow.

Our results from comparing first year and surviving seedlings indicate that microtopography also appears to affect seedling survival. When seedlings were categorized by age, seedlings older than one year of age were sensitive to microtopography, while seedlings in their first year were not. This result is likely reflective of the differences in conditions needed to germinate a seed compared to the conditions needed to ensure a seedling's survival into higher vegetation strata. We can see evidence of these differences in studies which focus on seedling survivorship; the mortality rate of seedlings is typically very high in the first year (Cleavitt et al. 2014). The initial rates of dieoff can be attributed to many seeds being germinated successfully, but not all on sites which can support the seedlings' growth into a higher vegetative layer.

When looking at WIS groups, it appears that of the groups which were sensitive to microtopography, the FAC and FACW grow best in the hummock and mixed microtopography groups, and OBL and Poaceae have the opposite distribution, with the most cover being in the hollow and mixed microtopography groups. The FAC and FACW results connect back to the relative range of heights between our hummocks and hollows, which shows that even our tallest hummocks still likely have a high soil moisture content. For the OBL group, Davis et al (2017) noted there were several species of OBL plants which were not present prior to the treatment implementation but were present after implementation. The presence of more OBL species after treatment along with the OBL group's distribution amongst microtopography groups is consistent because they are most competitive in inundated soils, which means the increase in water level has been most beneficial to them on a microtopographic scale.

1.5.3 Interactions between Microtopography and Treatments

Total seedling count was only sensitive to microtopography in the control and girdle treatments, likely because water levels dropped below the soil surface in only those treatments for a portion of the growing season. However, the ash-cut treatments never had their water levels drop fully below the soil surface, which may have created fewer differences between the hummock microtopography groups and the hollow microtopography groups at those sites (Figure 1.13). In other words, the hummock areas in the ash-cut sites may have been much closer in microsite characteristics (soil moisture, soil temperature) to the hollow areas in either of the other two treatments for most of the growing season. When assessing seedling diversity, girdle was the only treatment where seedling diversity was correlated with microtopography, possibly due to the combination of sunlight availability paired with water levels dropping below the soil surface. When examining the first-year seedling group, the results do not appear to be consistent with the rest of the seedling findings. This inconsistency is likely another example of how seeds can often be germinated in many conditions but may not survive to recruit in those same conditions (Cleavitt et al. 2014). Germination and recruitment differences are further enforced when we assess the surviving seedling group, which had the highest counts in the hummock groups for both control and girdle treatments. The pattern seen in both treatments indicates that the most surviving seedlings can be found in hummock microtopography groups, suggesting that hummock microtopography groups have better microsites for seedlings to persist.

The results for the herbaceous cover are harder to interpret when comparing between microtopography groups within treatments. Only total cover could really be analyzed statistically due to small sample sizes when split by WIS, treatment and microtopography group. In the control the results appear to be straightforward; like many of the other results, many plants do better on top of hummocks at our sites. At the girdle sites, the mixed and hollow groups both had more cover than the hummock group, possibly due to the competitive advantage some wetland plants may have in the intermediate and wet areas, such as with OBL plants which can handle higher inundation. Generally, the highest cover of OBL was found in the hollow microtopography group within the girdle treatment (Figure 1.12). However, there were too few cases to run statistical testing between the WIS groups within treatments, so this is only speculation. The lack of difference between microtopography groups in the ash-cut treatments could be attributed to once again the water level never dropping below the soil surface. The water level in the ash-cut treatment paired with the OBL group having significantly more cover in the ash-cut treatment than the control points to the ash-cut sites being covered more evenly with OBL plants as a whole, with little differences brought about by microtopography.

Pulling together all of the results from the seedling layer and the herbaceous layer given microtopography and treatment effects, we postulate that microtopography at our sites is functioning to buffer the hydrologic effects of the simulated EAB treatments. Microtopography appears to have a more consistent and profound effect on both vegetative layers, and though there are still direct hydrologic impacts from the treatments to be found, the hummocks could be allowing for many plants to escape the sustained inundation. If the hummocks at our sites were even higher in elevation compared to the hollows, this buffering effect may be emphasized even more, like the "bet-hedging" strategy described in Diefenderfer et al. (2018). It appears likely that in the future our sites will have a continued increase in OBL herbaceous cover, as well as persisting yellow birch, red maple, and grey alder canopy cover replacing the black ash.

1.6 Conclusions

Many sources have voiced concerns about herbaceous cover and increased water tables impeding the continued regrowth of the woody regeneration layer following an EAB infestation of black ash wetlands. We found that our herbaceous cover did increase in girdle and ash-cut treated sites, but seedlings appear to be unaffected even six years after the treatments. The future of our sites appears to have continued tree canopy in the form of yellow birch, red maple, and grey alder, with increased OBL group herbaceous plants on the ground layer. Our specific sites have attributes which may allow them to continue to stay forested following an EAB infestation. The attributes include the combination of how water levels change (which in our sites was not extremely dramatic, likely due to groundwater connectivity and surface wetland outflow) coupled with the microtopography at our sites. That being said, if the water levels increase following a black ash dieoff event to the point of never dropping below the soil surface and covering a larger area of each hummock's surface (such as in areas with shorter microtopography or less groundwater-connected hydrology), there may be a higher likelihood of woody regeneration decrease and a shift instead to a shrub-scrub habitat. We suggest that if forest managers wish to keep black ash wetlands forested following and EAB infestation, they may have better opportunities to do so by planting on or encouraging seedling growth on hummocks, especially in wetlands that have high groundwater connectivity and good outflow to buffer the effects of loss of evapotranspiration from the overstory black ash.

1.7 Tables

Table 1.1. Pre-treatment 2012 overstory information for each individual site in the Ottawa National Forest of Michigan's Upper Peninsula. Site area, black ash overstory basal area and density, as well as non-ash overstory basal area and density are given.

Site	Treatment	Area	Ash BA ($m^2 \cdot ha^{-1}$)	Ash Density ($stems \cdot ha^{-1}$)	Non-Ash BA ($m^2 \cdot ha^{-1}$)	Non-Ash Density ($stems \cdot ha^{-1}$)	Species Richness
135	Control	0.3	6.2 ± 1.3	225 ± 90	18.2 ± 2.2	500 ± 50	7
151	Girdle	0.29	16.1 ± 3.6	442 ± 131	12.3 ± 1.7	325 ± 52	8
077	Ash-Cut	0.61	13.9 ±2.5	758 ± 121	17.8 ± 2.5	567 ± 94	9
157	Control	0.23	23.8 ± 1.9	483 ± 58	5.2 ± 1.3	200 ± 29	6
119	Girdle	0.33	20.5 ± 3.6	458 ± 51	4.5 ± 2.1	125 ± 138	5
156	Ash-Cut	0.35	28.0 ± 2.0	742 ± 22	4.8 ± 1.4	183 ± 30	4
152	Control	0.81	27.5 ± 4.0	700 ± 46	9.1 ± 1.2	300 ± 54	10
140	Girdle	0.61	20.5 ± 3.8	608 ± 88	8.4 ± 1.9	258 ± 109	7
009	Ash-Cut	1.19	16.1 ± 2.6	590 ± 42	5.3 ± 1.1	275 ± 58	4

Table 1.2 Total seedling count comparisons by treatment for all vegetation plots with estimated marginal means for each treatment, standard error, and p-value. The response data is in ln(x+1) transformed format from fitting linear mixed models.

Total Seedling Count by Treatment

Treatment	Estimated Marginal Mean	Standard Error
Control	0.861	0.383
Girdle	0.941	0.941
Ash Cut	0.923	0.923

Treatments	p-value
Ash Cut - Control	0.993
Ash Cut - Girdle	0.999
Control - Girdle	0.988

Table 1.3 Total seedling count comparisons by treatment given age for all vegetation plots with estimated marginal means for each treatment by age, standard error, and p-value. In this case, "age" refers to any seedlings identified to be a newly germinated or "first year" seedling or not newly germinated and therefore in the "surviving" age group. The response data is in ln(x+1) transformed format from fitting linear mixed models.

Total Seedling Count by Treatment given Age		
Treatment and Seedling Age	**Estimated Marginal Mean**	**Standard Error**
<1yr		
Control	0.734	0.397
Girdle	0.980	0.399
Ash Cut	1.000	0.391
>1yr		
Control	0.847	0.391
Girdle	0.903	0.399
Ash Cut	0.988	0.397
Treatment Comparisons		**p-value**
<1yr		
Ash Cut - Control		0.884
Ash Cut - Girdle		0.999
Girdle - Control		0.902
>1yr		
Ash Cut - Control		0.966
Ash Cut - Girdle		0.995
Control - Gridle		0.988

Table 1.4 Total seedling diversity by treatment with estimated marginal means for each treatment, standard error, and p-value. The diversity index used is the Shannon's diversity index.

Total Seedling Diversity by Treatment		
Treatment	**emmean**	**SE**
Control	0.302	0.228
Girdle	0.644	0.228
Ash Cut	0.649	0.232
Treatment Comparisons		**p-value**
Ash Cut - Control		0.5656
Ash Cut - Girdle		0.9999
Control - Girdle		0.5695

Table 1.5 Mean number of *Fraxinus* seedlings per hectare per site for each treatment calculated from 1m^2 vegetation subplot counts.

Site	Treatment	Seedlings (no/ha)
077	ash cut	22222
009	ash cut	6667
156	ash cut	0
135	control	4444
152	control	3333
157	control	7778
119	girdle	1111
140	girdle	7778
151	girdle	8889

1.8 Figures

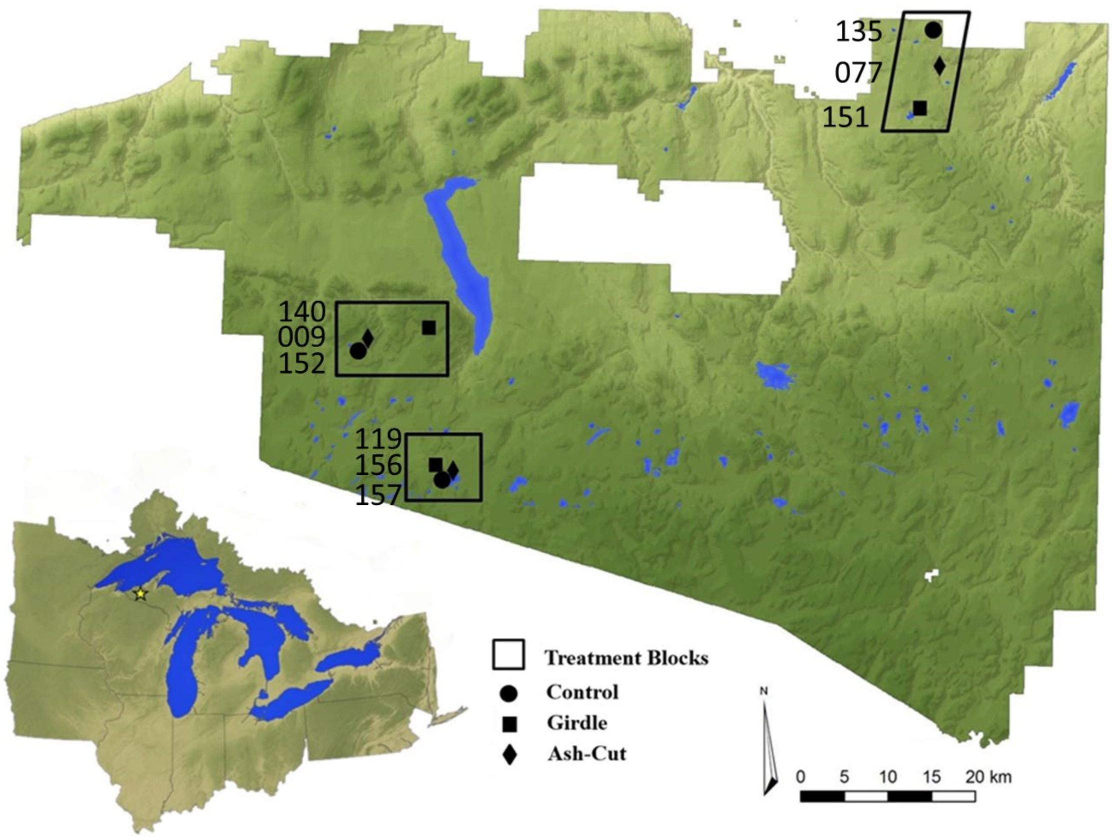

Figure 1.1 The three treatment blocks containing each treatment site in the Ottawa National Forest of Michigan's Upper Peninsula in the Great Lakes Region of North America.

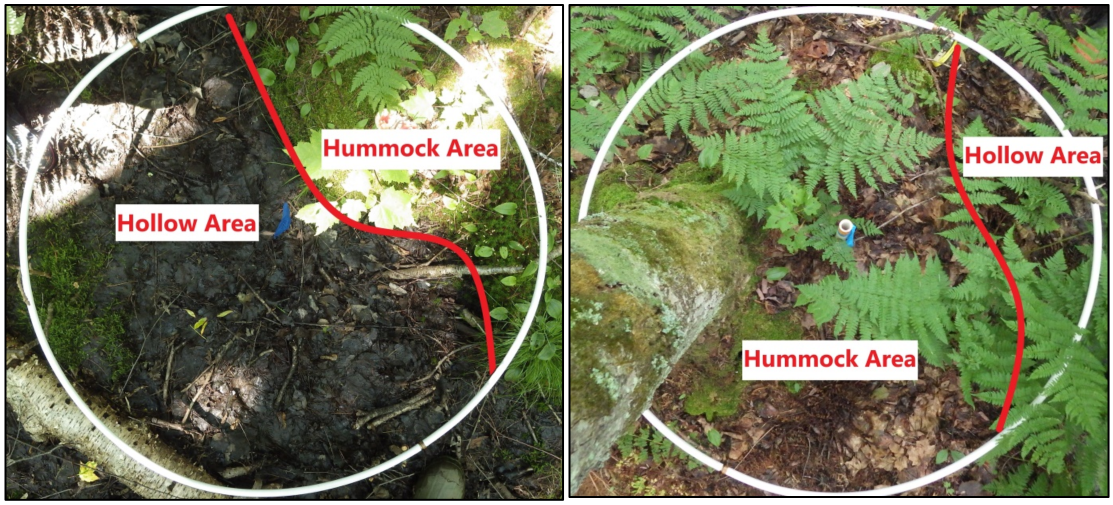

Figure 1.2 A visual representation of what cues were used to identify hummock or hollow cover at each subplot. On the left, a plot which would be in the “wet” category due to having mostly (>50-75%) hollow cover. On the right, a plot which would be in the “dry” category due to having mostly hummock cover (< 5-10% hollow).

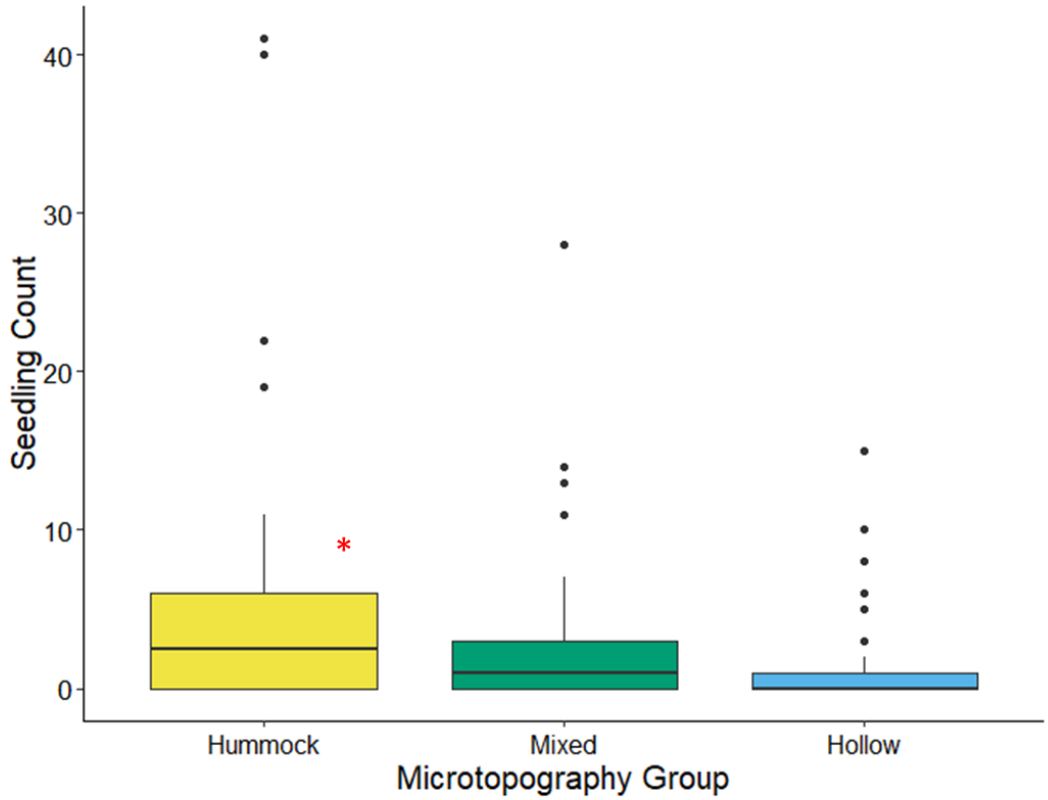

Figure 1.3 Comparisons of seedling counts between microtopography groups across all treatments. Boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers.

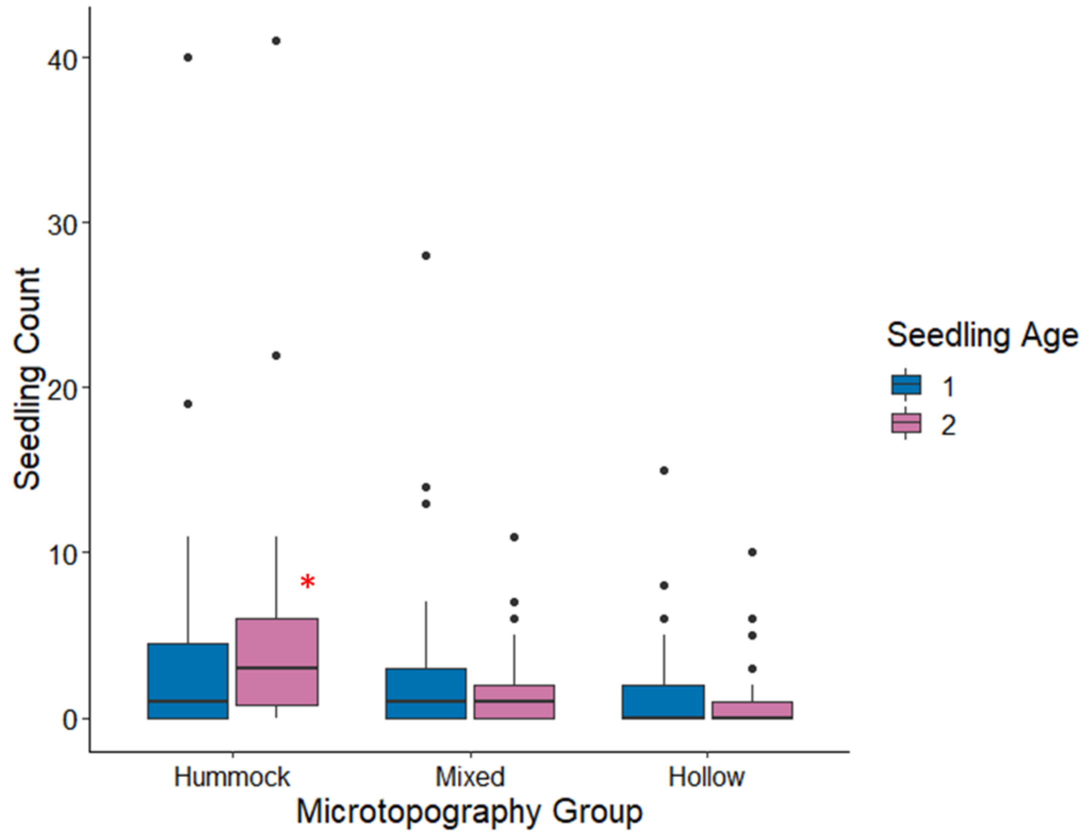

Figure 1.4 Comparisons of seedling counts between seedling age groups given microtopography group across all treatments. The diversity index used here is the Shannon's diversity index. Boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers.

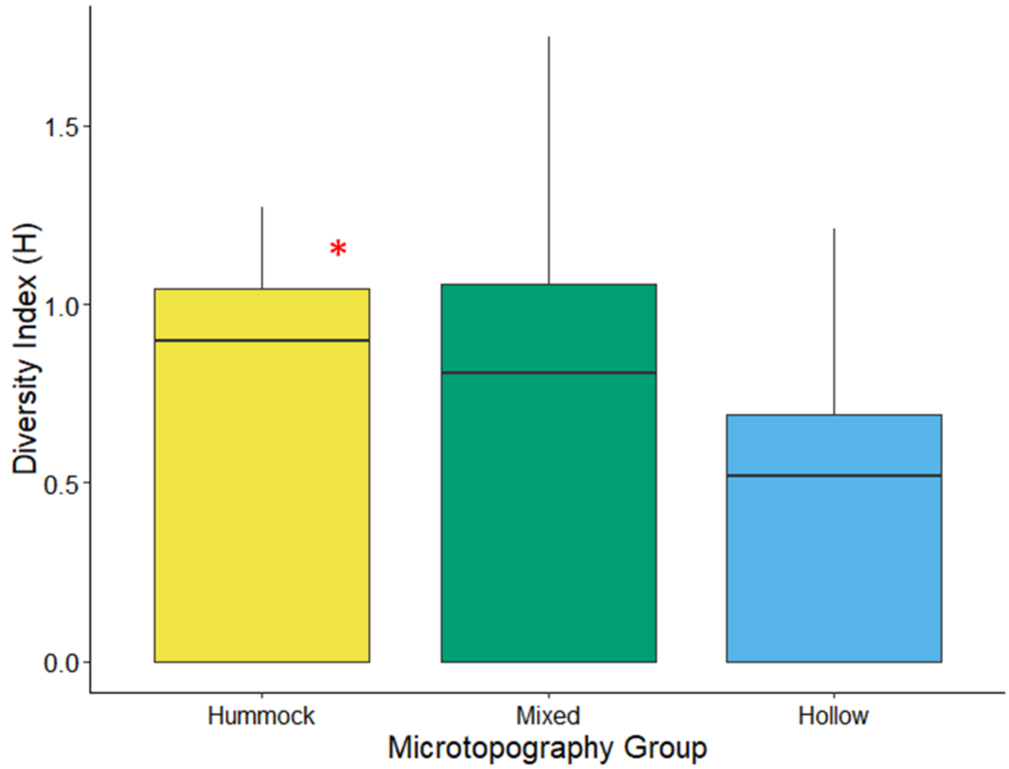

Figure 1.5 Comparisons of seedling diversity index between microtopography groups given seedling age across all treatments. Boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers.

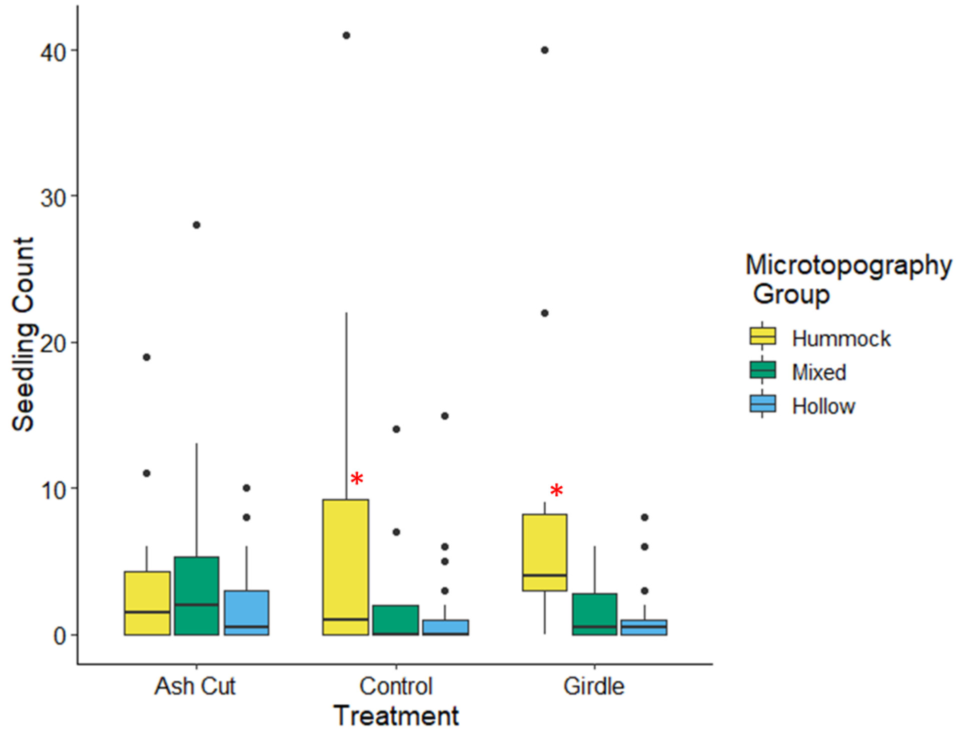

Figure 1.6 Comparisons of seedling count between microtopography groups given treatment. Boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers.

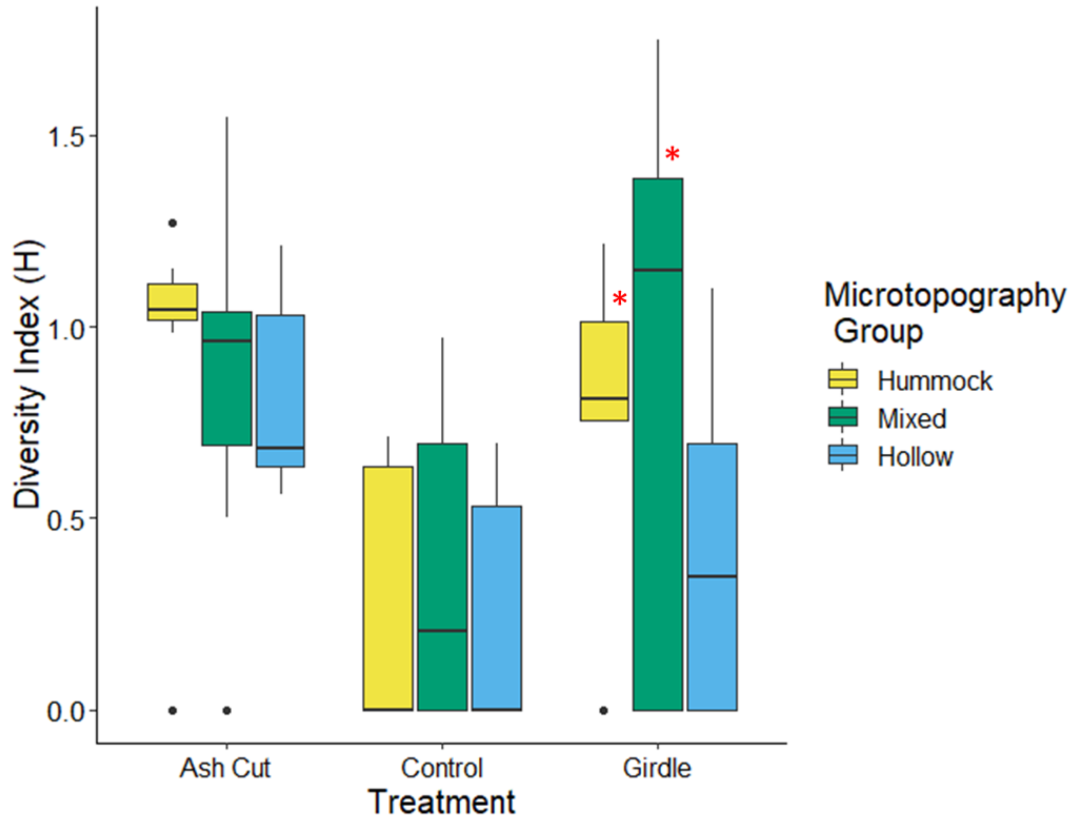

Figure 1.7 Comparisons of seedling diversity index by microtopography group given treatment. The diversity index used here is the Shannon's diversity index. Boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers.

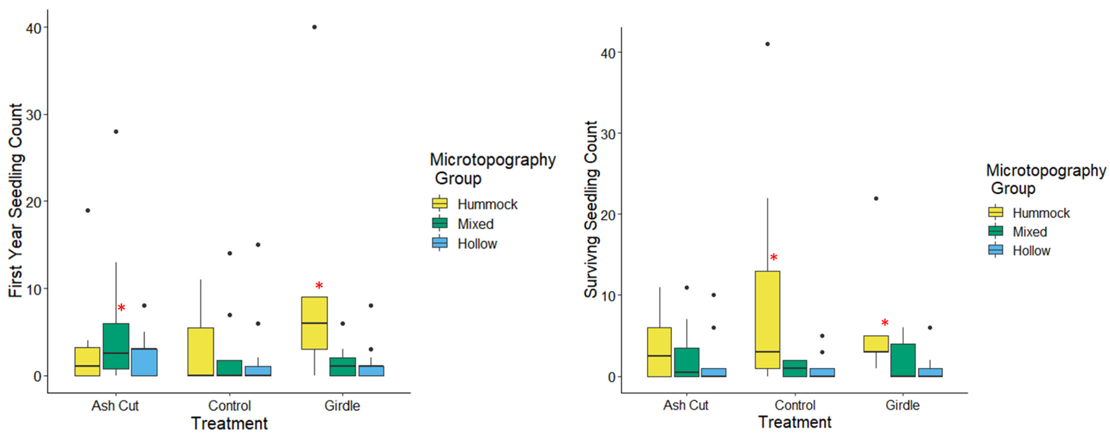

Figure 1.8 Left, comparisons of newly germinated (first year) seedling counts by microtopography group given treatment. Right, comparisons of surviving (> 1 year old) seedling counts by microtopography group given treatment. Boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers.

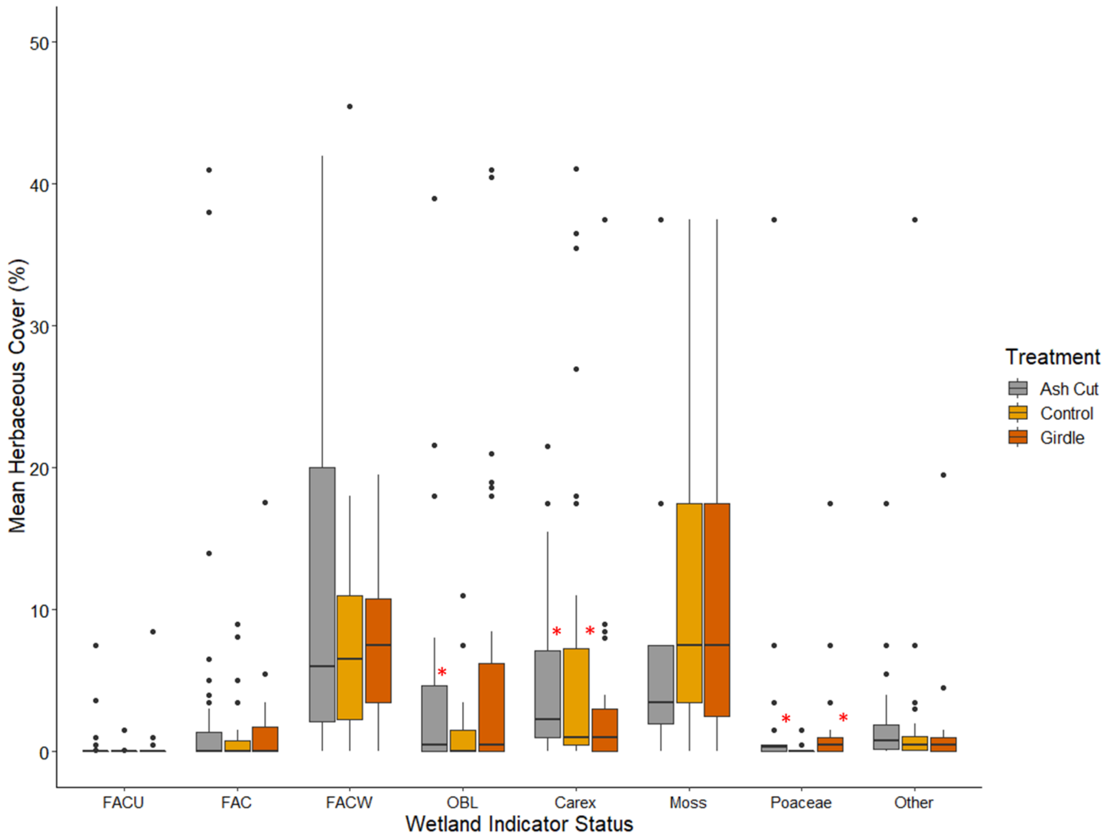

Figure 1.9 Comparisons of mean herbaceous cover (%) between treatments given wetland indicator status. Carex, Moss, and Poaceae are included here as they were the identified plant groups with the largest percent cover and no wetland indicator status group. Boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers.

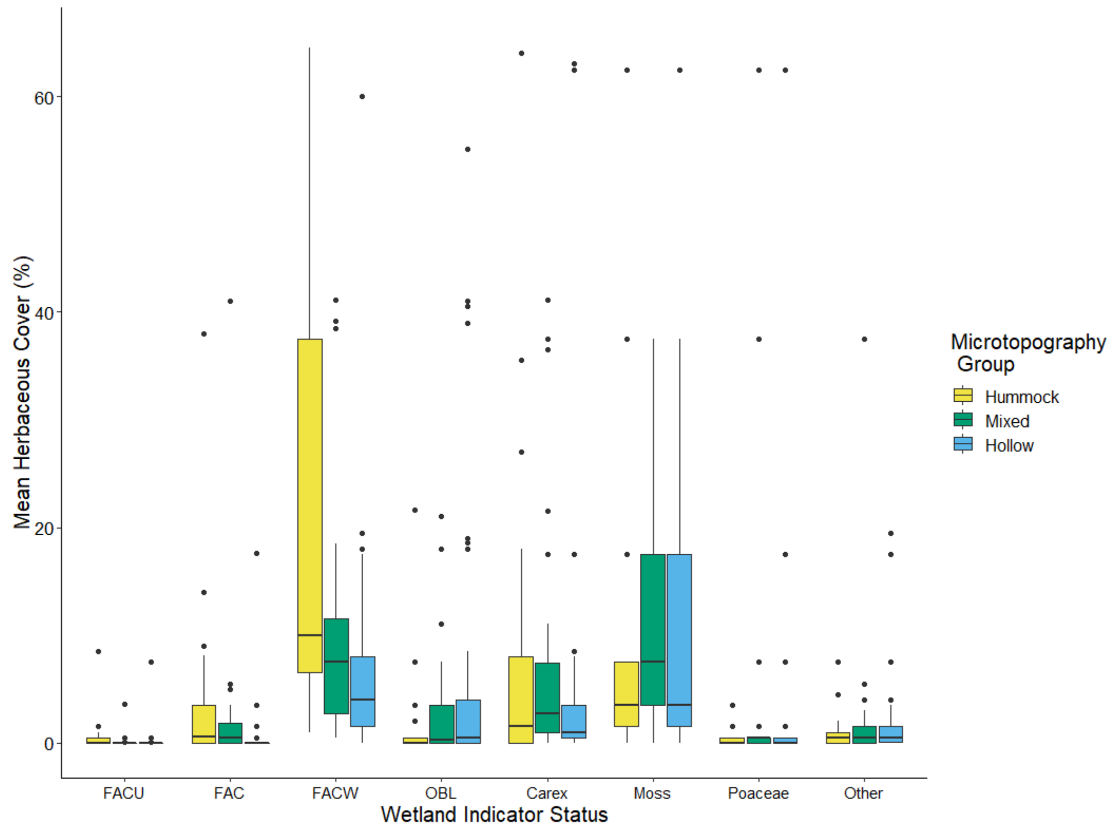

Figure 1.10 Comparisons of mean herbaceous cover (%) between microtopography group given wetland indicator status. Carex, Moss, and Poaceae are included here as they were the identified plant groups with the largest percent cover and no wetland indicator status group. Boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers.

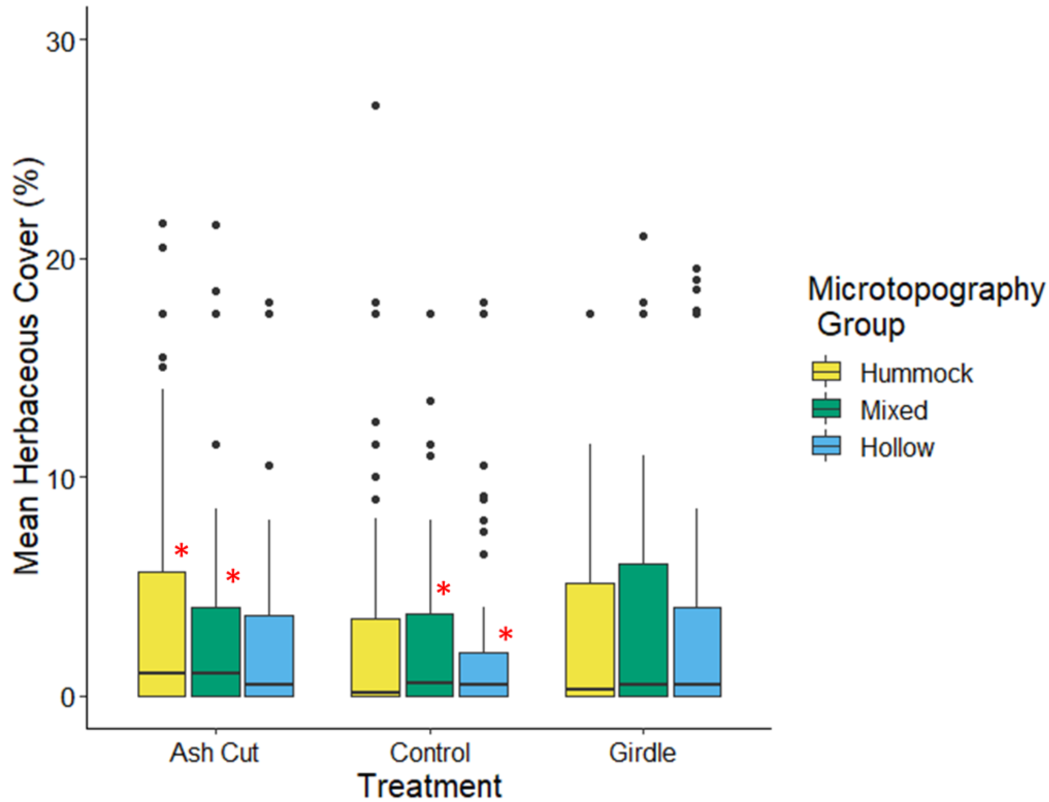

Figure 1.11 Comparisons of mean herbaceous cover (%) between microtopography group given wetland indicator status. Boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers.

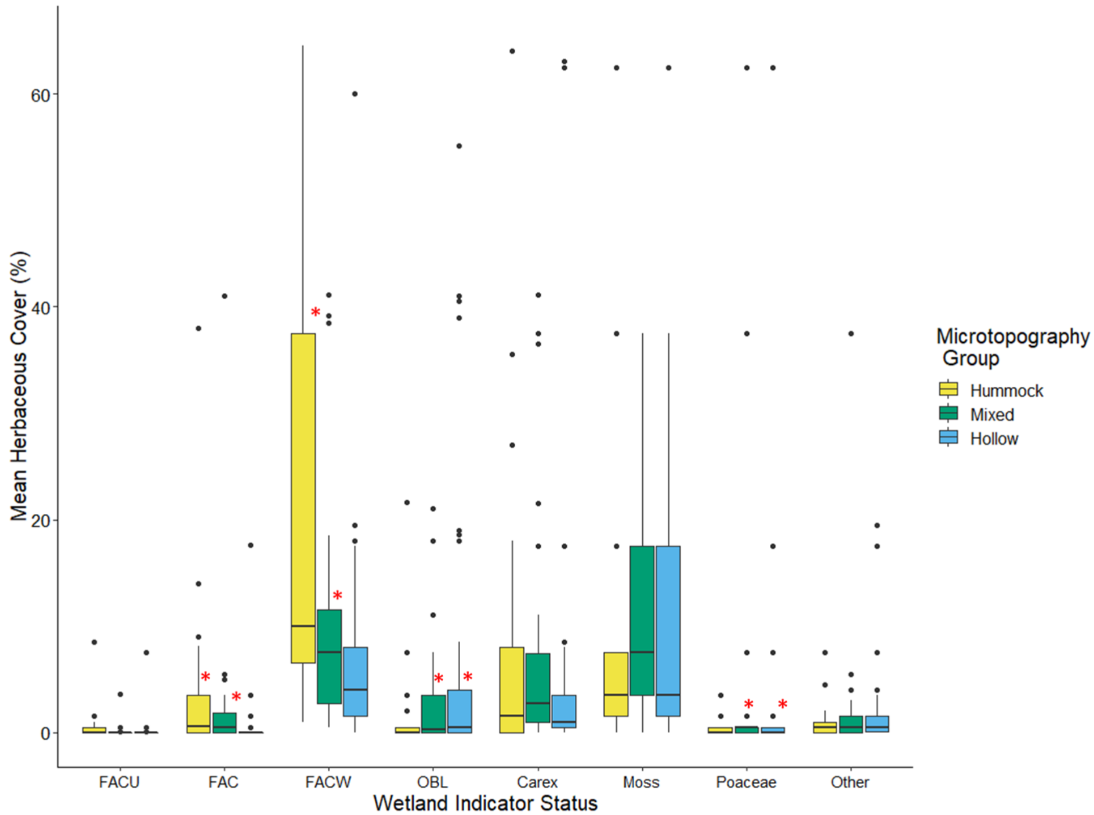

Figure 1.12 Comparisons of mean herbaceous cover by microtopography group given treatment for wetland indicator status groups that showed the highest sensitivity to microtopography group within treatments. Statistical analyses could not be performed on this data split due to low replicates. Boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers.

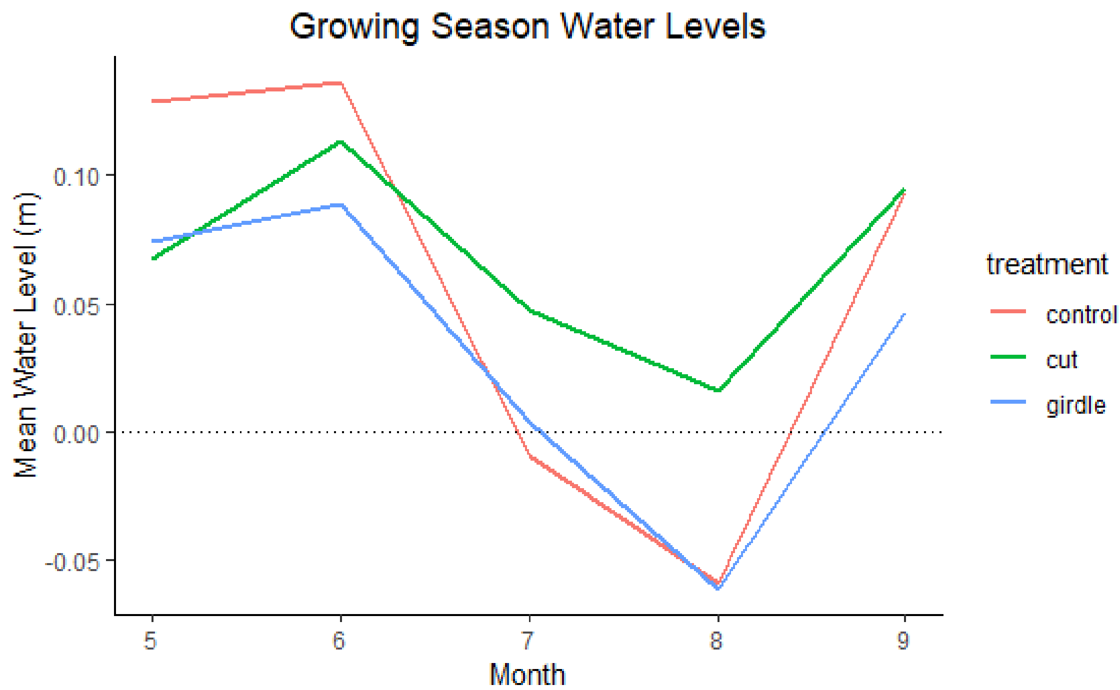

Figure 1.13 Mean monthly water levels compared to ground surface for each treatment throughout the growing season of 2018. Water levels were averaged between all control, girdle, or ash-cut treatments for each month.

2 The Influence of Microtopography on Landscape-Scale Soil Gas Fluxes in Black Ash Wetlands Treated with Simulated Emerald Ash Borer Infestations

2.1 Abstract

Wetlands soils are known to be an important source of the greenhouse gases (GHGs) carbon dioxide (CO_2) and methane (CH_4). Black ash wetlands are currently at risk for significant ecological and functional changes due to the invasive emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)), which may impact the amount of GHGs they release from their soils. We revisited a simulated emerald ash borer infestation in the Ottawa National Forest of Michigan's Upper Peninsula in the Great Lakes Region of North America six years after initial treatments to determine what differences still exist in GHG fluxes between treatments and if there are any differences in fluxes between microtopography classes. We also sought to more accurately scale chamber flux measurements by incorporating weighted microtopography (hummock and hollow) flux estimates into a total landscape estimate. We found that CO_2 fluxes were higher in the control sites and on top of hummocks, which can most likely be attributed to depth to water table allowing more aerated soil to decompose as well as more root respiration due to a higher volume of living trees. CH_4 fluxes were not different between treatments or hummocks and hollows, which may be due to the low output of CH_4 caused by lack of standing water during this growing season and groundwater connectivity. These results contrast our original hypotheses and suggest that during dry years, water table has a higher influence on gas flux than insect disturbance effects caused by EAB. Furthermore, landscape estimates with microtopography fluxes incorporated compared to those without suggest that traditional estimates without microtopography could be potentially overestimate CO_2 landscape fluxes and underestimate CH_4 landscape fluxes.

2.2 Introduction

Understanding the amount of carbon stored or released from different ecosystems in the form of greenhouse gasses (GHGs) has become important in recent years due to a need for a better understanding of our global carbon budget. Wetland ecosystems are known to be an important carbon source of GHGs such as carbon dioxide (CO_2) and methane (CH_4). Many studies have been carried out in wetlands to explore how different ecosystem components contribute to overall wetland GHG release. Different components which contribute to overall wetland gas release include coarse woody debris, tree stems, respiring plants, and soils (Noh et al. 2018; Maier et al. 2011; Bubier et al. 1995). Of these components, a heavy focus is often put on soil gas fluxes due to their function as an immense terrestrial carbon pool (Oertel et al. 2016). While the majority of studies to be found on wetland soil GHGs seem to be focused on peatlands, northern wetland forests give off a substantial amount of CO_2 and CH_4 as well (Trettin et al. 2006). Black ash (*Fraxinus nigra*) wetlands are unique northern forested wetlands with high densities of black ash overstory due to the species' high tolerance to flooding, and are one such northern forested wetland whose carbon fluxes are not as well studied.

Black ash wetlands are currently at risk for significant ecological and functional changes due to the invasive emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae)), which may impact the amount of GHGs they release. The wood-boring beetle larvae kill ash trees by consuming their nutrient transport tissue, girdling them. EAB can kill an ash tree in as few as 3-4 years following infestation, and can infest trees as small as 2.5 cm diameter at breast height (DBH) (Flower et al. 2013). Because the beetle can infest ash trees at such a small size, the likelihood of functional regeneration of the species following an infestation is low. Few other tree species can fill in this flooded niche, which means that these wetlands are likely to transition from forested into shrub-scrub wetlands (Erdmann et al. 1987). This major canopy death also causes a significant loss of evapotranspiration, which in turn causes the basal water level of the site to increase (Van Grinsven et al. 2017). The changes in temperature and water level caused by EAB infestations have been shown to increase CO_2 and CH_4 soil fluxes from these systems. Furthermore, specific site characteristics such as depth to water table, soil temperature, and groundwater connectivity can have a large effect on how much gas is released (Van Grinsven 2018).

Although the overall ecosystem changes caused by an EAB infestation would be site-wide, the other site characteristics in a black ash wetland contributing to soil gas flux are not always evenly distributed throughout a site. Black ash wetlands have surface microtopography including hummocks and hollows which have been shown to give off different amounts of CO_2 and CH_4 relative to water level, soil temperature, and insolation (Dinsmore et al. 2008; Kim and Verma et al. 1992; Jauhiainen et al. 2005; Munir et al. 2014). On hummocks, the conditions are often ideal for higher CO_2 release due to warmer temperatures and increased oxidation. In hollows, there is typically higher CH_4 production and release due to standing water and anoxic conditions. These generalizations are not always the case, as different characteristics at some sites such the presence of alternate electron acceptors in soil water can cause decreased CH_4 fluxes even with higher water levels, indicating that generalizations about GHG production do not fit every site and situation. This variation within sites makes it difficult to accurately scale up chamber-based gas measurements to the landscape level if spatial heterogeneity is not accounted for in some way (Dinsmore et al. 2008; Dinsmore et al. 2017). Forbrich et al. (2011) found success in accurately scaling up chamber-based gas measurements by using a high-resolution landcover map to determine microsites and then applying weighted microtopography-based CH_4 fluxes to model total ecosystem CH_4 flux.

In the Ottawa National Forest of Michigan's Upper Peninsula, previous work at depressional black ash wetlands explored the importance of different environmental controls on GHG fluxes, including the effects of a simulated emerald ash borer infestation. GHG fluxes were found to be sensitive to water level, temperature, and disturbance, with higher overall CO_2 fluxes and more frequent high CH_4 fluxes in disturbed sites (Van Grinsven et al. 2018). However, the effects of microtopography on

CO_2 and CH_4 fluxes have yet to be explored at our sites and total site estimates do not currently factor in small-scale ground-level spatial flux variations.

In an effort to quantify total site soil CO_2 and CH_4 flux from black ash wetlands affected by EAB including potential flux differences created by microtopography, we sought to (1) determine what differences in CO_2 and CH_4 fluxes exist between treatments that simulate EAB infestation and between hummocks and hollows at our sites, and (2) integrate potential microsite flux differences into total wetland flux estimates in order to create a more accurate estimate of gas flux from wetlands affected by EAB. Based on the findings specific to our sites from Van Grinsven et al. (2018), we would expect both CO_2 and CH_4 release to be higher in the girdle treatment sites due to the known sensitivity to disturbance created by the simulated emerald ash borer infestations. We would also expect to see higher CO_2 fluxes at hummocks due to drier, warmer soils, and higher CH_4 at hollows because of depth to water table and higher soil moisture. Combining both treatment and microtopography effects on flux will ultimately give us a better site-wide flux estimate for each individual site.

2.3 Methods

2.3.1 Study Sites

Six study sites were located in the Ottawa National Forest (ONF) of Michigan's Upper Peninsula in the Great Lakes Region of North America (Figure 2.1). Soils at our sites consisted of woody peat Histosols of depths between 5 cm and more than 690 cm with a clay lens or poorly sorted clay-loam underneath (Davis et al. 2017). All but one site have a high connectivity with the groundwater in the surrounding landscape (Van Grinsven et al. 2017). Sites were selected to be similar in terms of hydrology (all in first-order watersheds), canopy composition (all with dominant canopy tree species being *F. nigra*), landscape position (all in isolated depressions), and size (between 0.23 and 1.19 ha). The average annual precipitation our sites receive is 1010 mm year^{-1} (1981-2010 NOAA climate normals), and temperatures range from a monthly average of -11.3°C in January up to 18.2°C in July (Arguez et al. 2012). Monitoring wells are located in each wetland and record water levels at 15-minute intervals during ice-free periods.

2.3.2 Simulated EAB Infestation

Two treatments types from the simulated EAB infestation were used for the study: control sites and girdle sites. The six sites fell into three locations blocks, with each block containing one site of each treatment type (Figure 2.1). To simulate an early emerald ash borer infestation, in a given "girdle" site all ash trees above 2.5 cm DBH were girdled in a 15-30 cm band by hand at breast height with a draw knife. The "control" treatment consisted of sites with no manipulation of the overstory trees. Each treatment has three replicates, and treatments were carried out in the winter of 2012-2013 (Davis et al. 2017; Van Grinsven et al. 2017).

2.3.3 Instrumentation and Monitoring

2.3.3.1 Gas Measurements

Two 25.4 cm diameter PVC collars were placed within 2 m of one another at six random locations 5 m within the site boundary at each site for a total of 12 collars per site or 24 collars per treatment. Random collar locations were determined using ArcGIS (Esri, Redlands, CA, USA). Collars were 13 cm tall and set in the soil so that approximately 5 cm of the collar was below the soil surface. Each pair included one collar placed on top of a hummock and one collar placed in a hollow. Hummocks or hollows were determined based on visual cues such as contextual ground elevation differences, signs or presence of standing water, and presence of a thick partially decomposed leaf mat (indicating a hollow). Collar elevations relative to each site's monitoring well were recorded using a Leica TS11 total station.

CO_2 and CH_4 flux measurements were recorded during the snow-free months of 2019 (June-October) approximately twice per month using an ultra-portable greenhouse gas analyzer (UGGA) (LGR model 915-0011). A portable dynamic dark chamber made from a 25.4 cm ID PVC slip cap was placed onto collars one at a time for 180 seconds each, during which CO_2 and CH_4 was measured in 1-second intervals in tandem. An airtight seal between the chamber and the collar was ensured with a ring of foam adhered to the inside of the PVC cap. If the water table elevation exceeded 12 cm above the soil surface, cap extensions of 50 cm long and 28 cm in diameter made from high-density polyethylene were temporarily fitted to the collar to avoid water intake into the analyzer. Measurements at all 72 collars were taken within a four-day period between the hours of 10:30 and 17:30 (EDT) to reduce variation in insolation. Measurements were not collected on rainy days or when more than 6.4 mm of precipitation fell within the previous 8 hours as described by Van Grinsven et al. (2018).

Soil moisture, water depth (cm) at each collar location, soil temperature (°C) and air temperature (°C) were also recorded at each collar location during every measurement occasion. A Delta-T Devices HH2 moisture meter connected to an ML3 ThetaProbe was used for soil moisture measurements. The moisture meter was calibrated using a linear regression of the volumetric water content (calculated off-site using bulk density) over observed moisture readings (Gnatowski et al. 2018). To quantify insolation, canopy openness was measured at each collar pair once in late summer before leaf-off using a digital camera at 1.5 m above the soil surface and fish-eye lens (Nikon Fisheye Converter FC – E8 0.21x) and gap-light analyzing software (Gap Light Analyzer version 2.0). Precipitation data for the sites were obtained from the National Oceanic and Atmospheric Administration's National Centers for Environmental Information (NOAA-NCEI) station in Ironwood, Michigan.

While traditional methane measurements using a gas chromatograph are typically 60 – 90 min measurements (Oertel et al. 2016), the UGGA used for this study allows the user to watch the changing slope of the concentration measurement in real time on a wificonnected screen. We concluded during preliminary testing that three minutes was long enough to detect a visible slope change in CH_4 concentration. The brief sampling period also allowed us to get more sample points per site per 4-day measurement window.

Gas flux estimations were calculated using the following equation:

$$CO_2\,Flux = \frac{dCO_2}{dt} \times \frac{PV}{ART}$$

where CO_2 is the gas species (interchanged with CH_4 for CH_4 flux estimates), dCO_2/dt is the regression slope of change in concentration over time, P is the atmospheric pressure, V is the volume of air within the chamber (m^3), A is the area of soil surface within the gas collar (m^2), R is the ideal gas constant, and T is air temperature (K). Volume calculations were corrected when water tables were above the soil surface within the collar and when cap extensions were used. Flux calculations using the equation above were carried out using the Ecoflux package in R (Shannon 2019). Gas measurements were corrected for any measurement error caused by the UGGA with a linear regression calibration curve using standardized CO_2 and CH_4 gasses in laboratory conditions. Water levels for each site were recorded every 15 minutes (Levelogger Junior M5, Solinst Canada Ltd., Georgetown, ON, Canada) inside each site's steel monitoring well as described by Van Grinsven et al. (2017).

2.3.3.2 Total Station Surveying

In order to assess the microtopographic elevation variation within sites, a total station was used to survey randomly placed grids within each site along with the monitoring well present at each site. Grids were placed at 6 random locations within each site and each grid was 4 x 4 m with survey points every meter for a total of 25 survey points per grid or 150 points per site. Grids locations were determined using ArcGIS and were located at least 5 m within site boundaries. The points were then referenced back to the monitoring well soil surface elevation so microtopography elevation could be connected to site water level.

2.3.4 Statistical Analysis

2.3.4.1 Gas Flux Comparisons

Comparisons of CO_2 flux between treatments and microtopography location were completed using a linear mixed-effects model in R. CO_2 flux was log-transformed to better fit assumptions of normality after an initial density plot showed a right-skewed distribution. In the model, treatment and microtopography class (hummock or hollow) were fixed effects, and collar location was a random effect. Visual checks of residual plots from the model showed no violations of assumptions of normality and homogeneity of variance.

Comparisons of CH_4 flux between treatments and microtopography location required a different type of linear mixed-effects model due to a different distribution. CH_4 fluxes that were determined to be insignificantly different from zero using an f-test and the fluxes with negative values were all given flux values of zero. Negative fluxes were changed to zeros due to the fact that our sampling periods were likely too short for negative fluxes to be caused by methanotrophs, so they were highly likely erroneous and due to low CH_4 production. Along with the zero flux rates, the data was highly inflated with flux rates close to zero, so a general linear mixed model with family distribution of gamma was determined to best fit the data. The fixed effects were treatment and microtopography and collar location was a random effect. Only CH_4 fluxes > 0 were used for analysis in the model for the gamma distribution's requirement of positive values.

To test the effects of two more environmental controls on gas fluxes which have been shown to be significant in other studies (soil temperature and depth to water table), a final mixed effects model was created for CO_2 with treatment, microtopography, 5 cm soil temperature, and depth to water table (m) as fixed effects, and collar location as a random effect. We attempted to create a similar model with CH_4, but found the model to be unusable due to convergence errors.

2.3.4.2 Total Area Flux Calculations

To connect with our microtopography flux rates measured at the collars and to more accurately estimate total wetland soil gas production, each random grid point surveyed with the total station was given a microtopography bin of hummock or hollow. Grid point microtopography bin ranges were determined on a site-by-site basis. For each site, the range between the highest elevation in relation to the monitoring well and the lowest elevation in relation to the monitoring well was determined. That range was then cut into two even bins, with the lower range being "hollow" and the higher range being "hummock". Microtopography bin elevations were then cross-referenced with points that were visually estimated to be hummocks or hollows in the field (the collar locations) and the bins were found to have > 90% accuracy. By putting each grid point into a hummock or hollow group, we were able to apply the mean flux estimated from the hummock or hollow collars to the percentage of each site which was determined to be a hummock or a hollow. This flux rate with weighted microtopography fluxes factored in should better estimate the total wetland area flux by acting as a simplified proxy to depth to water table.

2.4 Results

2.4.1 CO_2 and CH_4 Fluxes

CO_2 fluxes were significantly different between treatments ($p = 0.05$) and microtopography groups ($p < 0.001$). CO_2 fluxes were higher in the control sites than the girdle sites (Figure 2.2) and higher on top of hummocks than hollows (Figure 2.3). This is in contrast to our hypothesis of girdle sites having higher CO_2 fluxes. The trend appears to be consistent even when split into individual sites (Figure 2.4). When soil temperature and depth to water table were added to the model as environmental controls, CO_2 fluxes were no longer significantly different between treatments ($p = 0.45$), but were still significantly different between hummocks and hollows ($p < 0.0001$). Throughout the growing season, CO_2 fluxes increased from June to September where they peaked (Figure 2.5) and then decreased in October. Statistical analyses could not be done on seasonal changes due to equipment malfunction in October which allowed for only one set of measurements at two sites. However, the visual trends seen in CO_2 fluxes from month to month match well with the timing of when the water level drops below and then returns to the soil surface (Figure 2.6). No obvious strong correlations between soil temperature and CO_2 flux throughout the season are visible (Figure 2.7). Soil moisture appears to follow a similar trend to water table level, but peaks in August rather than September (Figure 2.8).

CH_4 fluxes were not found to be significantly different between treatments ($p = 0.89$) or microtopography groups ($p = 0.56$). Other than June, CH_4 fluxes were consistently low throughout the growing season (Figure 2.9). CH_4 fluxes showed no obvious significant correlations with either soil moisture or soil temperature.

2.4.2 Wetland Area Flux

Soil flux weighted by percent of area in each microtopography class scaled up to landscape-scale showed a potential overestimation of mean CO_2 soil flux and a potential underestimation of mean CH_4 soil flux when microtopography is not included (Tables 1 and 2). However, the differences between flux estimates with and without microtopography were not completely consistent across all sites. Site 135 showed a potential underestimation of CO_2 soil flux when microtopography is not factored in, while site 119 showed a potential overestimation of CH_4. Overall, sites had higher estimated amounts of hollow area than hummock area (Tables 1 and 2).

2.5 Discussion

2.5.1 Microtopographic Influence on GHG Fluxes

2.5.1.1 CO_2 Flux

The CO_2 results support our hypothesis of higher fluxes on top of hummocks, and can most likely be attributed to depth to water table. Compared to our other measurements (5 cm soil temperature in Figure 2.8 and soil moisture in Figure 2.7) water levels had the closest matching trend to CO_2 flux increases and decreases throughout the growing season (Figure 2.6). The CO_2 treatment results, however, do not support our original hypothesis and also contrast the findings from Van Grinsven et al. (2018), who found higher CO_2 gas fluxes in the girdle sites than the control sites. Depth to water table is likely a partial explanation for this result as well. However, they did note that a substantial amount of CO_2 fluxes may have been attributed to the decomposition of fine roots following initial treatments. There is a possibility that these roots are still decomposing, but the loss of root respiration is very likely outweighing any amount of CO_2 being released from further decomposition.

Furthermore, Van Grinsven et al. (2018) found that at our specific sites, depth to water table was one of the site characteristics which gas fluxes were most sensitive to. The importance of water table depth at our sites is important to note because this was a particularly dry summer. During the previous study, the precipitation recorded was 25% higher in 2013 and 15% higher in 2014 than the 30-year normal (Van Grinsven et al. 2018). The 30-year precipitation normal for the growing season (June, July, August) in Ironwood, MI, is 28.27 cm. In 2019, the total precipitation for the growing season was 21.01 cm, which is almost 25% less than the 30-year normal (Arguez et al. 2012). The precipitation was not only lower than the 30-year normal, but water levels were also significantly lower during this study than in 2013 and 2014 during the Van Grinsven et al. (2018) gas flux study, as you can see when looking at the lower tenth percentile of water levels measured (Figure 2.10). These conditions likely caused a larger difference between treatments in amount of time water levels were above the soil surface (Figure 2.5). The higher CO_2 in control sites suggest that water levels must have a higher influence at our sites on overall soil gas release than soil temperature differences caused by canopy openness due to the simulated EAB disturbance. The larger influence of water levels during this growing season may be explained by standing water's ability to decrease soil temperatures (Van Grinsven et al. 2018). We postulate that during high precipitation years, water levels and their impacts between treatments would be similar, meaning canopy openness could make girdle sites have higher soil temperatures and therefore higher CO_2 fluxes. During low precipitation years, however, the lack of excess soil water allows for warmer conditions in the control sites, causing the control sites to have higher CO_2 fluxes. .

Other studies such as Alm et al. (1999) have also found that drier soil conditions including those found on hummock microtopography sites produce more CO_2 when compared to wetter microtopography sites, further supporting the importance of water levels on gas fluxes. Similarly, Dinsmore et al. (2008) found that lowered water tables increased CO_2 fluxes as well, and Gutenberg et al. (2019) reported decreased CO_2 emissions as soil moisture increased. Hummocks create soil microclimates which are raised higher than the rest of the ground surface, meaning they have lower soil moisture and water levels than hollows (Table 3). These drier conditions create warmer, oxic soils which release increased amounts of CO_2. Further support for this idea can be seen in Jauhiainen et al. (2005) and Kim et al. (1992), who both found higher CO_2 release on hummocks and lower CO_2 release in hollows, and attributed these results to water table level. Lastly, although we did not statistically measure temporal variation, the trend seen in the boxplot figures of a peak in CO_2 flux during mid-summer matches the trends seen other studies as well, which commonly found a peak CO_2 flux in July and August when water levels were deepest below the soil surface (Alm et al. 1999; Gutenberg et al. 2019).

2.5.1.2 CH_4 Flux

The response of CH_4 differed from what we had originally hypothesized. The lack of difference between treatments or microtopography, and the overall low output of CH_4 can most likely be attributed to the low precipitation for the growing season of 2019. Typical CH_4 flux rates for our sites during a year of higher precipitation are closer to 6.6 mg m^{-2} d^{-1} – 11.9 mg m^{-2} d^{-1} (Van Grinsven 2018). Furthermore, Van Grinsven et al. (2018) hypothesized that the connectivity to groundwater at our sites may function to decrease the production of CH_4 as well, as the site water has more oxygen and nutrients to act as electron acceptors than if it were a precipitation-only fed system. Other studies, which showcase the importance of standing water to CH_4 production, include Alm et al. (1999) who found that their CH_4 "hotspots" were wet microsites, such as hollows or wet lawns. Dinsmore et al. (2008) also found a trend of higher CH_4 concentrations in treatments with higher water tables. Gutenberg et al. (2019) is another study which attributed much of their variations in CH_4 flux to water depth. In contrast, other studies such as Dinsmore et al. (2017) found a general decrease in CH_4 production as water table level increased, which they credited to availability of alternate electron acceptors in their water. These variable outcomes show how important site-specific factors such as water source and water chemistry can be to the response of CH_4 fluxes.

The high spikes of CH_4 in two of the sites for June were at sites where there is typically higher standing water visible at the soil surface throughout the growing season, and particularly after snowmelt (Figure 2.9). Our regional precipitation was also higher (31.62 cm) than the 30-year average (19.69 cm) for the spring months of March, April, and May. June in Michigan's Upper Peninsula is a month when the effects of snowmelt are typically still present, so there is a possibility that higher precipitation and snowmelt water levels combined with rapid drawdown of water caused these pulses. Dinsmore et al. (2008) found CH_4 pulses can happen immediately following significant water table drawdown. The high pulses of CH_4 recorded during June are also the cause for the high mean flux amounts calculated in Table 2. We estimate site 119 may have also had higher CH_4 production in early June as well because it is another site with typically higher water levels overall (Figure 2.6), but collars could not be installed in the heavily inundated part of that site until near the end of June when the surface water levels had already begun to recede. We also speculate that site CH_4 may have increased following leaf-off in October for several of the other sites when water returned to levels above the soil surface, but due to mechanical failure of the UGGA we were only able to obtain one set of measurements from two sites in the month of October. Other studies that measured CH_4 seasonal variation found that CH_4 flux typically decreases from spring to summer and then increases again in the fall (Bubier et al. 2005).

There is also a possibility that our 180-second sampling time was not long enough to capture accurate estimates of CH_4 flux, although other studies have successfully measured CH_4 for shorter periods, such as Bubier et al. (2005), who measured CH_4 for less than 20 minutes at a time and Gutenberg et al. (2019) who measured CH_4 for only 10 minutes using a UGGA. Our sampling time amount was determined to be sufficient during standards gas testing as well as early season data collection preparations. However, both of these times had higher CH_4 concentrations to be measured than during the middle of the season when no standing water was present. We postulate that if we had more measurements during periods of higher water levels there would be higher fluxes of CH_4 at hollows than in hummocks based on how many possible days water was calculated to be above or below the water's surface (Table 3), similar to the results found in other studies (Jauhiainen et al. 2005; Alm et al. 1999; Munir et al. 2014).

2.5.2 Scaling Wetland Area Flux

Extrapolating soil flux can be difficult to do using chamber measurements due to the high amounts of variation seen at small spatial scales within a wetland. There are concerns with both the seasonal and spatial variation captured in gas flux via the chamber measurements as well as the surface variation captured when surveying a site for spatial variation (Dinsmore et al. 2008). Our use of two bins (hummock or hollow) was primarily to get as many measurements as possible during the growing season while still accounting for differences in elevation. Other studies broke their microtopography down into three or four bins (Dinsmore et al. 2008; Forbrich et al. 2011). Had this study spanned multiple years, it may have been more effective to use more microtopography classifications as there would be more points in both space and time to analyze from a higher sample pool (n). However, weighted elevation flux models have been shown to be more accurate than a base model not integrating microtopography (Forbrich et al. 2011), and we speculate that even with only two bins our estimates are likely more accurate than they would be without the inclusion of microtopography.

Another point of uncertainty in flux extrapolation is the method by which you classify and scale up landscape features. While we used a grid system to get our elevation point cloud, other studies used total station transects (Alm et al. 1999) or satellite imagery (Bubier et al. 2005; Dinsmore et al. 2017). Overall, there are many possible ways to scale chamber fluxes to the landscape level, and there is currently no single accepted method on the most accurate way to do so. What our study and other similar studies show collectively is that flux estimates in these systems can be overestimated or underestimated depending on gas species if microsite influence is not accounted for (Alm et al. 1999; Bubier et al. 2005; Dinsmore et al. 2017). In systems where water table depth information is available and collars have been measured at varying relative elevations, fine-scale LiDAR data could also be included as a tool to scale-up elevation fluxes to more easily get higher accuracy estimates of total wetland flux. Bubier et al. (2005) is one such study where 3 m compact airborne spectrographic imager data was used to scale up from point measurement gas fluxes. Terrestrial laser scanning is yet another even higher detailed fine-scale measurement tool to quantify microtopography (Diamond et al. 2018), which could be used to scale up elevation-specific wetland flux rates.

2.6 Conclusions

This study highlights the difference in GHGs fluxes between hummocks and hollows and the importance of including small scale surface variability when scaling chamber gas measurements. Managers seeking to get the most accurate landscape-scale flux estimates should take into account ground-level site variation when calculating wetland-level fluxes. We also suggest that the effects of an emerald ash borer disturbance may be less influential on gas flux during dry precipitation years when a sufficient amount of time has passed since initial canopy dieoff. We found that CO_2 fluxes were highest where water level was farthest below the soil surface, such as in control sites and on hummocks. It is also likely that CO_2 fluxes were higher in control sites than in treated sites because of higher root respiration due to greater biomass of living canopy trees. CH_4 estimates were less predictable during a dry precipitation year, and while we speculate fluxes would have been higher in hollows, they may have been decreased even if there were higher water levels due to groundwater connectivity. Our results portray how the interactions between yearly climatic conditions and insect disturbance effects can change the present importance of different predictors such as depth to water table and insolation in estimating soil gas flux release.

2.7 Tables

Table 2.1 Average wetland CO_2 soil flux with and without weighted microtopography flux amount, along with percent of hummock and hollow area determined for each site. The difference is calculated from the average wetland soil flux without and with microtopography factored in.

Site	Treatment	% Hummock Area	% Hollow Area	Average Wetland Flux ($mg\ m^{-2}\ d^{-1}$)	Average Wetland Flux with Microtopography ($mg\ m^{-2}\ d^{-1}$)	Difference
135	Control	31	69	1335.3	1352.7	-17.4
152	Control	38	62	1670.6	1564.9	105.7
157	Control	25	75	1547.4	1300.9	246.5
119	Girdle	26	74	1603.4	1323.8	279.6
140	Girdle	39	61	1087.9	1016.5	71.4
151	Girdle	39	61	1257.4	1185.5	71.9
			Mean	**1417.0**	**1290.7**	**126.3**

Table 2.2 Average wetland CH_4 soil flux with and without microtopography flux amount along with percent of hummock and hollow area determined for each site. The difference is calculated from the average wetland soil flux without and with microtopography factored in.

Site	Treatment	% Hummock Area	% Hollow Area	Average Wetland Flux ($mg\ m^{-2}\ d^{-1}$)	Average Wetland Flux with Microtopography ($mg\ m^{-2}\ d^{-1}$)	Difference
135	Control	31	69	2.42	3.20	-0.78
152	Control	38	62	0.05	0.08	-0.03
157	Control	25	75	40.02	49.12	-9.10
119	Girdle	26	74	5.75	4.71	1.04
140	Girdle	39	61	15.21	16.35	-1.14
151	Girdle	39	61	0.88	1.10	-0.22
			Mean	**10.72**	**12.43**	**-1.71**

Table 2.3 The mean percent of days that water level was estimated to be above the soil surface at hummock or hollow collars. Water level at each individual collar was determined using the 15 minute water level data at each site's monitoring well. Site 156 not shown due to an offset benchmark used during elevation surveying which would give inaccurate estimates of % of days water above or below the soil surface at that location.

Site	Collar Position	Mean % Days Water Above Soil Surface
119	Hummock	3.4
	Hollow	17.4
135	Hummock	0.0
	Hollow	14.7
140	Hummock	0.0
	Hollow	11.1
151	Hummock	1.3
	Hollow	13.0
152	Hummock	0.9
	Hollow	25.9

2.8 Figures

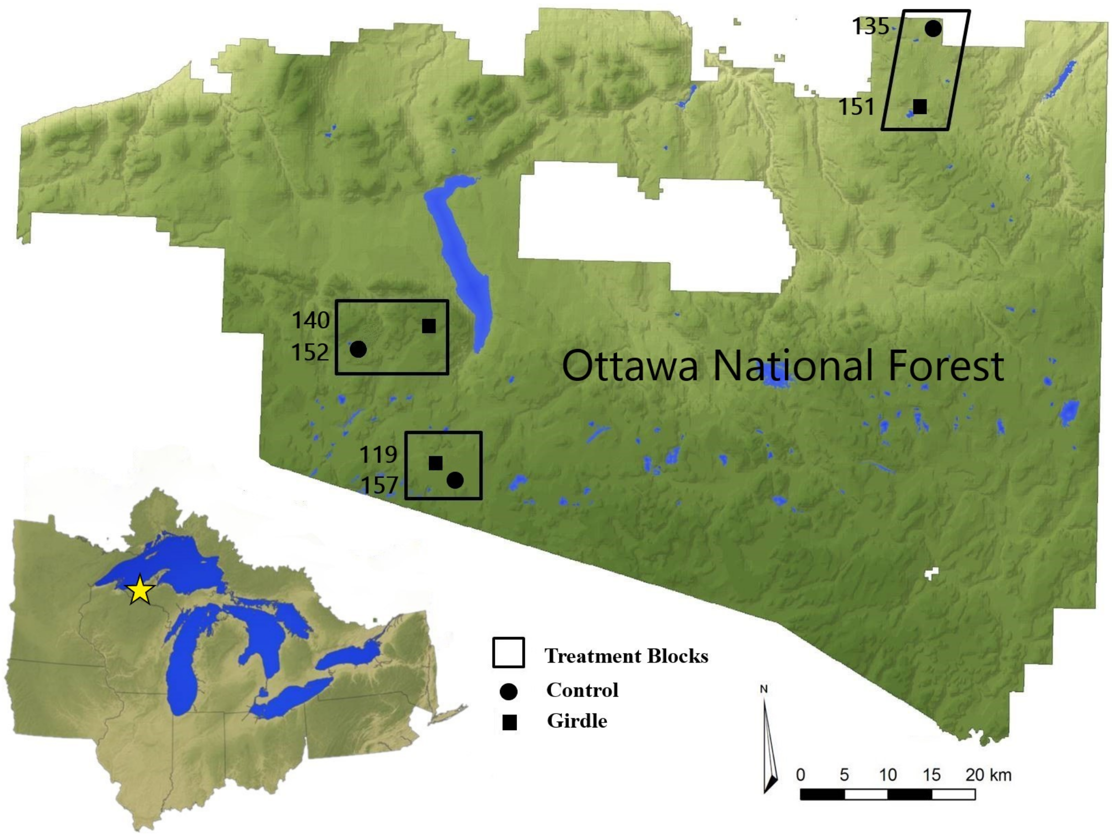

Figure 2.1 Treatment sites located in the Ottawa National Forest of Michigan's Upper Peninsula in the Great Lakes Region of North America. Each of the three geographic treatment blocks contains a control site and a girdle site.

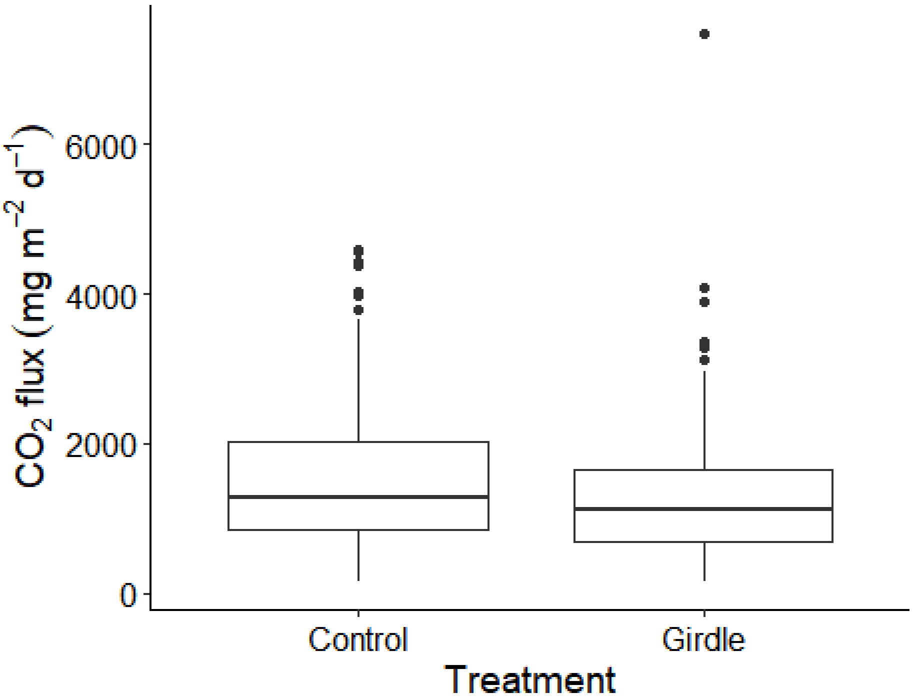

Figure 2.2 Comparison of carbon dioxide fluxes in mg/m^{2}d^1 between treatments; boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers. Control is significantly higher ($p < 0.05$) than girdle.

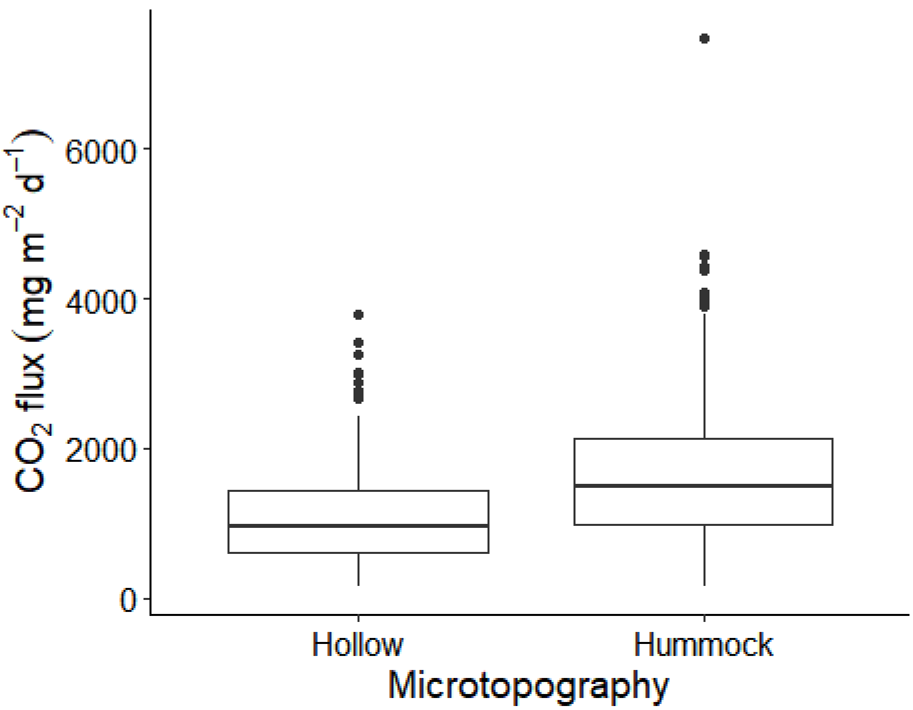

Figure 2.3 Comparison of carbon dioxide fluxes in mg/m^{2}d^1 between microtopography classifications; boxes show the 25th quartile, median, and 75th quartile, while whiskers show scores outside the middle 50% range, and dots are outliers. Hummock is significantly higher ($p < 0.05$) than hollow.

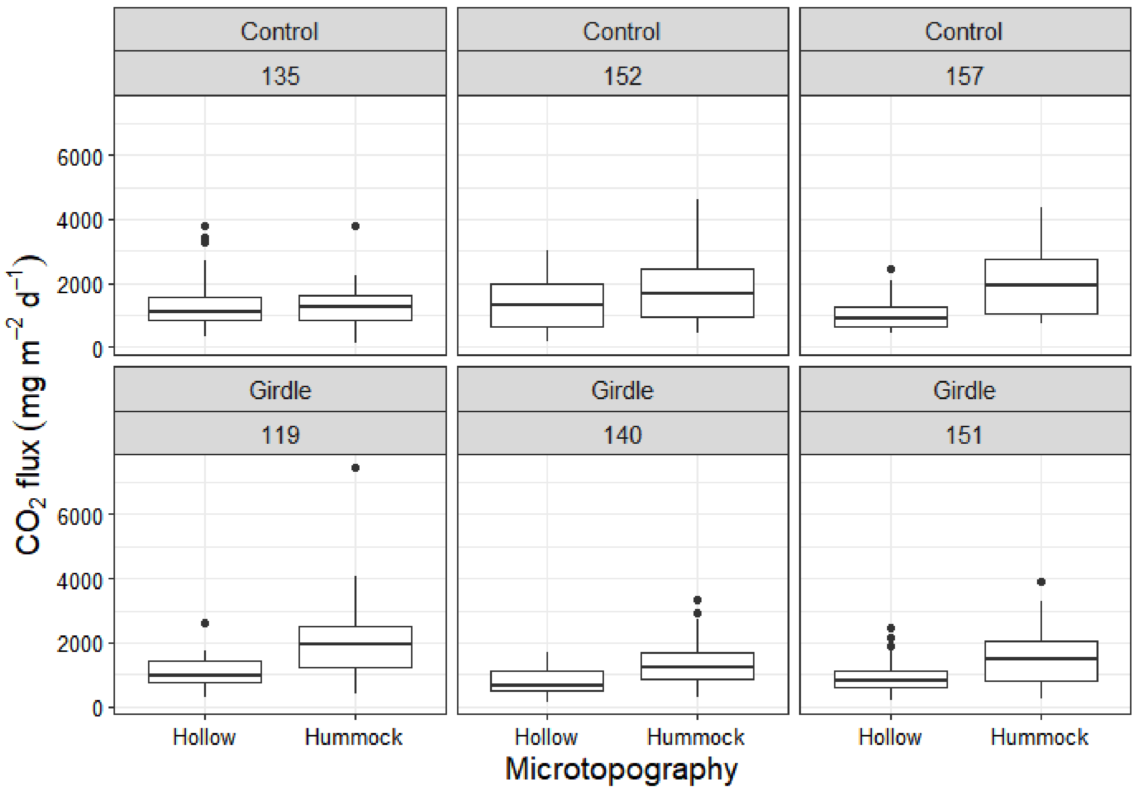

Figure 2.4 Carbon dioxide fluxes for each site along with treatment type between hummock and hollow microtopography classifications; boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers. Hummock is significantly higher ($p < 0.05$) than hollow.

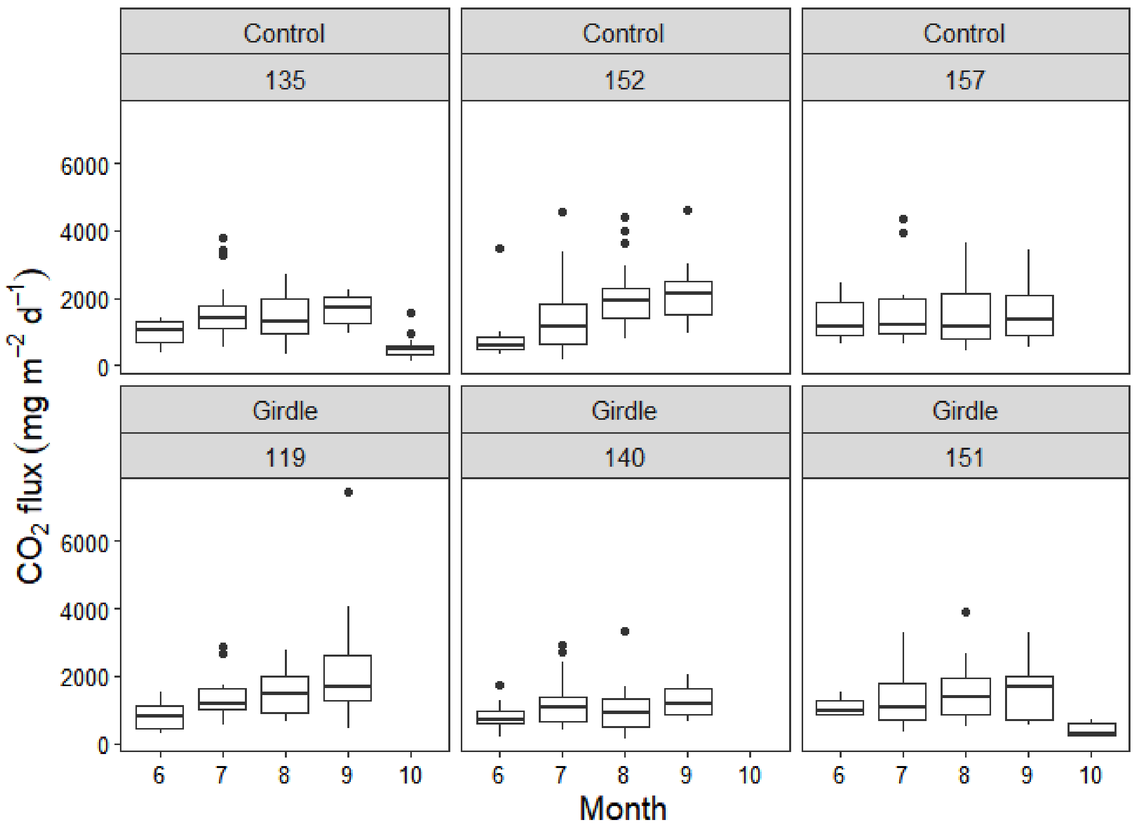

Figure 2.5 Carbon dioxide fluxes by site along with treatment type for the months of June – October; boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers. Control is significantly higher ($p < 0.05$) than girdle, but no significance calculated between months.

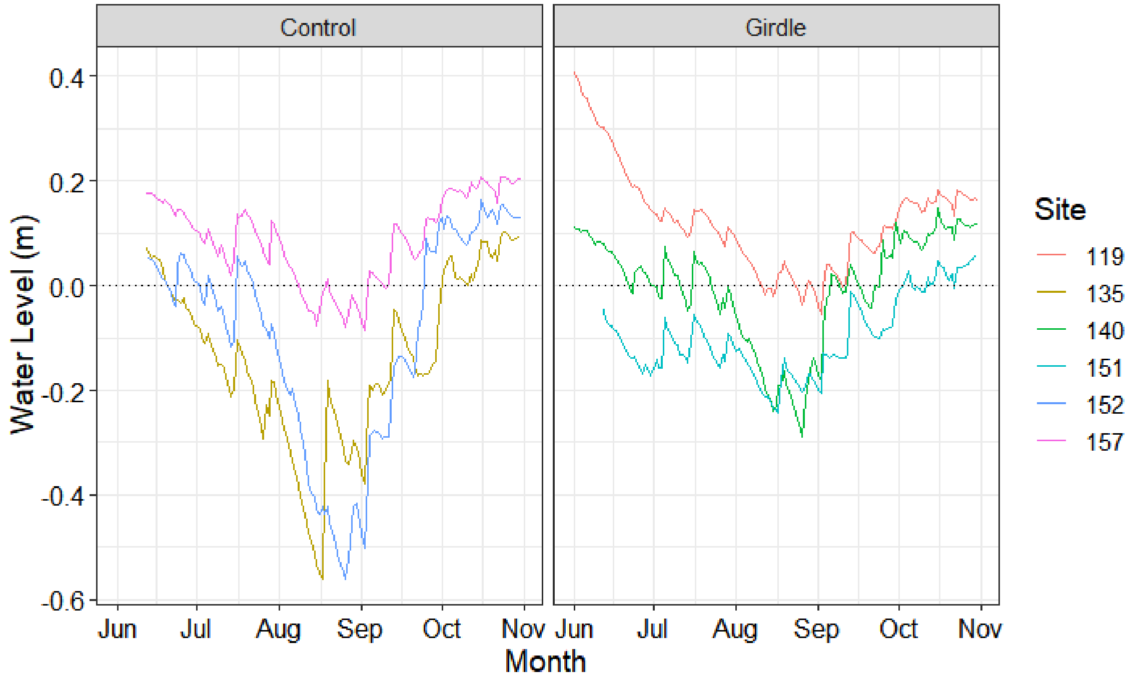

Figure 2.6 Fifteen-minute water levels by site within the control and girdle treatment groups. Water levels for each site are in reference to the soil surface at each site's individual monitoring well, which is denoted by the dashed line where water level is zero.

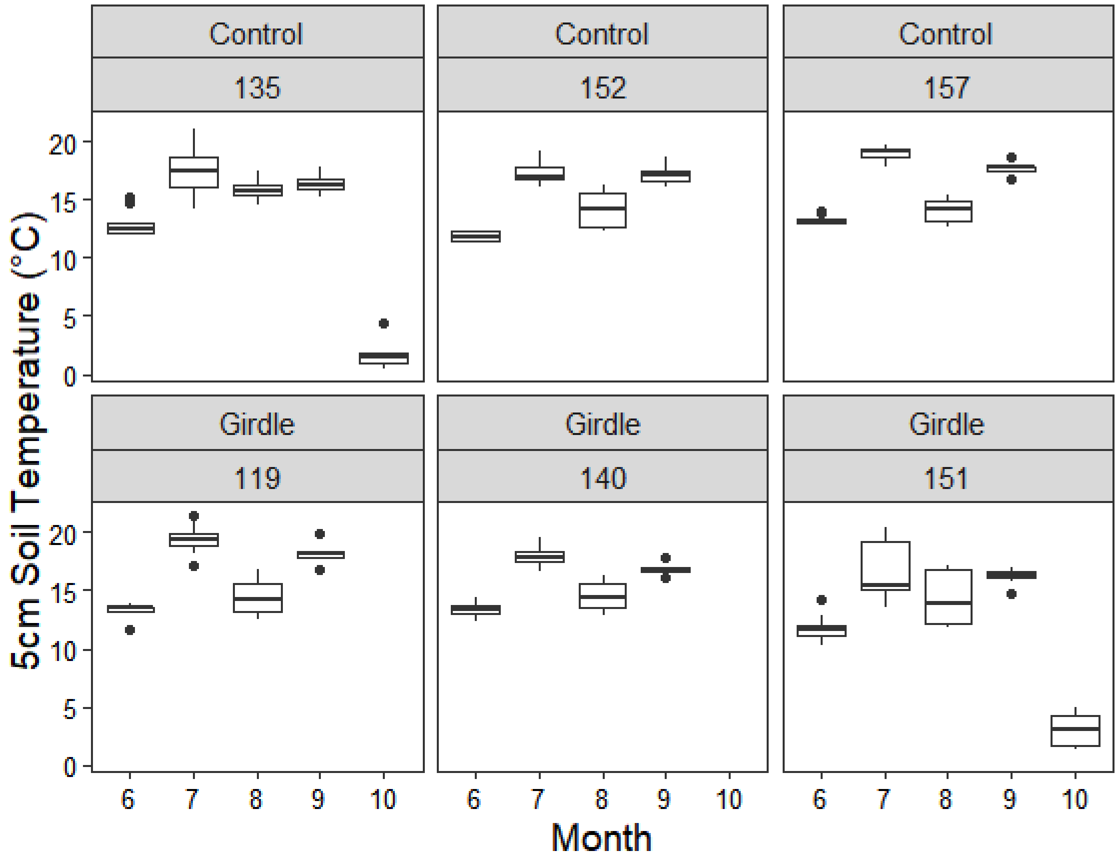

Figure 2.7 Monthly soil temperature for June – October for each site along with site treatment. Soil moisture was only available for two sites in October due to equipment failure. Boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers. No significance calculated between treatments or months.

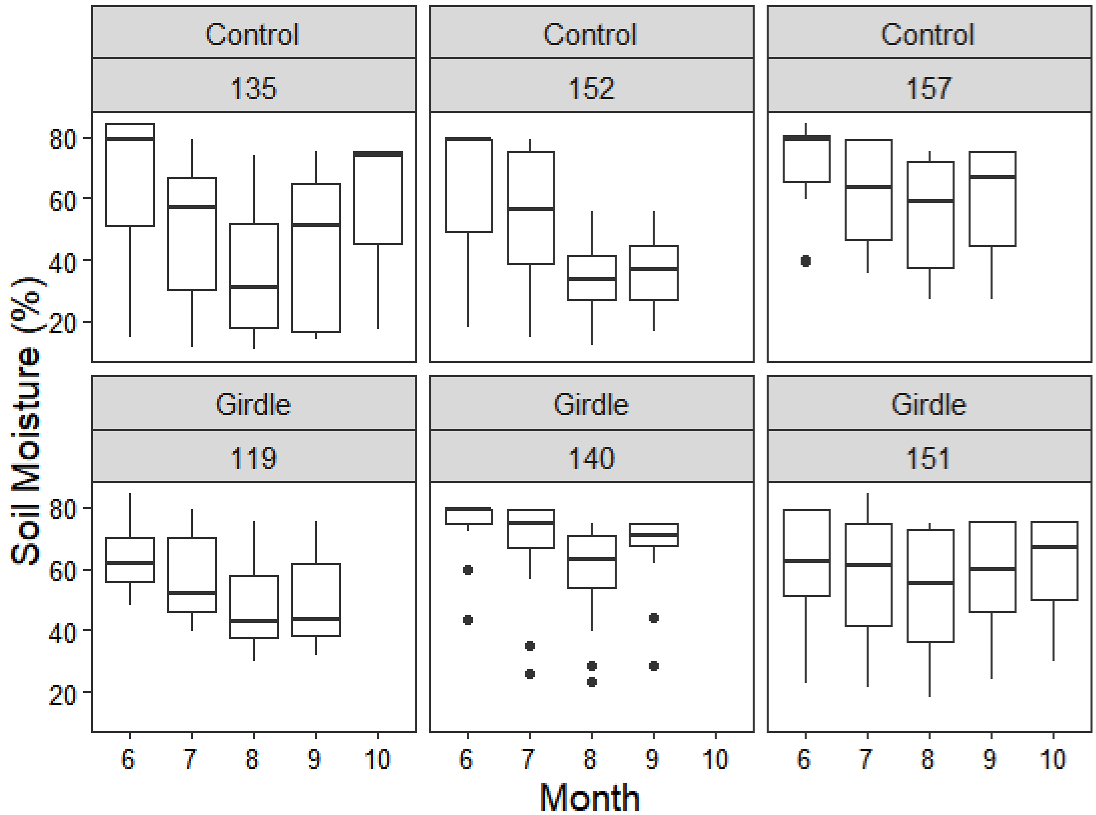

Figure 2.8 Monthly soil temperature for June – October for each site along with site treatment. Soil moisture was only available for two sites in the month of October due to equipment failure. Boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers. No significance calculated between treatments or months.

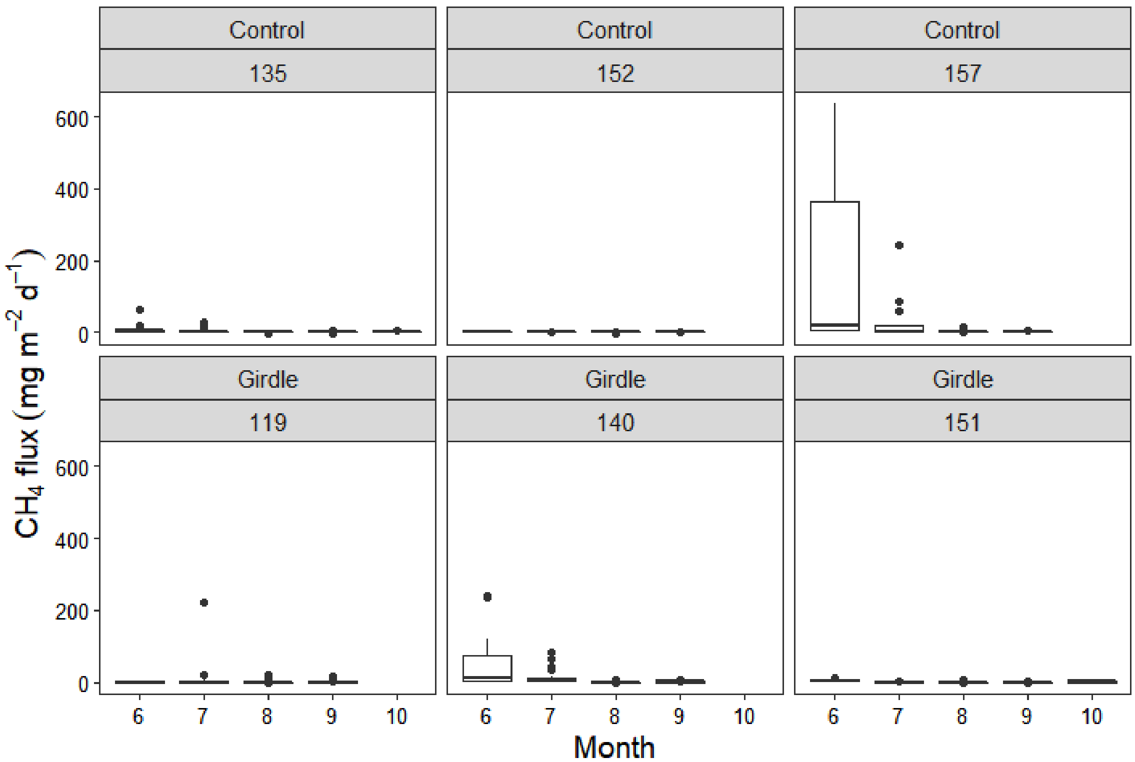

Figure 2.9 Methane flux by site along with treatment type for the months of June – October. Boxes show the 25th quartile, median, and 75th quartile, while whiskers show the interquartile range ± 1.5, and dots are outliers. No significance found between treatments.

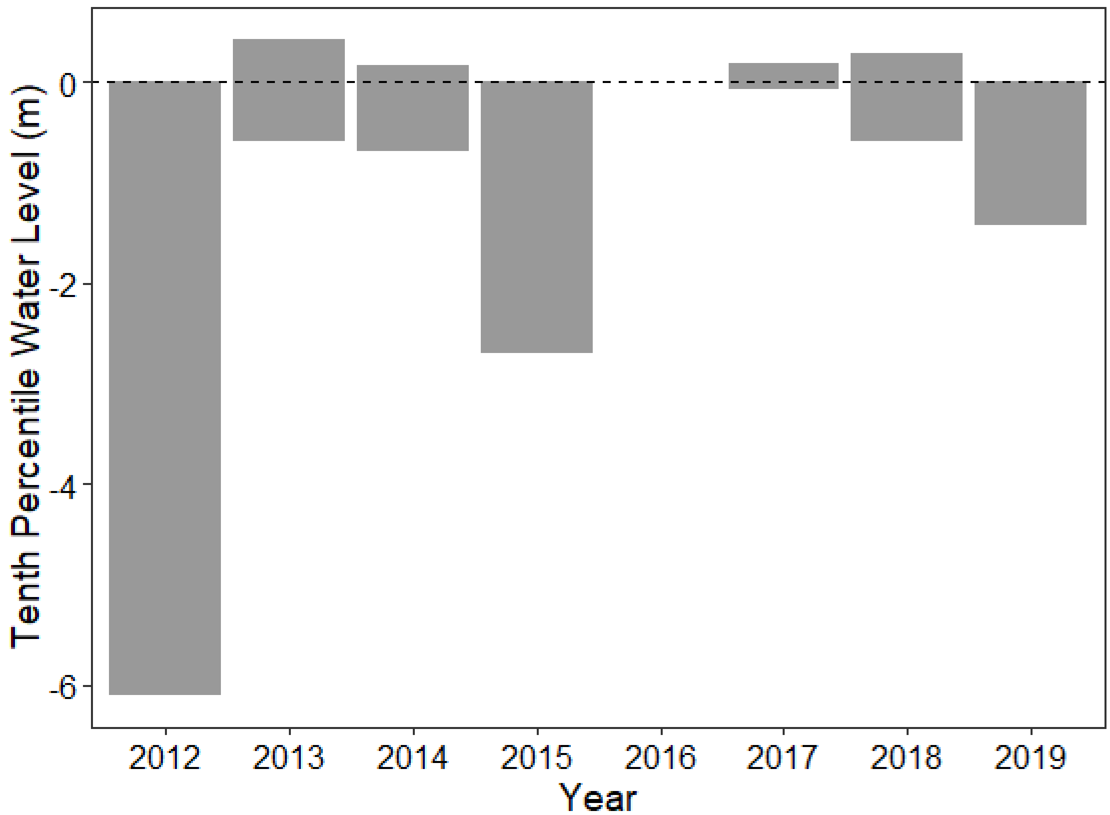

Figure 2.10 Tenth Percentile water levels in meters for all sites combined from pre-treatment year 2012 through to 2019. No data for 2016 due to equipment failure.

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