Effect of simulated emerald ash borer infestation on nitrogen cycling in black ash (*Fraxinus nigra*) wetlands in northern Minnesota, USA

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ABSTRACT

Black ash (*Fraxinus nigra*) wetlands are an important economic, cultural, and ecological resource in the northern Great Lake States, USA, and are threatened by the invasive insect, emerald ash borer (*Agrilus planipennis* Fairmmaire [EAB]). EAB-induced ash mortality can increase air temperatures, alter understory vegetation communities, and modify wetland hydrology by elevating the water table following canopy dieback, which may, in turn, alter nitrogen cycling in wetland soils and affect plant N uptake, N export to watersheds, and N emissions to the atmosphere. We sampled soils in replicated 1.6-ha plots six years after the following treatments were applied: girdling all ash greater than six cm diameter to simulate EAB-induced mortality, clearcutting with removal of all trees, and an unharvested control. In situ samples were collected from soil cores and from adjacent bulk areas influenced by vegetation to measure the concentration and rates of accumulation of inorganic nitrogen over the 2017 growing season. In the soil cores, the greatest nitrogen accumulation occurred in the unharvested control treatment and nitrate/nitrite were the dominant species of inorganic N. In contrast, in bulk soils, the clearcut treatment had greater overall nitrogen mineralization than both the control and girdle treatments, and ammonium had a greater relative abundance than nitrite/nitrate. Potential N mineralization (assessed with a lab incubation) was not affected by treatment, indicating that treatment effects observed in the field were likely due to changes in the microenvironment. Overall, the effect of simulated EAB on net nitrogen mineralization rates was minimal as mineralization was greatly constrained by ecosystem conditions compared to suitable, laboratory conditions, under which potential N mineralization was an order of magnitude greater than measured in the field. However, canopy treatment influenced water table levels, soil temperature, and vegetation communities, all of which influenced the relative abundance of inorganic N species. Our findings indicate limited indirect effects of EAB on net N mineralization in black ash wetlands.

1. Introduction

In the Great Lakes region, many deciduous wetland forests have significant components of ash (*Fraxinus* spp.) in the overstory, and are especially vulnerable to changes in composition and successional development following emerald ash borer (*Agrilus planipennis* Fairmaire [EAB]) invasion (D'Amato et al., 2018; MacFarlane and Meyer, 2005). Since its discovery in the United States in 2002, EAB has spread quickly across the country, generally top-killing all ash trees within six years of initial infestation (Herms and McCullough, 2014; Knight et al., 2013; Smitley et al., 2008). The invasion of EAB and large-scale ash dieback can decrease litter input, increase amounts of large woody debris, and cause canopy gap formation. These effects can lead to large-scale changes in ecosystem structure, food web interactions, and altered biogeochemical cycling (Kolka et al., 2018). Black ash (*F. nigra*) wetlands are especially prone to ecosystem-scale changes because ash makes up 40–100% of the canopy (Looney et al., 2015; Van Grinsven et al., 2017) and serves as a foundational species with large control on ecosystem function (Youngquist et al., 2017). Previous work has shown that simulated EAB-induced black ash mortality can alter wetland

hydrology (Slesak et al., 2014), demonstrating that large scale, rapid dieback of ash from EAB would result in an elevated water table and saturated soils following stand mortality. The resulting elevated water table, coupled with canopy loss, leads to a transition in understory vegetation from moderately dense herbaceous understory species to extremely dense sedges, grasses, and other open marsh species (Looney et al., 2015; Slesak et al., 2014). Altered hydrology has persisted with little evidence of recovery, and it is possible that ash dieback will result in loss of ecosystem function and permanent transition to an open marsh ecosystem (Diamond et al., 2018; Youngquist et al., 2017).

The microbial processes controlling nitrogen (N) cycling in wetland soils are regulated by soil moisture, soil temperature, nutrient availability, and interactions with vegetation. All of these factors are likely to change following EAB caused mortality in black ash wetlands, potentially leading to changes in N cycling and availability. Ammonification or N mineralization, the conversion of organic-N to ammonium (NH_4^+), occurs under both aerobic and anaerobic conditions, but occurs more rapidly under aerobic conditions (Vepraskas et al., 2016). Nitrification, the conversion of NH_4^+ to nitrite (NO_2^-) and then to nitrate (NO_3^-), only occurs in aerobic soils, or oxygenated zones surrounding plant roots growing in anaerobic soils (Vepraskas et al., 2016). Changes in the rate of N mineralization and the amount of inorganic N (NH_4^+, NO_2^-, and NO_3^-) available in soil affects microbial communities and N uptake essential for vegetation growth (Schimel and Bennett, 2004). Nitrogen export may also be influenced, as excess NO_3^- in wetland soils is vulnerable to gaseous loss to the atmosphere via denitrification (Toczydlowski et al., Under Review) and greater rates of N mineralization can increase N leaching from forested watersheds adversely affecting water quality downstream (Fitzhugh et al., 2003; Lawrence et al., 2000). Furthermore, black ash litter is nutrient rich, having one of the highest N concentrations among hardwood species and is decomposed more quickly than many associated species (Kolka et al., 2018). The loss of ash litter input may result in a lower quality wetland ecosystem.

Previous research has shown that N mineralization in wetland soils depends on groundwater levels. Bohlen and Gathumbi (2007) observed that nitrification dominated N cycling during the dry period of seasonally flooded pasture wetlands, ammonification dominated during prolonged flooding, and denitrification was greatest immediately following water table drawdown when soils were wet and partially aerobic. Others observed similar patterns in riparian wetlands and floodplains, and noted that NH_4^+ is the main end-product of N mineralization under waterlogged conditions (Hefting et al., 2004; Noe et al., 2013). Multiple studies have shown that rates of N cycling and removal in treatment wetlands are positively correlated with temperature (Huang et al., 2013; Kadlec and Reddy, 2001) and Pavel et al. (1996) saw a similar temperature response in a laboratory experiment using riparian wetland soils.

Field experiments simulating EAB-induced ash mortality in black ash wetlands have measured changes in water table, tree regeneration, ground layer plant communities, soil temperature, and gaseous carbon fluxes (Davis et al., 2017; Looney et al., 2015; Slesak et al., 2014; Telander et al., 2015; Van Grinsven et al., 2018, 2017), but the effects of these changes on N cycling are not clear. There are several methods for evaluating the impacts of EAB infestation on N cycling (Hanselman et al., 2004; Hatch et al., 2000; Noe, 2011). The covered-cylinder method is a simple approach to assess N mineralization in soil cores incubated in-situ while removing the influence of vegetation uptake. Nitrogen could potentially diffuse out of the open bottom of the cylinder, but this method encompasses the microclimatic and hydrologic influences at the treatment plot level (Hanselman et al., 2004). Incubating sieved, homogeneous soils in a controlled laboratory setting removes the influence of microsite conditions and allows N mineralization rates to be compared among variable soil substrates. In combination, in-situ and laboratory techniques of measuring N mineralization can be used to identify mechanisms controlling N cycling in

complex, black ash wetland ecosystems.

Many of the hypothesized impacts of emerald ash borer on forested wetlands have not been directly assessed (Kolka et al., 2018; Youngquist et al., 2017) and there is an urgent need to understand the cascading impacts of EAB on ecosystem functions such as nitrogen cycling. The objectives of this experiment were to identify effects of overstory treatments simulating EAB disturbance on inorganic nitrogen cycling rates in black ash wetland soils that were (1) influenced by vegetation under field conditions, (2) isolated from the influence of vegetation under field conditions, and (3) in a controlled laboratory incubation eliminating overstory treatment effects on the microclimate. We hypothesized that (1) N mineralization would be greatest in the girdled or clearcut treatment plots because of altered microclimate effects, (2) removing the influence of vegetation would increase N concentrations, and (3) treatment effects would be smaller, but still significant, on potential N mineralization in the laboratory because of altered substrate inputs. This experiment builds on previous research studying a critical system of feedbacks stemming from the imminent threat of emerald ash borer invasion and a subsequent cascade of ecosystem-scale changes (Looney et al., 2015; Slesak et al., 2014; Youngquist et al., 2017).

2. Methods

2.1. Study area

The study site was a black ash wetland complex located in the Chippewa National Forest in northern Minnesota, USA (N47.5°, W94°) at an approximate elevation of 380 m. The region receives 70 cm mean annual precipitation, much as snow, and has a continental climate with a mean growing season (May-Oct.) temperature of 14.3 °C (Slesak et al., 2014). Forest stands are classified as wet (type WFn55) or very wet (type WFn64) *Fraxinus* swamps based on the Minnesota habitat classification system (Aaseng, 2003) and contain poorly to very poorly drained soils with either loam to sand textures derived from glacio-fluvial parent material, or clay to silty clay texture formed from glacio-lacustrine parent material overlain with approximately 30 cm of muck (Slesak et al., 2014), primarily Morph series, frigid Typic Glossaqualfs. The forest composition is primarily black ash (75%-100% basal area) with small components of American elm (*Ulmus americana*), quaking aspen (*Populus tremuloides*), American basswood (*Tilia americana*), and bur oak (*Quercus macrocarpa*). The black ash wetlands in this region occur in flat, low regions in a gently rolling to flat, complex glacial landscape. Most of the wetlands are underlain by a hydrologic confining layer of lacustrine clay at a depth of 10–150 cm, but sandier upland soils are scattered throughout the landscape acting as groundwater recharge zones (Slesak et al., 2014).

This experiment utilizes the 1.6 ha experimental plots installed by the USDA Forest Service Northern Research Station and the Chippewa National Forest, as described in Slesak et al. (2014) and Looney et al. (2015) in the winter of 2011–2012 to study the impacts of simulated EAB disturbance on black ash wetland ecosystems. The study design was a complete block factorial with four treatments each replicated eight times. The treatments were (1) clearcut with removal of all trees, (2) group selection with 0.04 ha gaps constituting 20% of the treatment unit, (3) girdling of all black ash greater than six cm diameter to simulate EAB mortality, and (4) control (unharvested forest). All treatments were applied under frozen ground conditions resulting in virtually no soil disturbance. For this study, we utilized five replicates each of the control, clearcut, and girdle treatment plots, six years after they were installed.

Looney et al. (2017) surveyed vegetation in the treatment plots as recent as one year prior to this study. They reported significantly greater regeneration of woody species (seedlings ≤ 2.54 cm basal diameter) in the clearcut treatment than the control or girdle treatments. The clearcut, control, and girdle treatments had multi-species

mean stem counts of 22520 ± 4351 (standard error), 7629 ± 3054 and 3631 ± 700 stems ha^{-1} respectively. In all treatments, black ash composed the strong majority of regeneration with approximately 89%, 76%, and 50% black ash in the clearcut, control, and girdle treatments respectively. Other common species included *Ulmus americana, Quercus macrocarpa, Populus balsamifera, Populus tremuloides,* and *Tilia americana.* In 2015, Looney et al. (2017) measured herbaceous layer vegetation height and cover. The reported mean herbaceous layer height was significantly greater in the clearcut treatment (95.2 ± 2.5 cm) than in the girdle (76.4 ± 2.8 cm) or control (40.9 ± 1.5 cm) treatments. Herbaceous layer cover did not vary significantly by treatment with reported means of greater than 200% in all treatments. According to the results of a PERMANOVA analysis the herbaceous community composition did not differ significantly between the girdle and control treatments, but responded to the clearcut treatment (Looney et al., 2017).

2.2. In-situ experiment

Nitrogen mineralization rates were measured in-situ over the 2017 growing season (May-September) in both soil cores and in bulk soil in the treatment plots. In early May, four 3-inch polyvinyl chloride (PVC) tubes (7.7 cm inner diameter by 30 cm long) were driven into each plot to a depth of approximately 27 cm and covered loosely with PVC caps to create isolated soil cores with a similar effect to a trench plot (Hanselman et al., 2004). The tubes were inserted into hollows with similar microtopographic settings cleared of vegetation and surface litter, following an east-west transect with approximately two-meter spacing and were located near the center of each plot. Driving the cores in compacted the soil 5.7 cm on average, resulting in a lower soil surface inside the cores. Temperature sensors (iButton® Embedded Data Systems LLC, Lawrenceburg, KY, USA) enclosed in PVC capsules were buried in the center of each transect at a depth of 10 cm, and recorded soil temperature at 4-hour intervals for the duration of the experiment.

Soils were sampled monthly from May to September. The first sample, collected in May, was a bulk soil sample collected when the cores were installed, providing a pre-field incubation baseline. The cores were then sampled sequentially from east to west, with one entire core collected from each transect (plot) and taken to the lab for extraction in June, July, August, and September. For comparison, bulk soils, which were not isolated within cores and therefore subject to vegetation uptake and precipitation inputs, were also sampled in May, July, and September approximately one meter north of the sampled core. Bulk soil samples were collected and analyzed at the 0–5 cm, and 10–15 cm soil depth. Based on preliminary sampling, these increments were chosen in an attempt to characterize the organic soil horizon at the surface and the sandy to clayey mineral soil beneath it. However, on average the organic horizon depth across all samples was 9.7 cm (n = 30) and extended into about one-third of the 10–15 cm depth samples.

Each month, soil samples were collected from the plots and immediately processed in the laboratory. The soils were homogenized by hand and 10.0 g subsamples were measured into sealed jars, refrigerated overnight and extracted the following day with 2 M KCl for analysis of extractable NH_4^+-N (referred to as NH_4^+) and the sum of NO_2^--N + NO_3^--N (referred to as NO_{2+3}) using a Lachat QuikChem 8500 series 2 flow-through analyzer (Hach Company, Loveland, CO) and standard colorimetric methods (Mulvaney, 1996). For both depth increments of each sample, 10.0 g of field-wet soil was shaken with 40.0 ml 2 M KCl for one hour, allowed to settle for one hour, then the solution was decanted and filtered using Whatman no. 42 filters. Six random soils (three from each depth) were extracted in duplicate, and four blanks (2 M KCl) were included in each set of extractions (n = 40). The extracts were frozen in 20-ml poly scintillation vials until analysis at the conclusion of the experiment. Soil water content was determined by oven-drying an additional 20–30 g wet soil at 105 °C for 30 h.

2.3. Laboratory experiment

In August, soil samples were collected at 0–5 cm and 10–15 cm depths from two hollows with similar microtopographic settings near the core transects in each plot and composited by depth. The soil was air-dried, sieved through 2 mm mesh, and used for a soil incubation assessing potential rates of net nitrogen mineralization. The experiment used pairs of 10.0 g subsamples of soil from both depths within the 15 treatment plots. For both soil depths, three plots were randomly selected and run in duplicate to assess within-sample variation. From each pair (n = 36), one subsample was used for a pre-incubation analysis and one following incubation. Each pre-incubation subsample was extracted with 2 M KCl using a similar procedure as above. For the 0–5 cm samples, which were organic soils, 80.0 ml of 2 M KCl was used per 10.0 g soil due to the absorbance of the dry soil. The remaining subsamples were incubated for 14 days at 20 °C with optimal water content. Due to variability in soil texture, a different water content was selected for sandy (n = 6), clayey (n = 4), and organic soils (n = 20) based on textural analysis and visual assessment starting with mean field soil moisture contents and adjusting until the soil was completely wetted, but with no glistening water. All 15 of the 0–5 cm samples were considered organic as well as five of the 10–15 cm samples; the remaining 10–15 cm samples were considered either sandy or clayey. We added 8.0 ml, 3.0 ml, or 2.0 ml of deionized water to 10.0 g of air-dried organic, sandy, or clayey soil, respectively, corresponding to average water contents of 96%, 21%, and 36% respectively. The wetted soils were incubated in 8-oz glass jars with a large headspace and sealed lid to limit evaporation (Breuillin-Sessoms et al., 2017). Each jar was opened for approximately three minutes every few days to refresh the air in the headspace and maintain aerobic conditions. The jars were incubated in a temperature-controlled chamber set at 20 °C in the dark. Four additional jars were positioned throughout the chamber with temperature sensors (iButton® Embedded Data Systems LLC, Lawrenceburg, KY, USA) sealed in them, recording at 15-minute intervals. The mean temperature for the incubation was 20.3 °C. Due to evaporation, the soils had an average 2.0% reduction in moisture content over the incubation. Following the experiment, 80.0 ml or 40.0 ml 2 M KCl was added directly to each incubation jar for organic and mineral soil respectively, the soils were extracted using the method described above, and the extracts frozen until analysis. Each soil was also analyzed for total C and N on a 1-g pulverized subsample with dry combustion using a LECO Dumas combustion technique on a Fisons NA1500 NCS Elemental Analyzer (ThermoQuest Italia, Milan, Italy).

2.4. Data analysis

All statistical analyses were conducted using R statistical software (R Core Team, 2017) with an alpha level of 0.1. Ammonification (NH_4), nitrification (NO_{2+3}), and total net mineralization (total inorganic N [TIN]) were each analyzed separately by soil depth. For the field samples, N concentration was analyzed using a mixed effects repeated measures analysis from the 'nlme' R package with an auto regressive level (1) (AR[1]) covariance matrix, including overstory treatment and sample month as fixed effects and treatment block as a random effect (Pinheiro et al., 2017). Nitrogen concentration was log transformed to stabilize variance based on results from the "powerTransform" tool in the 'car' R package (Fox and Weisberg, 2011). When significant treatment effects were identified using a two-way ANOVA, significant differences were tested with an LSM test from the 'lsmeans' R package (Lenth, 2016). For the soil incubation lab experiment, N accumulation rates were calculated directly as the change in concentration over the experiment and compared among overstory treatments using a one-way ANOVA.

Total time-integrated inorganic nitrogen accumulation was also calculated for both the field and lab experiments using trapezoidal integration of the un-transformed N concentrations versus time as done in

previous studies (Alexander et al., 2019; Engel et al., 2010). The time-integrated values were log-transformed to stabilize variance. Overstory treatment effects were tested within each soil depth using a one-way ANOVA and identified with an LSM test when significant (Lenth, 2016). For easier visual comparison between the in-situ and laboratory results, the time-integrated values were normalized to the incubation duration period by dividing by the number of days in the experiment, 124 days and 14 days for the in-situ and lab experiments respectively.

Total soil carbon and nitrogen contents of the soil used for the lab incubation were compared among overstory treatments within depths, and among depths within overstory treatment using one-way ANOVA's. When identified, significant treatment effects were tested with an LSM test (Lenth, 2016).

3. Results

3.1. Soil cores

In the soil cores, uninfluenced by vegetation, there were no significant interaction (treatment by month) effects, but there were significant main (overstory treatment) effects for NH_4^+ in the 10–15 cm depth, NO_{2+3} in both depths, and TIN in the 0–5 cm depth (Table 1). In cases where treatment effect was significant, the control treatment had 2.7–6.8 times greater NO_{2+3} than the clearcut treatment at both depths and TIN at the 0–5 cm depth (Fig. 1). In contrast, the clearcut treatment had 2.5 times greater NH_4^+ than the control treatment in the 10–15 cm depth. The girdle treatment did not differ from either the control or clearcut except for NO_{2+3} in the 0–5 cm depth where the girdle treatment was 2.8 times greater than the clearcut treatment but did not differ significantly from the control treatment.

When evaluating total inorganic N accumulation (time-integrated values) in the soil cores uninfluenced by vegetation over the study, the control treatment accumulated six times more NO_{2+3} and 2.5 times more TIN than the clearcut treatment at 0–5 cm depth, but did not differ significantly from the girdle treatment in either case (Table 2). In the 10–15 cm depth, total NO_{2+3} accumulation was four to seven times greater in the control than in the clearcut and girdle treatments, but NH_4^+ and TIN accumulation did not differ among treatments.

3.2. Bulk soils

In the bulk soil analysis (soils influenced by vegetation), there were

no significant interaction effects and overstory treatment effect was a significant factor only for NH_4^+ (Table 1). In that instance, N concentrations in the clearcut treatment at both depths were 5.5 to 14 times significantly greater than the control treatment (Fig. 2). In addition, NH_4^+ in the 0–5 cm depth was 2.6 times significantly greater in the girdle treatment than the control treatment; the girdle and clearcut treatments did not differ. In general, inorganic N concentrations increased over the growing season (Fig. 2); however, since there were no significant treatment by month interaction effects, rates of N accumulation did not differ by overstory treatment. In the total inorganic N accumulation data (Table 2), significant differences among treatments were only observed in the bulk soil for NH_4^+ at the 10–15 cm depth, where the clearcut treatment accumulated approximately 15 times more NH_4^+ than the control treatment, but did not differ from the girdle treatment. Total N accumulation did not differ significantly among treatments in the 0–5 cm depth bulk soils.

3.3. Laboratory experiment

In the 14-day laboratory incubation experiment, overstory treatment was not a significant predictor of N accumulation rates (Table 1). Under the controlled conditions of the experiment, there was no difference in N accumulation rates among soils from different overstory treatments (Table 3). Rates of NH_4^+ accumulation were generally about four to 12 times greater in the 0–5 cm soil than the 10–15 cm soil. Concentration of NO_{2+3} decreased over the experiment in the 0–5 cm soil, and only increased minimally in the 10–15 cm soil. There was no significant effect of overstory treatment on total inorganic N accumulation in the incubated soils; however, the N accumulation over the 14-day lab incubation experiment was an order of magnitude greater than accumulation over the entire growing season in the field (Table 2).

3.4. Total soil C and N

The soils collected in August and used for the lab incubation experiment had 32.1% (2.1% standard error) and 7.8% (2.1% SE) total soil C for the 0–5 cm and 10–15 cm depths, respectively. Total soil N was 2.3% (0.2% SE) and 0.5% (0.1% SE) for the 0–5 cm and 10–15 cm depths, respectively. Both total C and total N differed significantly by depth and total N differed among overstory treatments only at 10–15 cm depth where the control treatment was significantly greater than the girdle treatment ($p = 0.07$) but the clearcut treatment did not

Table 1

N analysis ANOVA results: Two-way ANOVA results summary for soil N concentration in in-situ experiments based on mixed effects repeated measures analysis with overstory treatment (OT) and sample month as fixed effects, one-way ANOVA results summary for laboratory experiment N accumulation rates, and one-way ANOVA results for total cumulative N mineralization for all experiments (values shown in Table 2). The numerator degrees of freedom (DF) and p-values are presented for each model term.

| Factor | DF | NH_4^+-N | | NO_2^- + NO_3^--N | | Total Inorganic N | |
| | | p-value | | p-value | | p-value | |
		0–5 cm	10–15 cm	0–5 cm	10–15 cm	0–5 cm	10–15 cm
In-situ Experiment: Soil Cores							
Overstory Treatment	2	0.5061	0.0066	0.0116	0.0507	0.0601	0.3390
Month	4	0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
OT:Month	8	0.1708	0.7119	0.2858	0.1511	0.4920	0.1253
Overstory Treatment (Cumulative N-Min)	2	0.5800	0.2160	0.0027	0.0560	0.0092	0.2875
In-situ Experiment: Bulk Soil							
Overstory Treatment	2	0.0348	0.0340	0.7672	0.5800	0.2038	0.2719
Month	2	0.9962	0.0100	< 0.0001	0.0001	0.0007	0.0035
OT:Month	4	0.2893	0.1835	0.6917	0.3800	0.3519	0.1656
Overstory Treatment (Cumulative N-Min)	2	0.1093	0.0157	0.5675	0.6234	0.1978	0.4249
Laboratory Experiment							
Overstory Treatment	2	0.8636	0.1386	0.9065	0.2245	0.8953	0.1519
Overstory Treatment (Cumulative N-Min)	2	0.8636	0.1386	0.9065	0.2245	0.8953	0.1519
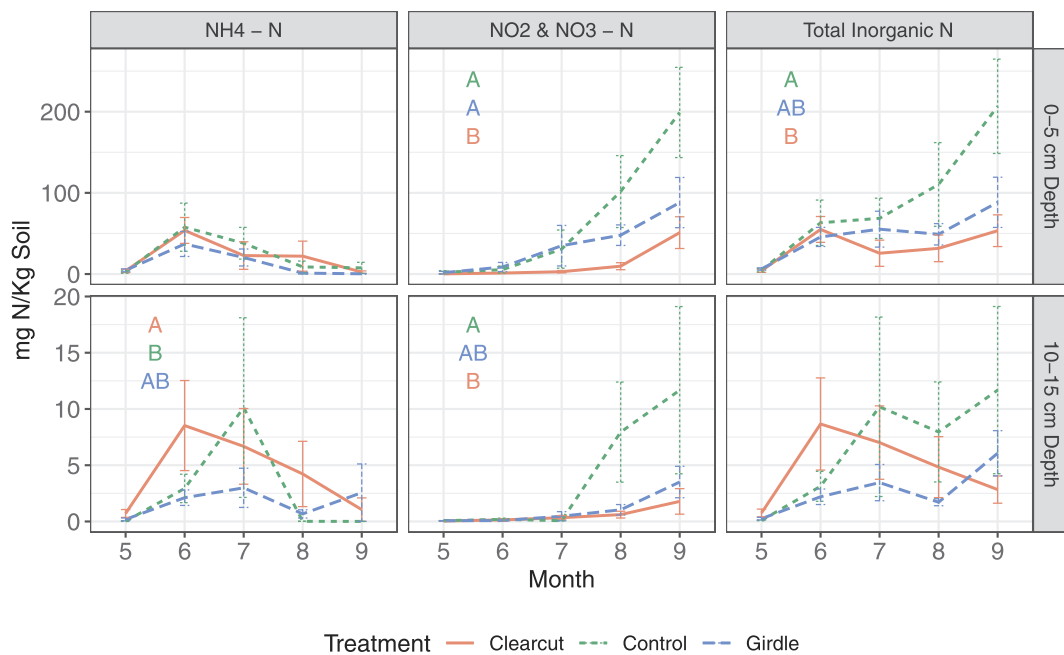
Fig. 1. In-situ inorganic nitrogen concentration observed in the soil cores, not influenced by vegetation, from May to September 2017. Uppercase letters indicate significant differences among overstory treatments in panels with significant treatment effects (p < 0.1). There were no significant overstory treatment by month interaction effects. Note the large difference in scales between soil depths.

Table 2

Time-integrated inorganic nitrogen accumulation (mg N kg^{-1}) calculated via trapezoidal integration of the N concentration curve and normalized by number of days in experiment (124 days for in-situ and 14 days for lab). Standard error shown in parentheses. Superscript letters indicate significant differences (p < 0.1) among overstory treatments within a depth.

Soil Depth	Treatment	NH$_4^+$-N	NO$_2^-$ + NO$_3^-$-N	Total Inorganic N
In-situ Experiment: Soil Cores				
0–5 cm	Control	26.49 (7.7)	62.94 (11.1)[a]	89.42 (11.3)[a]
	Clearcut	24.79 (9.8)	10.27 (1.9)[b]	35.05 (10.9)[b]
	Girdle	14.47 (5.3)	35.64 (11.2)[a]	50.12 (7.4)[ab]
10–15 cm	Control	3.28 (1.9)	3.75 (1.5)[a]	7.04 (3.0)
	Clearcut	5.02 (1.5)	0.52 (0.1)[b]	5.54 (1.5)
	Girdle	1.78 (0.7)	0.88 (0.3)[b]	2.67 (0.6)
In-situ Experiment: Bulk Soil				
0–5 cm	Control	1.14 (0.4)	5.39 (0.9)	6.53 (0.7)
	Clearcut	7.50 (2.5)	4.29 (0.6)	11.79 (2.6)
	Girdle	3.82 (1.3)	6.42 (1.8)	10.24 (2.8)
10–15 cm	Control	0.06 (0.05)[a]	0.64 (0.2)	0.70 (0.2)
	Clearcut	0.93 (0.4)[b]	0.65 (0.3)	1.58 (0.6)
	Girdle	0.15 (0.06)[ab]	0.52 (0.3)	0.67 (0.3)
Laboratory Experiment				
0–5 cm	Control	206.9 (14.6)	−5.8 (14.8)	201.1 (9.6)
	Clearcut	205.9 (13.6)	−10.8 (9.1)	195.1 (11.4)
	Girdle	218.4 (36.0)	−13.5 (26.1)	204.9 (20.7)
10–15 cm	Control	23.7 (3.9)	7.2 (4.4)	30.9 (8.0)
	Clearcut	46.9 (16.8)	6.9 (3.1)	53.8 (19.2)
	Girdle	17.9 (8.3)	1.0 (1.6)	18.9 (7.0)

differ from either. There was 0.76% (0.3% SE), 0.44% (0.2% SE), and 0.21% (0.1% SE) total N at 10–15 cm depth in the control, clearcut, and girdle treatments, respectively. Total soil C did not differ among treatments in the 0–5 cm depth (p = 0.48) or the 10–15 cm depth (p = 0.12) soil.

3.5. Soil temperature and moisture

The study-wide mean soil temperature was 14.4 °C (2.1 °C standard error) at 10 cm depth. On average, the control treatment had the greatest mean soil temperature, significantly warmer than the clearcut and girdle treatments (Table 4). The control treatment also had significantly warmer soil temperatures in July and the greatest maximum recorded soil temperature. The clearcut plots had the coolest temperatures during the 2017 growing season.

The saturated muck surface soils had high soil moisture. Mean soil moisture at 0–5 cm depth in the in situ soil cores, as measured during sampling, was 424% by mass (18% standard error), consistently decreasing from 561% (42% SE) in May to 312% (21% SE) in September. Based on a two-way ANOVA with canopy treatment and sample month as factors, the clearcut treatment was significantly more wet than the girdle or control treatments (p = 0.01) and the girdle and control treatments did not differ (Toczydlowski, 2018). The mean soil moisture for the clearcut, girdle, and control treatments was 490% (27% SE), 397% (29% SE), and 384% (33% SE). At 10–15 cm depth, mean soil moisture was 63% (7% SE) and remained relatively stable from May through September with no significant treatment effects present.

4. Discussion

Black ash wetland ecosystems are especially threatened by the impending invasion of emerald ash borer because of the regulatory role ash-dominated canopies have on local groundwater levels via transpiration (Telander et al., 2015). Field experiments indicate that the loss of ash from the canopy will also result in warmer air temperatures and drastic changes in understory vegetation communities (Davis et al., 2017; Looney et al., 2017; Slesak et al., 2014; Van Grinsven et al., 2017). We expected that the elevated water table, increased temperature, lack of ash litter fall, and dense sedges and grasses in the girdle and clearcut treatments would alter rates of inorganic N cycling in the wetland soils. The results do suggest clearcutting depresses N mineralization, but the effect is only manifested when plant uptake is removed. Observed changes in the field were largely associated with differences in N forms among treatments, likely due to changes in the microenvironment. Overall, wetland ecosystem conditions depressed rates of inorganic N cycling compared to the controlled conditions of the laboratory incubation experiment.

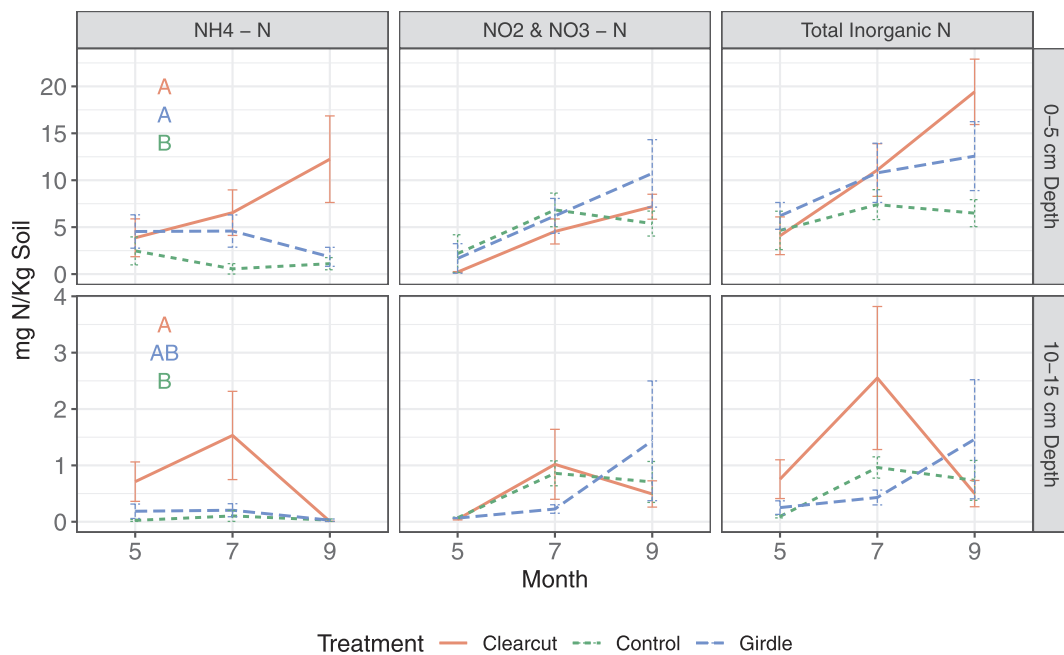Contradictory to our first hypothesis (1), nitrogen concentrations in

Fig. 2. In-situ inorganic nitrogen concentration observed in bulk soil in May, July, and September 2017. Uppercase letters indicate differences among overstory treatments in panels with significant treatment effects (p < 0.1). There were no significant overstory treatment by month interaction effects. Note the large difference in scales between soil depths.

Table 3

Inorganic N accumulation rates (mg N Kg^{-1} day^{-1}) measured in the laboratory incubation experiment. Standard error shown in parentheses. There are no significant treatment effects within either soil depth.

Soil Depth	Treatment	NH_4^+-N	NO_2^- + NO_3^--N	Total Inorganic N
0–5 cm	Control	29.4 (1.9)	−1.5 (1.3)	27.9 (1.6)
	Clearcut	29.6 (2.1)	−0.8 (2.1)	28.7 (1.4)
	Girdle	31.2 (5.1)	−1.9 (3.7)	29.3 (3.0)
10–15 cm	Control	6.7 (2.4)	1.0 (0.4)	7.7 (2.7)
	Clearcut	3.4 (0.6)	1.0 (0.6)	4.4 (1.1)
	Girdle	2.6 (1.2)	0.1 (0.2)	2.7 (1.0)

Table 4

Soil temperature (°C) at 10-cm depth monitored 5/11/17 through 9/18/17. Superscript letters indicate significant differences (p < 0.1) based on one-way ANOVA and LSD test. One temperature sensor malfunctioned in a girdle plot.

Treatment	Growing Season Mean	July Mean	Growing Season Max
Control (n = 5)	14.80[a]	16.28[a]	20.50
Clearcut (n = 5)	13.95[b]	15.72[b]	18.75
Girdle (n = 4)	14.28[c]	15.75[b]	18.00
Study-Wide Mean	14.35	15.93	

the in situ soil cores, uninfluenced by vegetation, indicated greater rates of inorganic nitrogen accumulation in the control treatment compared to the clearcut and girdle treatments (Table 2, Fig. 1). These soils were isolated from the effect of vegetative roots and precipitation, but still experience the temperature and groundwater regimes created by the overstory treatments simulating EAB disturbance. When the effects of vegetation are excluded, nitrogen cycling is controlled by microbial communities, which are driven primarily by C and nutrient availability, soil moisture, and temperature (Schimel and Bennett, 2004). Total soil C did not differ among overstory treatments and total N differed minimally, only at 10–15 cm depth where the control treatment had more total N than the girdle treatment and did not differ from the clearcut treatment. This suggests that treatment effects were not driven by differences in total soil C and nutrient availability in the plots. Soil

temperature and moisture, however, most likely influenced N mineralization. For the majority of the growing season, the control treatment had significantly warmer soil temperatures at 10 cm depth (Table 4) and lower soil moisture at 5 cm depth than the clearcut and girdle treatments (Slesak et al., 2014; Toczydlowski, 2018). We suspect these warmer soil temperatures are due to the water table being much lower in controls relative to the other treatments, decreasing the specific heat of the soil and allowing it to warm more rapidly. Additionally, understory layers in the girdle and clearcut treatments were characterized by dense sedges and grasses with greater than 200% cover (Looney et al., 2017), which may shade and insulate the soil more effectively than the intact ash canopy and sparser understory vegetation in the control treatment. Microbial processes that control both ammonification and nitrification occur more rapidly at higher temperatures, which would have contributed to significantly greater accumulation of NO_{2+3} and TIN in the 0–5 cm depth soils in the control treatment compared to the clearcut treatment (Table 2). Lower water tables in the control treatment allowed for greater N mineralization because although ammonification can occur in both aerobic and anaerobic soils, it is more rapid in aerobic soils (Vepraskas et al., 2016). Nitrification, the production of NO_2^- and NO_3^- from NH_4^+, also occurs rapidly in aerobic conditions and likely accounts for greater NO_{2+3} accumulation in the control treatment where aerobic conditions were more common (Diamond et al., 2018; Slesak et al., 2014). Similarly, Hefting et al. (2004) noted that riparian wetlands with water tables below −30 cm facilitated net nitrification and the accumulation of NO_3^- in the topsoil. While the control treatment had water tables near the surface in spring, they were below −30 cm for much of the growing season (unpublished data) and had greater accumulation of NO_{2+3}, especially later in the growing season (Fig. 1, Table 2). Without plant uptake, NH_4^+ in the soil is available to nitrifiers to produce NO_2^- and NO_3^-. This idea is reinforced by the inverse relationship between NH_4^+ and NO_{2+3} over the growing season (Fig. 1). As microbes use up available nutrients in the soil, the production of NH_4^+ wanes and NO_3^- accumulates in the soil (Schimel and Bennett, 2004), especially in the control plots where lower soil moisture limits denitrification. Microenvironment conditions created by the clearcut treatment appear to repress overall N mineralization.

In support of our second hypothesis (2), in soils not isolated in PVC cores, the effect of vegetation appeared to predominate treatment effects of soil moisture and temperature. In bulk soil samples collected near the isolated soil cores, there was generally an order of magnitude less inorganic N than in the isolated cores, indicating that the PVC cores did exclude plant uptake. In support of our first hypothesis (1), compared to the control and girdle treatments, the clearcut treatment had greater NH_4^+ accumulation, likely because the production of NO_3^- in the soil was inhibited by wetter, more anaerobic conditions. These conditions also favor denitrification, the reduction of NO_3^- to N_2O or N_2 gasses transferring N from the soil to the atmosphere (Hefting et al., 2004). The greater relative abundance of NH_4^+ in the clearcut treatment could also be linked to plant uptake, as most plants preferentially uptake NO_3^-, a more oxidized form of nitrogen, quickly depleting the NO_3^- pool, especially when its production is inhibited by anaerobic conditions (Vepraskas et al., 2016). The dense sedges and grasses in the clearcut and girdle treatments compete directly with microbes for N in the soil, particularly in nitrogen-poor ecosystems where plants must be adapted to scavenge N as a limiting nutrient (Schimel and Bennett, 2004). It is possible that the sedges and grasses in the clearcut and girdle treatments are less competitive against microbes, or simply have less demand for N than the ash trees in the control treatment, contributing to greater NH_4^+ accumulation in the clearcut treatment compared to the control.

Similar to our observations, and those reported by Looney et al. (2017), Trettin et al. (2011) observed changes in understory vegetation following whole-tree removal in a forested mineral soil wetland and noted that the understory plants became the primary source of C and organic matter additions to the forest floor. They cited others' work to enforce their own conclusions that C and N cycling was driven by understory vegetation communities and water table level. Hefting et al. (2004) however, did not find differences in nitrification or denitrification among vegetation types in riparian wetlands, but attributed N dynamics primarily to water table position. The authors concluded that sites with water tables within -10 cm of the soil surface favored ammonification and the accumulation of NH_4^+ in the topsoil. Similarly, the clearcut treatment, with the highest water table, had the greatest accumulation of NH_4^+ (Table 2). Sites with water tables between -10 and -30 cm from the surface favored denitrification (Hefting et al., 2004), potentially explaining why the girdle and clearcut treatments had less total N at 10–15 cm depth after six years of altered water tables and increased denitrification. The relative increase in NH_4^+ in bulk soil from the clearcut plots indicates that N demand of the herbaceous vegetation in that treatment was less than N demand of intact ash stands in the control treatment.

In the controlled laboratory setting, we removed differences in microclimate and their influence on N mineralization to assess treatment effects on N substrate and the microbial community. Counter to our third hypothesis (3), similar N mineralization rates across all treatments indicated no influence of canopy treatments on substrate-induced changes in inorganic N cycling six years post-treatment. However, there was a significant treatment effect on total soil N in the 10–15 cm soil depth. The continuously aerobic conditions and warmer temperature of the soil incubation experiment resulted in much greater N cycling rates (Table 3) and greater inorganic N accumulation in the soil (Table 2) compared to both in-situ portions of the experiment. In most instances, these increases were more than an order of magnitude higher, suggesting that microbes are not limited by nutrient availability, and that soil temperature, water content, and vegetation uptake are greatly constraining rates of inorganic N accumulation in the field. This may account for the limited treatment effects in the in-situ experiments. Notably, N accumulation was dominated by NH_4^+ and there was little accumulation of NO_{2+3} in the incubation experiment. This may be a result of the short, 14 day incubation period. Provided a longer incubation, more NH_4^+ might have been converted to NO_2^- and NO_3^-.

In both the laboratory and in situ settings, there was a drastic difference in N cycling rates (Table 3) and N concentrations (Figs. 1 and 2) between the organic horizon in the 0–5 cm depth and the mineral soil at 10–15 cm depth. The 0–5 cm soil had more total N and C than the 10–15 cm soil and this was reflected in the N cycling rates observed in both the in-situ and laboratory studies. Based on a subset of the soils sampled, the organic horizon had an average depth of 9.7 cm, but varied spatially ranging from 5 cm to 19 cm. This spatial variability in organic matter could have masked potential treatment effects.

While overstory treatment had minimal effects on soil N concentration and cycling rates in a laboratory setting, the combined effects of changes in soil moisture, soil temperature, and understory vegetation in black ash wetlands will likely result in altered N cycling dynamics. The bulk soil samples are the most representative of ecosystem conditions following EAB disturbance, and those samples indicated NH_4^+ dominated N cycling with slightly greater accumulation in the clearcut treatment. The exact mechanism behind the increased rates of NH_4^+ accumulation remains unclear but is likely influenced by the increased soil moisture and denser understory vegetation in the clearcut plots. Since this study is only a snapshot of one growing season, six years after treatment installation, it is difficult to predict the long-term future of N cycling following EAB invasion. Five years after whole-tree harvest and site-prep in a forested, mineral soil wetland in Michigan, McLaughlin et al. (2000) reported elevated soil NO_3^-, NH_4^+, and dissolved organic N (DON), associated with soil disruption (mixing in of organic matter) and relative shift towards inorganic N with the loss of nutrient uptake by mature trees. However, similar to our results, McLaughlin et al. (2000) did not detect a difference in N mineralization rates after 4 years or after 11 years (Trettin et al., 2011), and after 14 years C and N pools remained similar to that of an uncut stand (Mclaughlin et al., 2010). It is important to note that McLaughlin et al. (2010) and Trettin et al. (2011) are studying replanted ecosystems with ample regeneration whereas black ash ecosystems without alternative management will likely have no natural regeneration and divert to an open marsh type ecosystem. Regardless, our in-situ and laboratory experiments in tandem indicated ash mortality from EAB would not greatly influence net N cycling rates in black ash wetlands in northern Minnesota.

5. Conclusion

This study identified minor, but potentially compounding effects of overstory treatment simulating EAB caused tree mortality on nitrogen cycling in black ash wetland soils. Our results indicate that complex interactions between vegetation communities, soil moisture, soil temperature, and organic matter drive N cycling, especially in the top five cm of soil in black ash wetlands. Each of these ecosystem components was altered by canopy treatments simulating EAB infestation. The influence of ash mortality from EAB may not alter overall N mineralization rates over the short term, but might shift N cycling from NO_{2+3}-centered to NH_4^+-centered influencing microbial communities and plant uptake in the ecosystem.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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