Responses of Red-Backed Salamander (Plethodon cinereus) Abundance in a Northern Hardwood Forest

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Responses of Red-Backed Salamander (*Plethodon cinereus*) Abundance

One Year After Application of Wood Ash in a Northern Hardwood Forest

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Abstract

Wood ash may be an effective soil amendment in North America to restore acidified and low nutrient forest soils, but little research exists beyond its effects on soil and plants. Eastern red-backed salamander (*Plethodon cinereus*) abundance was assessed in a northern hardwood forest 1 year following an ash addition field trial. Plots were established with fly and bottom ash treatments of 0, 1, 4, and 8 Mg ha\(^{-1}\) (n=4), and cover boards were positioned both with and without ash beneath. One year following ash additions salamander abundance had increased under boards with fly ash beneath, and bottom ash had no effect. Soil pH and EC increased under cover boards with ash beneath them and for uncovered soil, and the effects were strongest under cover boards with ash beneath. The effects of ash were generally stronger at higher dosages, and fly ash was stronger than bottom ash. The moisture holding capacity of fly ash was 60% higher than the soil and for bottom ash was 63% lower, but they had little effect on moisture of the forest floor. These results suggest that ash altered salamander abundance via soil pH and moisture, and would not inhibit their movement over the forest floor.

Key words: fly ash, bottom ash, liming, cover boards, Haliburton Forest and Wildlife Reserve

Running title: Salamander abundance following ash additions
1. Introduction

Forest soils in eastern North America have experienced acidification and nutrient depletions resulting from acid rain (Likens & Bormann, 1974; Watmough & Dillon, 2003; McLaughlin, 2014). Low soil pH and nutrient imbalances from acid rain persist (Driscoll et al., 2001; Gradowski & Thomas, 2006), and forest harvesting also has the potential to deplete nutrients in certain forest soils (Phillips & Watmough, 2012). Declines in the growth and health of sugar maple (*Acer saccharum* Marsh.) have already been recorded in response to soil acidification and Ca declines in northern hardwood forests (Horsley et al., 2002; Long et al., 2011), and there is growing interest in using soil amendments to restore soil pH and nutrient status in these ecosystems (Long et al., 2011; Moore et al., 2012).

Wood ash is produced in significant quantities in pulp and paper and lumber mills in North America (Elliott & Mahmood, 2006), and may be a practical forest soil amendment as it has a high pH, and other than N, contains nutrients in similar proportions to those needed by growing trees (Mandre et al., 2010). In certain European countries ash is already commonly applied to intensively managed forests to raise soil pH and reinstate depleted nutrients (Emilsson, 2006; Stupak et al., 2008), and is also used as a soil amendment in certain agricultural settings (Elliott & Mahmood, 2006). In Canada and the USA, ash is typically sent to landfill, but interest has been growing in using ash as a forest soil amendment (Elliott & Mahmood, 2006; Pugliese et al., 2014). Production of ash is likely to increase in the future due to a growing bioenergy sector (use of bioenergy has been projected to triple by 2050), with Canada as a major supplier of biomass fuel (IEA, 2013).

Although ash is already used to increase soil pH and address nutrient deficiencies in agriculture and intensively managed forests in Europe, its use in North American forests is not well studied. European forests where ash additions are employed are intensively managed for timber production, and applying ash to less intensively managed forests in North America may pose more significant risks. There are several properties of ash that could harm susceptible forest species, especially when applied at high dosages. These include a high pH, and retention of salts and traces of heavy metals (Pitman, 2006;
Augusto et al., 2008). Current literature focuses on soil and plant responses to ash additions, and little is known regarding its effects on other components of forest ecosystems.

Biomass boilers typically produce both fly ash - a volatile, lighter, more reactive ash extracted from flue gasses, and bottom ash – heavier, less reactive particles that fall to/remain on the bottom of combustion chambers or grates. These ashes vary in their physical and chemical properties (Ingerslev et al., 2011), and conclusions regarding their suitability as soil amendments vary. Precautionary research is required to investigate the effects of these ashes on other components of forest ecosystems beyond plants and soils before they are endorsed as forest soil amendments in North America.

Eastern red-backed salamanders (Plethodon cinereus) are one of the most abundant amphibian species in northern hardwood forests, and their importance in many forest ecosystem functions such as litter decomposition, carbon cycling, and biodiversity has been widely explored in the literature (Wyman, 1998; Homyack et al., 2010; Hocking & Babbitt, 2014). Several factors make them good indicator species and their responses to soil conditions and disturbances convenient to study in forests of northeastern North America. First, they are well-distributed (Moore & Ouellet, 2015) and abundant; and Burton & Likens (1975) suggested that in some forests their total biomass can exceed that of all other small vertebrates combined. Second, they live beneath cover objects such as fallen logs, and their relative abundances can be easily monitored with cover object surveys (Welsh & Droege, 2001). Third, red-backed salamander populations are reasonably stable from year to year, and do not drastically fluctuate in the absence of disturbances (Moore, 2005). Fourth, salamanders are relatively central in the forest food web, and can be used as indicators of forest ecosystem health and integrity (Welsh & Droege, 2001; Davic & Welsh, 2004). Finally, they respond quickly to environmental disturbances and changes, as they occupy the LFH layer of soil and are both ectothermic and lungless, breathing directly through their skin (Welsh & Droege, 2001). Key environmental factors that influence the presence and abundance of red-backed salamanders include soil pH, Ca, and moisture, and moist soils with a circumneutral pH and high Ca levels are generally preferred (Sugalski & Claussen, 1997; Beier et al.,
However, contradictions exist in the literature regarding the tolerance of salamanders to soil acidity, with Moore & Wyman (2010) finding healthy and abundant populations of red-backed salamanders in a forest with a pH of 3.7 +/-0.4.

Although amending acidic northern hardwood forest soils with wood ash presents a practical alternative to landfilling, research is needed regarding the impacts of ash on ecologically important forest species such as red-backed salamanders. To our knowledge, no studies exist regarding the effects of ash on salamander abundance, or the abundances of any other vertebrate communities. The neutralizing properties and Ca content of ash may increase red-backed salamander abundance (Sugalski & Claussen, 1997; Beier et al, 2012); however, potential toxicants could have negative effects at large dosages. This study assessed how fly and bottom ashes influenced soil pH, moisture, salinity, and eastern red-backed salamander abundance (using cover board surveys), 1 year after application in a northern hardwood forest. We also measured whether salamanders favored or avoided ash by residing under cover boards with or without ash beneath.

2. Methods

2.1. Site description

The study site was located in Haliburton Forest and Wildlife Reserve, Central Ontario (45’11N, 78’35W), a mixed-deciduous Great Lakes-St. Lawrence forest (Rowe, 1972) dominated by sugar maple (Acer saccharum) and American beech (Fagus grandifolia Ehrh.). The mean annual temperature in the region is 5.0°C, ranging from -9.9°C in January to 18.7°C in July, with an average of 1074mm of annual precipitation (Environment Canada, 2014). Soils are shallow, rocky, and medium to coarse-textured and belong to the Orthic or Eluviated Dystric Brunisol subgroups based on the Canadian System of Soil Classification (Soil Classification Working Group, 1998). Much of the soil parent material is derived from granite or granitic-gneiss deposits atop the Precambrian shield, with pH values ranging from 4 to 5.5. The study site was harvested using the single tree selection silviculture system (OMNR, 2000) in
2003, and subsequently salvage logged for beech due to the arrival of beech bark disease in the stand.

Mean basal area in the study site in 2013 for trees >8cm DBH was 16.4 m$^2$ ha$^{-1}$, canopy openness ranged from 12-60% (mean 33%; median 33%), and the moisture content of the LFH horizon ranged from 34-259% (mean 93%; median 59%).

2.2. Plot setup and experimental design

Bottom and fly ashes were each applied at rates of 1, 4, and 8 Mg ha$^{-1}$, with one control treatment (0 Mg ha$^{-1}$ ash). In the summer of 2012 an area of forest was selected and 28 20m×20m plots (including a 2.5m buffer) were established in a grid. The plots were then assessed for canopy openness and soil moisture content (see field and laboratory sampling and analyses for details), and assigned into four blocks: high soil moisture and high canopy openness, low soil moisture and high canopy openness, high soil moisture and low canopy openness, low soil moisture and low canopy openness. Each treatment was replicated four times by randomly assigning 1 replicate plot from each block to each treatment.

Ash was added over 4 days in August, 2013. To spread the ash evenly, each plot was sectioned into a 5m×5m grid, and the amount of ash needed for each square was weighed, carried to the plot using buckets, and manually spread as uniformly as possible. Ashes for the trials came from a large RotoStoker VGC biomass boiler system fired with bark after bole de-barking for pulp production (Detroit Stoker Company, Monroe, Michigan, USA). The furnace had air and feedstock injected from above, and was equipped with a horizontal vibrating stoker grate to move the fuel bed through the combustion chamber and remove the bottom ash, which comprised of heavier, coarse, sandy particles. Fly ash represented a mix of material recovered from both cyclone and electrostatic precipitators emissions control systems on the boiler.

Cover boards were cut to 30×30×2cm from locally harvested, rough cut, untreated hemlock, and placed in each plot in August of 2013. Four cover boards were placed in each plot 1 week prior to ash additions, evenly spaced along a diagonal transect running between the north-south corners of each plot. Another 4 were placed immediately after ash additions along the opposite transect, making 8 cover
boards per plot: 4 with ash beneath, and 4 without (Figure 1). By incorporating cover boards both with and without ash beneath in each treated plot we were able to determine whether the salamanders were residing under cover objects in order to avoid the ash, or because they favored the ash. Cover boards were placed flat against the ground, avoiding rocks and logs, and at least 4 m from the edge of the plot, then left to weather for 9 months before being monitored for salamanders. Red-backed salamanders have home ranges of approximately 4m², although are also able to disperse much larger distances in short time periods (Kleeberger & Werner, 1982). Thus, placing the boards a minimum of 4m from the edge of the plot could have minimized edge effects. Although the cover boards were systematically distributed within each plot and not randomized (Figure 1), we do not expect that this introduced significant confounding effects that would not have been present had the boards been randomized.

2.3. Field and laboratory sampling and analyses

The plots were assessed for canopy openness in July of 2013 and for the moisture content of the LFH soil horizon in August of 2014, to be used as covariates in statistical analysis. Soil moisture was determined by pooling 4 randomly located soil cores from the top 5cm of the LFH layer of soil in each plot. Samples were sieved through a 2mm mesh, 10g was weighed out, then dried in a drying oven at 90°C for 48 hours. The soil was reweighed, and moisture content was calculated as: \((\text{wet mass} - \text{dry mass}) / \text{dry mass}\) \times 100. Canopy openness was determined by taking 5 photographs at evenly spaced locations in each plot with a digital camera facing vertically upwards and at 1.3m above the ground, on standard settings. Photographs were then imposed with a 5×5 grid, and assessed to determine the percentage of canopy cover compared to open sky.

Prior to application, three samples each of fly and bottom ashes were placed in sealable plastic bags for pH and elemental analysis. Ash chemistry was determined using a NCS combustion analyzer (Vario EL III, Elementar Americas, Mt. Laurel, NJ) for total C, N and S and with an inductively coupled argon plasma (ICAP) spectrometer (Varian Analytical Instruments, Walnut Creek, CA) following high
temperature microwave acid digestion for P, K, Ca, Mg, Al, Na, S, Fe, Cu, Mn, As, Zn, V, Cd, Co, Cr, Mo, Ni, Pb, Ba, Be, Si, La, Li, Se, and Sr (EPA standard method 3052).

Salamander sampling occurred 8 times between May-October, 2014, always at least 1 week apart. Each sampling event was completed within 1 day, and the order of plots visited was reversed every second sampling to avoid temporal sampling bias. To check for salamanders each board was lifted, the number of salamanders was recorded, and the board was returned to its original position. All sampling methods complied with the Guide to the Care and Use of Experimental Animals (CCAC, 1993), and were approved by the University of Toronto’s Animal Care Committee.

In August of 2014, a 1cm diameter soil core of the surface 5cm of the organic (LFH) layers of soil was sampled from beneath each cover board, and from 4 randomly located soil cores in each plot. Soil cores were pooled to obtain 3 samples per plot, providing 1 each from under boards with ash beneath, without ash beneath, and from uncovered soil. Soil moisture content was determined as described previously. pH and electrical conductivity (EC) were measured after sieving soil using a 2mm mesh, then making a water:soil slurry using a 4:1 ratio. The slurry was stirred, allowed to sit for 30 minutes, and stirred again before taking pH and EC readings using an Accumet pH and EC meter (ThermoFisher, Waltham, MA, USA). The pH and EC probes were calibrated after every 14 samples.

To assess the moisture holding capacity of ash, fly and bottom ashes were first oven dried at 90°C for 48 hours. Ten fine mesh bags (mesh opening size approximately 0.01mm) were each then filled with approximately 50g of fly ash, and another 10 with 50g of bottom ash. In October, 2014, 10 cover boards were placed approximately 5m apart along a transect near the ash addition experiment. One bag each of fly and bottom ash were placed beneath each cover board, and left for 2 weeks. The bags were then retrieved, a 1cm diameter soil core of the surface 5cm of the LFH layers of soil was taken from beneath each board, and the moisture content of each ash and soil sample was determined using the same methods described above.

2.4. Statistical analysis
The average number of salamanders observed per survey based on the pooled number of salamanders found in each survey, for each plot, was used to represent salamander abundance. Salamander abundance, soil pH, soil EC, and soil moisture content were the dependent variables. Normality was assessed using normal probability plots of residuals, frequency histograms, and the Kolmogorov-Smirnov, Lilliefors, and Shapiro-Wilks tests, and homoscedasticity of variances was assessed using the Cochran, Hartley, and Bartlett tests (p>0.05). To achieve normality, salamander abundance was transformed using the square root function, soil pH was transformed using $1/x^2$, and soil EC and moisture content were log transformed.

For salamander abundance, ash type (fly and bottom ash), ash dosage (1, 4, and 8 Mg ha$^{-1}$), and cover boards (with or without ash beneath) were tested in an ANOVA, with all interactions. Because the experimental units for the cover board variable (each cover board within the plot) were not the same as for the ash type and dosage variables (the whole plots), and the plots were randomized within experimental blocks, a split plot design was used (Montgomery, 1991). To account for this in the model, block was included as a random variable, with an interactive term between ash type, ash dosage, and block (Pinheiro & Bates, 2000; Steel and Torrie, 1980). The controls were assigned a ‘0’ level for ash type, ash dosage, and cover board, and thus were not tested in any of the fixed effects. Dunnett’s post-hoc test was used to test for significant differences to the control, and Tukey’s HSD post-hoc was used to test between all other levels of the significant variables.

For soil pH, EC, and moisture content, the same analyses as described for salamander abundance was used except that the variable cover boards included an extra level (uncovered soil), and Dunnett’s post hoc test was performed twice - once using soil from beneath cover boards in the control treatment as the reference, and again using uncovered soil in the control treatment as the reference. To assess differences between the moisture holding capacity of ash and soil, a one-way ANOVA was used with ash/soil moisture as the dependent variable and sample type (fly ash, bottom ash, or soil) as the
independent variable. Tukey’s HSD test was then used to assess all pairwise comparisons. Statistica 7 (Statsoft inc, 2007) was used for all analyses, and all results were considered significant at p<0.05.

3. Results

Ash dosage and ash type did not have a significant effect on soil pH or EC, but cover boards did, and the interactive effects between cover boards and ash dosage, and cover boards and ash type were significant (Table 1). Cover boards with ash beneath exhibited the largest increases in soil pH and EC, minor increases occurred on uncovered soil, and cover boards without ash beneath did not change significantly from the controls (Figures 2,3; Tables 2,3). For cover boards with ash beneath and for uncovered soil there was an increasing trend in soil pH and EC as ash dosage increased, and the effects of fly ash were stronger than those of bottom ash (Figures 2,3; Tables 2,3). When fly and bottom ash treatments were pooled, at 8 Mg ha\(^{-1}\) and when ash was beneath the cover boards, soil pH increased from 4.9 to 6.5 compared to cover boards without ash beneath, and soil EC increased from 64 to 174 ms m\(^{-1}\) (Table 3). When ash dosages were pooled, fly ash increased soil pH and EC from 4.9 to 6.5 and 63 ms m\(^{-1}\) to 199 ms m\(^{-1}\) respectively when comparing cover boards with ash beneath to those without, and bottom ash increased soil pH from 4.9 to 5.5 and EC from 67 ms m\(^{-1}\) to 99 ms m\(^{-1}\) (Table 3).

The main significant experimental effect on the moisture content of the LFH horizon was that moisture was generally higher under cover boards when compared to exposed soil (Figure 4; Tables 1,2). The crossed effects of cover board and ash type were also significant, and it appeared that when there was ash beneath the cover boards bottom ash had a decreasing effect on soil moisture whereas fly ash slightly increased moisture (Figure 4, Table 2). However, post hoc tests did not find any significant differences between the soil moisture content under cover boards with either fly or bottom ash beneath compared to those without (Table 2). When the moisture holding capacity of the ash was determined in mesh bags, bottom ash was 63% lower than the soil (which had a moisture holding capacity of 72%) and fly ash was 60% higher (p<0.001).
One hundred and eighty two red-backed salamanders were counted in total. Ash type and presence of ash beneath the cover boards were significant predictor variables for red-backed salamander abundance, and the crossed effects between cover boards and ash type were also significant (Table 1). Fly ash had a positive effect on salamander abundance (Figure 5, Table 4), and there were no significant effects of bottom ash (Table 4). Although the effects of ash dosage were not significant, salamander abundance appeared to increase with fly ash dosage and at the highest dosage of 8 Mg ha\(^{-1}\) approximately 1 more salamander was found under cover boards with fly ash beneath compared to those without in the same plot, and also compared to the control plots (Figure 5). When all dosages were pooled, 0.4 more salamanders were found per plot per survey under cover boards with ash beneath compared to those without (Table 4).

4. Discussion

4.1. Effects of ash on soil

The pH of the ashes used in this experiment (Table 5) were low compared to previously reported averages (Vance, 1996; Pitman, 2006), but the observed liming effects are consistent with current literature (Demeyer et al., 2001; Pitman, 2006; Augusto et al., 2008; Ingerslev et al., 2011; Reid & Watmough, 2014), particularly for fly ash. In a meta-analysis of ash addition literature, Augusto et al (2008) report 1-5 year increases in soil pH to average 1.0 at dosages of 4-8 Mg ha\(^{-1}\), and a more recent meta-analysis by Reid & Watmough (2014) found pH increases in organic soil horizons to average 1.04. In this experiment, although the effects of ash on the pH of uncovered soil were not significantly different from the control (Table 2,3), a consistent increase in soil pH with ash dosage occurred on uncovered soil for both ash types, and at 8 Mg ha\(^{-1}\) fly and bottom ash increased soil pH by 0.8 and 0.5 units respectively compared to boards that did not have any ash beneath in the same treatment (Figure 2).

Larger and significant increases in soil pH were observed under cover boards with ash beneath (Figure 2; Tables 2,3), and this is because the soil had been protected from direct rainfall and subsequent...
leaching of base cations and alkalinity lower in the soil profile. Soil pH can also increase in conjunction with moisture, and in this experiment the soil moisture was also higher beneath the cover boards compared to uncovered soil (Figure 4). However, the increases in soil pH observed in this study were primarily an ash effect as opposed to a confounding effect of moisture, because the cover boards with ash beneath had higher soil pH values than those without, and for cover boards with ash beneath soil pH increased in conjunction with ash dosage.

The main effect of the experiment on the moisture of the LFH horizon was that moisture was higher beneath the cover boards compared to exposed soil, and this is because the soil beneath the cover boards had been protected from direct sunlight and subsequent drying. The moisture content was assessed by comparing dry and wet masses of 4 pooled samples from each plot. This method may bias the results because organic matter and soil texture can impact the measurement, and the relatively low number of replicates used for each sample could increase the variance. However, this was still useful for comparing differences between treatments and the control. The moisture holding capacity of bottom ash was also 63% lower than the soil whereas it was 60% higher for fly ash, but minimal effects of ash were seen on soil moisture (Table 2). The sandy consistency and low C content of the bottom ash (large particles with limited swelling capacity) and the finer consistency and higher C content of the fly ash (Table 5) explains their different moisture holding capacities.

The increases in soil EC in response to ash additions in this study are consistent with current literature (Staples & Van Rees, 2001; Chirenje & Ma, 2002), and occur because ash both supplies cationic nutrients to the soil and increases their availability by neutralizing acidity. The Na content of ash has been suggested as a potential toxicant in dry forest soils by causing excessive salinity (Pugliese et al., 2014), and a high EC can be indicative of excessive Na levels in soils. The typical Na values in ash vary highly, and in this study the Na values were 36,400 mg kg\(^{-1}\) and 16,300 mg kg\(^{-1}\) for fly and bottom ashes respectively (Table 5), which are on the higher end of the spectrum (Augusto et al., 2008; Karltun, 2015). The highest increases in soil EC were for fly ash at 8 Mg ha\(^{-1}\) where soil EC increased from 70 to
239 ms m$^{-1}$ (Figure 3). For forest soils an EC>400 ms m$^{-1}$ can be considered detrimental (Khan, 2013), which was not reached in any of the treatments in this study (Figure 3).

4.2. Effects of ash on salamander abundance

Red-backed salamanders prefer moist soil with a circumneutral pH (Sugalski & Claussen, 1997), although healthy populations have been reported on soils with a pH as low as 3.7 +/-0.4 (Moore & Wyman, 2010). The neutralizing effect of fly ash (Figure 2) and its higher moisture holding capacity compared to soil likely explain the observed increases in red-backed salamander abundance under cover boards with fly ash beneath (Figure 5). Although the effects of ash on the moisture content of the LFH horizon were not significant (Table 2), the moisture holding capacity of the ash itself may be more reflective of the salamander’s environment than the moisture content of the entire LFH horizon, as the salamanders occupying boards with ash underneath were found in direct contact with the ash. For bottom ash, non-significant but consistent increases in soil pH were seen (Figure 2) and its moisture holding capacity was lower than the soil, and we suggest that these 2 potentially competing factors controlling salamander abundance explain why there were not significant effects seen with bottom ash additions.

Although salamander abundance was significantly greater beneath boards with fly ash beneath compared to those without (Figure 5; Table 4), the boards without ash beneath represent a more likely scenario in terms of how forest management and operational ash applications would manifest on the ground. If forest application of ash is to be employed on a large scale it is likely to occur immediately after harvests, as this decreases the cost of application and would maximize the benefits to regenerating trees. During a harvest large amounts of downed woody debris are created but a comparatively small amount is added in subsequent years. Thus, if ash was applied following a harvest then there would be many logs without ash underneath (but concentrated around the edges) and very few with ash beneath. However, red-backed salamanders move through the forest floor to defend and mark their territories, and hunt for food. As they do not appear to avoid cover boards with ash underneath, we suggest that 1 year
following ash additions presence of ash on the forest floor would not impede their ability to move or
hunt.

The observed positive effects of ash on salamander abundance may have occurred only after its pH
had equilibrated with the soil, and the potentially high initial pH of other ashes (Pitman, 2006) could be
cautious to salamanders (and other ground fauna and vegetation) immediately after application. Until the
initial pH drops, salamanders may avoid ash by utilizing cover objects without ash beneath, or by
burying into the soil. This is an important future research question that should be addressed, for example
with behavioural “choice tests” in controlled soil mesocosm environments using salamanders or other
soil fauna (e.g. McTavish et al., 2013). In this study several rain events occurred in the weeks following
ash additions which likely accelerated leaching of base cations and alkalinity into the soil profile, and
shortened the duration of any possible negative effects from the high initial pH of the ashes. The effects
of ash on soil pH also dissipate over time, and some guidelines in Europe recommend re-applying ash
every 10 years to maintain soil pH (Stupak et al., 2008). This highlights the importance of longer term
studies to assess the full suite of temporal effects of ash on salamander abundance.

To our knowledge, no studies exist regarding the effects of wood ash on salamanders, or any other
vertebrate communities. There is a growing body of literature on the ecological effects of liming in sugar
maple dominated forests in eastern North America, which has been reviewed by Moore et al. (2014).
They conclude that low to moderate dosages of lime applied every 25-30 years would not have negative
effects on key fauna, and could benefit amphibians that reproduce in acidified bodies of water. Although
red-backed salamanders do not reproduce in water, in untreated northern hardwood forests their
abundance has been shown to increase in conjunction with soil Ca levels (Beier et al., 2012), suggesting
that liming could also benefit their population levels. Forest liming has been shown to indirectly benefit
songbird populations, as the increases in soil pH and Ca levels increase snail populations, which are a
staple food source of many songbirds. A similar effect of ash on red-backed salamanders could also
occur, as wood ash has been shown to increase abundances of collembolans, mites, and enchytraeid.
populations in soil (Nieminen et al., 2012), which are food sources of red-backed salamanders. Moore 
(2014) studied the effects of different textured forest liming agents on red-backed salamander body 
weight and mortality in closed microcosms in a hardwood forest in Eastern Canada, and found no effect 
of finely ground or sandy textured CaCO₃.

Heavy metals in ash could also have a detrimental effect on salamander health and abundance.
Concentrations of heavy metals in ashes used in this study (Table 5), and in ashes from a variety of other 
biomass boilers in Canadian mills (Pugliese et al. 2014) are generally low, and their mobility in forest 
ecosystems is not normally a concern as the higher pH of ash renders them less biologically available 
(Perkiomaki et al., 2003; Pugliese et al. 2014). Land application of industrial waste residues is regulated 
federally in the USA by the Environmental Protection Agency (EPA), and provincially in Canada by the 
Ministry of Environment and Climate Change (MOECC) in Ontario. For both ashes used in this study, 
metals were within EPA and MOECC limits (Table 5) and are unlikely to have been affecting 
salamander health and abundance. However, the mobility of metals may increase if soil pH begins to 
return to pre ash addition levels, again highlighting the importance of long term studies. It would also be 
beneficial for future studies to assess the health effects of ash on salamander populations alongside their 
abundances, by measuring body parameters such as snout-vent length and weight.

It is important to consider that this study was assessing relative abundance of red-backed 
salamanders between treatments and is not a representation of their absolute abundance in northern 
hardwood forests. As mentioned previously, the observed increases and decreases in salamander 
abundance could have actually been a redistribution of their location in the soil horizons. Changes in 
absolute abundance may take longer to be recognized, unless ash directly causes mortality of 
salamanders which was not observed in this study.

5. Conclusions
Fly ash beneath the cover boards increased soil pH and had a higher moisture holding capacity than the adjacent soil, causing increases in red-backed salamander abundance. Bottom ash beneath the cover boards also increased soil pH, but had a lower moisture holding capacity than the soil and no effect on salamander abundance. Smaller increases in soil pH were observed on uncovered soil. The salamander boards with ash beneath did not represent a common habitat scenario from a management perspective, but the finding that salamanders do not avoid boards with ash beneath suggests that ash would not prevent their movement on and through the forest floor. Both ashes had relatively high Na levels but soil EC did not indicate excessive soil salinity. Heavy metal concentrations in the ash were minimal and did not appear to negatively affect salamander abundance. These results tentatively support use of ash as a soil amendment in acidified hardwood forests of eastern North America, but more research is needed into the longer-term effects of ash and mobility of its potential toxicants. This study provides just one aspect of the evidence needed to make an informed decision on whether ash addition is an acceptable practice in North American forests.

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References


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Table 1. p values from GLMs for soil pH, EC, and moisture, and salamander abundance (average number counted per plot, per survey). Degrees of freedom in brackets are for the salamander abundance variable, as there was one fewer levels of the variable ‘cover boards’.

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<th>Variables</th>
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<th>DF</th>
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<th>EC</th>
<th>Moisture</th>
<th>Salamander abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Ash type</td>
<td>Fixed</td>
<td>1</td>
<td>0.145</td>
<td>0.059</td>
<td>0.853</td>
<td>0.007</td>
</tr>
<tr>
<td>2 - Ash dosage</td>
<td>Fixed</td>
<td>2</td>
<td>0.167</td>
<td>0.347</td>
<td>0.683</td>
<td>0.236</td>
</tr>
<tr>
<td>3 – Cover boards</td>
<td>Fixed (1)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>4 - Block</td>
<td>Random</td>
<td>3</td>
<td>0.343</td>
<td>0.022</td>
<td>0.001</td>
<td>0.058</td>
</tr>
<tr>
<td>5 - Ash type*Ash dosage</td>
<td>Fixed</td>
<td>2</td>
<td>0.790</td>
<td>0.565</td>
<td>0.436</td>
<td>0.779</td>
</tr>
<tr>
<td>6 – Ash type*Cover board</td>
<td>Fixed (1)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.014</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>7 – Cover board*Ash dosage</td>
<td>Fixed (2)</td>
<td>0.018</td>
<td>0.035</td>
<td>0.572</td>
<td>0.525</td>
<td></td>
</tr>
<tr>
<td>8 - Cover board<em>Ash dosage</em>Ash type</td>
<td>Fixed (2)</td>
<td>0.251</td>
<td>0.720</td>
<td>0.836</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>9 – Block<em>Ash type</em>Ash dosage</td>
<td>Random</td>
<td>18</td>
<td>&lt;0.001</td>
<td>0.029</td>
<td>&lt;0.001</td>
<td>0.021</td>
</tr>
</tbody>
</table>
**Table 2.** Mean values for soil pH, EC, and moisture content, grouped by ash type*cover board. Differing letters indicate statistically significant results from Tukey’s HSD test (between all bottom and fly ash treatments) and from Dunnett’s test (between all treatments and controls).

<table>
<thead>
<tr>
<th>Cover board</th>
<th>Ash type</th>
<th>pH</th>
<th>EC (units)</th>
<th>MC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover board - ash</td>
<td>Control</td>
<td>5.2</td>
<td>58.3 d</td>
<td>230 d</td>
</tr>
<tr>
<td>Exposed soil</td>
<td>Control</td>
<td>5.2</td>
<td>64.1 cd</td>
<td>349 bcd</td>
</tr>
<tr>
<td>Cover board - ash</td>
<td>Bottom</td>
<td>4.9</td>
<td>66.5 cd</td>
<td>245 bd</td>
</tr>
<tr>
<td>Cover board + ash</td>
<td>Bottom</td>
<td>5.5</td>
<td>99.3 b</td>
<td>189 bc</td>
</tr>
<tr>
<td>Exposed soil</td>
<td>Bottom</td>
<td>5.1</td>
<td>69.8 bcd</td>
<td>88 a</td>
</tr>
<tr>
<td>Cover board - ash</td>
<td>Fly</td>
<td>4.9</td>
<td>62.9 d</td>
<td>188 b</td>
</tr>
<tr>
<td>Cover board + ash</td>
<td>Fly</td>
<td>6.5</td>
<td>198.9 a</td>
<td>215 bcd</td>
</tr>
<tr>
<td>Exposed soil</td>
<td>Fly</td>
<td>5.3</td>
<td>85.4 bc</td>
<td>75 a</td>
</tr>
</tbody>
</table>
Table 3. Mean values for soil pH and EC, grouped by ash dosage*cover board variables. Differing letters indicate statistically significant results from Tukey’s HSD test (between all bottom and fly ash treatments) and from Dunnett’s test (between all treatments and controls).

<table>
<thead>
<tr>
<th>C</th>
<th>Soil location (cover board)</th>
<th>pH</th>
<th>EC (ms m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Cover board - ash</td>
<td>5 cde</td>
<td>64.1 d</td>
</tr>
<tr>
<td>0</td>
<td>Exposed soil</td>
<td>5 cde</td>
<td>58.3 cd</td>
</tr>
<tr>
<td>1</td>
<td>Cover board - ash</td>
<td>4.9 e</td>
<td>67.4 bcd</td>
</tr>
<tr>
<td>1</td>
<td>Cover board + ash</td>
<td>5.5 bcd</td>
<td>111.6 b</td>
</tr>
<tr>
<td>1</td>
<td>Exposed soil</td>
<td>5 e</td>
<td>65.5 bcd</td>
</tr>
<tr>
<td>4</td>
<td>Cover board - ash</td>
<td>4.9 e</td>
<td>62.9 cd</td>
</tr>
<tr>
<td>4</td>
<td>Cover board + ash</td>
<td>6.1 ab</td>
<td>161.8 a</td>
</tr>
<tr>
<td>4</td>
<td>Exposed soil</td>
<td>5.1 de</td>
<td>77.6 bcd</td>
</tr>
<tr>
<td>8</td>
<td>Cover board - ash</td>
<td>4.9 e</td>
<td>63.9 cd</td>
</tr>
<tr>
<td>8</td>
<td>Cover board + ash</td>
<td>6.5 a</td>
<td>174 a</td>
</tr>
<tr>
<td>8</td>
<td>Exposed soil</td>
<td>5.6 bc</td>
<td>89.6 bc</td>
</tr>
</tbody>
</table>
Table 4. Mean values for salamander abundance (average per plot, per survey), grouped by ash type*cover board. Differing letters indicate statistically significant results from Tukey’s HSD test (between all bottom and fly ash treatments) and from Dunnett’s test (between all treatments and the control).

<table>
<thead>
<tr>
<th>Ash over/under board</th>
<th>Ash type</th>
<th>Number of salamanders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control</td>
<td>0.6 b</td>
</tr>
<tr>
<td>Over</td>
<td>Bottom ash</td>
<td>0.4 b</td>
</tr>
<tr>
<td>Under</td>
<td>Bottom ash</td>
<td>0.4 b</td>
</tr>
<tr>
<td>Under</td>
<td>Fly ash</td>
<td>0.9 a</td>
</tr>
<tr>
<td>Over</td>
<td>Fly ash</td>
<td>0.5 b</td>
</tr>
</tbody>
</table>
Table 5. Elemental composition of fly and bottom ash (means; n=3), and untreated Haliburton soil* (0-20 cm depth including LFH), and EPA† and MOECC‡ limits for metals.

<table>
<thead>
<tr>
<th>Element</th>
<th>Fly ash</th>
<th>Bottom ash</th>
<th>*Untreated soil (0-20cm depth including LFH)</th>
<th>EPA limits for land application</th>
<th>MOECC limits for land application</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.63</td>
<td>9.67</td>
<td>4.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C (g kg⁻¹)</td>
<td>175.7</td>
<td>5.2</td>
<td>78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOI (g kg⁻¹)</td>
<td>204.2</td>
<td>4.3</td>
<td>137.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TN (g kg⁻¹)</td>
<td>0.9</td>
<td>0</td>
<td>4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P (g kg⁻¹)</td>
<td>3.8</td>
<td>1.7</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K (g kg⁻¹)</td>
<td>30.7</td>
<td>14.3</td>
<td>19.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na (g kg⁻¹)</td>
<td>36.4</td>
<td>16.3</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al (g kg⁻¹)</td>
<td>23.3</td>
<td>44.7</td>
<td>65.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe (g kg⁻¹)</td>
<td>15.4</td>
<td>28.2</td>
<td>3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca (g kg⁻¹)</td>
<td>101.1</td>
<td>43.6</td>
<td>17.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg (g kg⁻¹)</td>
<td>8.7</td>
<td>8.4</td>
<td>4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S (g kg⁻¹)</td>
<td>42.2</td>
<td>2.9</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn (g kg⁻¹)</td>
<td>8.0</td>
<td>3.2</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ba (g kg⁻¹)</td>
<td>1.7</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co (mg kg⁻¹)</td>
<td>10</td>
<td>13</td>
<td>15</td>
<td>340</td>
<td></td>
</tr>
<tr>
<td>As (mg kg⁻¹)</td>
<td>10</td>
<td>12</td>
<td>13</td>
<td>75</td>
<td>170</td>
</tr>
<tr>
<td>Be (mg kg⁻¹)</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>La (mg kg⁻¹)</td>
<td>10</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Li (mg kg⁻¹)</td>
<td>12</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu (mg kg⁻¹)</td>
<td>52</td>
<td>38</td>
<td>34</td>
<td>4300</td>
<td>1700</td>
</tr>
<tr>
<td>Pb (mg kg⁻¹)</td>
<td>21</td>
<td>15</td>
<td>55</td>
<td>840</td>
<td>1100</td>
</tr>
<tr>
<td>Cd (mg kg⁻¹)</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>85</td>
<td>34</td>
</tr>
<tr>
<td>Hg (mg kg⁻¹)</td>
<td></td>
<td></td>
<td>57</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Se (mg kg⁻¹)</td>
<td>10</td>
<td>3</td>
<td>5</td>
<td>100</td>
<td>34</td>
</tr>
<tr>
<td>Mo (mg kg⁻¹)</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>75</td>
<td>94</td>
</tr>
<tr>
<td>Zn (mg kg⁻¹)</td>
<td>691</td>
<td>140</td>
<td>74</td>
<td>7500</td>
<td>4200</td>
</tr>
<tr>
<td>V (mg kg⁻¹)</td>
<td>40</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr (mg kg⁻¹)</td>
<td>28</td>
<td>33</td>
<td>31</td>
<td>2800</td>
<td></td>
</tr>
<tr>
<td>Ni (mg kg⁻¹)</td>
<td>16</td>
<td>20</td>
<td>11</td>
<td>420</td>
<td>420</td>
</tr>
<tr>
<td>Sr (mg kg⁻¹)</td>
<td>387</td>
<td>218</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Pugliese et al., (2014); †EPA, (1993); ‡OFIA, (1999)
Figure 1. Plot setup, and placement of cover boards within each plot.

- Cover board with ash beneath
- Cover board without ash beneath
- Plot boundary
- Buffer boundary
Figure 2. pH of the LFH layer beneath cover boards with/without ash beneath, and for uncovered soil. 95% confidence intervals shown.
Figure 3. Electrical conductivity (EC; ms m\(^{-1}\)) of the LFH layer beneath cover boards with/without ash beneath, and for uncovered soil. 95% confidence intervals shown.
Figure 4. Moisture content (%) of the LFH layer beneath cover boards with/without ash beneath, and for uncovered soil.

95% confidence intervals shown and bound at 0.
Figure 5. Average number of salamanders found per sampling event, per plot. 95% confidence intervals shown and bound at 0.