

Ecological Monitoring 2012  
*rare* Charitable Research Reserve



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### Lists of Acronyms

| <b>Acronym</b> | <b>Description</b>                           |
|----------------|--|
| EMAN           | Ecological Monitoring and Assessment Network |
| ACO            | Artificial Cover Object                      |
| IN             | Indian Woods                                 |
| HO             | Hogsback                                     |
| CA             | Cliffs and Alvars                            |
| SVL            | Snout-Vent Length                            |
| VTL            | Vent-Tail Length                             |
| ANOVA          | Analysis of Variance                         |
| CPUE           | Catch Per Unit Effort                        |
| AIC            | Akaike's Information Criterion               |
| SD             | Standard Deviation                           |
| dbh            | Diameter at Breast Height                    |
| IV             | Importance Value                             |
| SARA           | Species at Risk Act                          |
| SARO           | Species at Risk in Ontario                   |
| ADR            | Annual Decay Rate                            |

## 1.0 Introduction

### 1.1 Ecological Monitoring

Ecological monitoring is the methodical collection of ecosystem data at regular intervals over time (Spellerberg 2005). The value of long-term monitoring has been long stressed in the literature (Wolfe *et al.* 1987; Jeffers 1989; Pimm 1991; Davis *et al.* 1992; Vos *et al.* 2000), as it provides vital information on ecosystem health. Collecting long-term ecosystem data not only provides baseline information for future reference, but also allows for observation of ecosystem changes, in response to both natural and human disturbances (Lindenmayer and Likens 2010). Closely tied with detecting changes is the desire to determine the cause of any observed changes (Vaughan *et al.* 2001), and the potential further impacts on the ecosystem.

Ideally, an extensive long-term monitoring program would look at all representative areas of an ecosystem, including all biotic and abiotic factors and the interactions between them (Davis 1992). Financial limitations (Caughlan and Oakley 2001) and limited manpower often make this difficult and generally infeasible. It is therefore advantageous to monitor one or several indicator species, which are particularly sensitive to environmental change and relatively easy and cost effective to monitor (Noss 1990). Monitoring indicator species can provide information on the health of a community, and can act as an early warning of environmental damage (Draper 2002).

### 1.2 Ecological Monitoring and Assessment Network (EMAN)

The Ecological Monitoring and Assessment Network (EMAN) was established in 1994 by Environment Canada to more closely monitor environmental changes across Canada and to promote prompt environmental planning decisions (Craig and Vaughan 2001). The network was a collaboration of academic, government, and private sector scientists with the collective goal of “*What is changing and why in Canadian ecosystems*” (Vaughan *et al.* 2001). A set of standardized monitoring protocols for terrestrial, marine, and freshwater systems have been developed and are available without cost to promote comparable long-term monitoring across Canada. Until September 2010, an electronic information catalogue system was available for data sharing and metadata analyses (Environment Canada 2012; Craig and Vaughan 2001). The EMAN Coordinating Office is currently closed, preventing data sharing, and the future of EMAN is unknown.

### 1.3 Ecological Monitoring at *rare* Charitable Research Reserve

The *rare* Charitable Research Reserve provides a unique opportunity for monitoring. Located at the confluence of the Speed and Grand River within Waterloo Region, it is 900+ acres of preserved land surrounded by expanding urban development. A high diversity of habitats supports a wide biodiversity of flora and fauna, providing a good representation of local species (Figure A.1).

An ecological monitoring program was established at *rare* in 2006 following EMAN protocols, with the goal of developing baseline data and the hope of creating a long-term protocol to observe changes over time. Due to limitations, such as funding and manpower, monitoring is restricted to indicator species, which are closely tied to environmental changes. Butterfly monitoring began in 2006 on two transects, Cliffs and Alvares and South Field, and was expanded in 2009 to include the newly

acquired Thompson's Tract, and again in 2010 to Blair Flats. Plethodontid salamander monitoring began in 2006 in Indian Woods and was expanded in 2008 to include the Hogsback forest. Benthic invertebrate monitoring occurred at Bauman and Cruickston creeks in 2006, and, continuing on a three year cycle, occurred again in 2009 and 2012 (see separate report). In 2009, the monitoring program was expanded to include forest canopy tree biodiversity plots in the Indian Woods and Cliffs and Alvars forests, with soil humus decay rate monitoring also occurring within the Cliffs and Alvars plot. In 2010, an additional forest health plot was added to the Hogsback forest, and soil humus decay rate monitoring was included in all forest plots. Here, the results of the 2012 monitoring year are reported and discussed.

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## 2.0 Plethodontid Salamander Monitoring

### 2.1 Introduction

#### 2.1.1 Salamander Taxonomy

Ontario is home to salamanders representing four different families (Proteidae, Salamandridae, Ambystomatidae, and Plethodontidae), of which two families are known to be present at *rare*. The mole salamanders (Ambystomatidae) are large burrowing salamanders with an aquatic juvenile phase and terrestrial adult phase (Conant and Collins 1998). Members of this family such as Yellow-spotted Salamanders (*Ambystoma maculatum*) and Blue-spotted Salamanders (*A. laterale*) are occasionally observed at *rare*. Potentially, *rare* may also be home to a population of Jefferson Salamanders (*A. jeffersonian*) however this has yet to be investigated (Table D.1).

The lungless salamanders (plethodontids) are the most frequently observed salamander family at *rare*. Primarily observed are Eastern Red-backed Salamanders (*Plethodon cinereus*), with occasional sightings of Four-toed Salamanders (*Hemidactylium scutatum*). Plethodontids are the largest family of salamanders worldwide representing 27 genera and over 370 recognized species (Larson et al. 2006). These salamanders are generally long and slender and are lungless, breathing through their thin, moist skin (Behler and King 1979). This reliance on cutaneous respiration across moist body surfaces makes plethodontid salamanders particularly sensitive to environmental changes in their micro-habitat (Zorn et al. 2004). Gas exchange requires skin to be moist (Welsh and Droegge 2001) resulting in high absorption rates potentially exposing the salamander to contaminants in the soil.

The Eastern Red-backed Salamanders are the most abundant plethodontid in Eastern Canada (Zorn et al. 2004) and at *rare*. They are completely terrestrial and therefore do not require ponds or vernal pools for development. They can generally be found in moist soil under downed woody debris in mature forests (Conant and Collins 1998). There are two main colour phases of the Eastern Red-backed Salamander- a red-backed morph that has dark grey sides and a rough edged red stripe down the back, and a lead-backed morph that lacks the red stripe and is completely grey.

#### 2.1.2 Plethodontid Salamanders as Indicator Species

Woodland plethodontids, which complete their entire life cycle on the forest floor, are useful indicator species for a forested ecosystem. This is due to their life history traits, sensitivities to anthropogenic stresses, and population sampling properties (Zorn et al. 2004).

Under normal conditions, plethodontid salamanders typically have stable population sizes due to long life spans (10+ years), high annual survivorship, and low birth rates. They have small home ranges (13m<sup>2</sup> for males and juveniles and 24m<sup>2</sup> for females (Kleeberger and Werner 1982)) and display site fidelity, with some species exhibiting occasional territorial behaviours (Peterson et al. 2000; Maerz and Madison 2000). Due to these traits, observed changes in population from long-term monitoring are more likely to be indicative of ecosystem stresses than typical home range shifts or population fluctuations. The role of plethodontid salamanders in the forest ecosystem is an important one. They are efficient predators and quickly metabolize insect and other invertebrate prey, which can result in plethodontid densities equaling or surpassing other vertebrate groups (Burton and Likens 1975). These

high densities provide an ample food source for predators such as snakes, rodents, and birds. Their role, therefore, in transferring energy up trophic levels is invaluable (Zorn et al. 2004).

Being lungless, plethodontid respiration is strongly affected by body moisture and the contact between their skin and contaminants (Welsh and Droege 2001). This sensitivity makes woodland plethodontids useful indicators of ecological stresses influencing their micro-climate and water and air quality. Potential stresses include both human activities (development, pollution, etc.) and natural disturbances (storms, fires, etc.) or any event that may alter soil moisture, quality, or sun exposure (Zorn et al. 2004).

Finally, monitoring and identifying plethodontid salamanders can be done with relative ease. With a limited number of salamanders inhabiting the area, accurate identification can occur with minimal training, and reliable data can be collected from year to year with varying observers and/or volunteers. Additionally, since woodland plethodontids are attracted to artificial cover boards (ACOs) they can be easily sampled avoiding destruction of habitat and unnecessary stress or harm to individuals. Since populations remain relatively stable, population trends can still be detected with small sample sizes (Zorn et al. 2004).

### 2.1.3 Plethodontid Salamander Monitoring at *rare*

In 2004, the Ecological Monitoring and Assessment Network (EMAN) and Parks Canada published a joint National Monitoring Protocol for plethodontid salamanders. The collective goals of this protocol were to work alongside a suite of other standardized protocols to act as an early detection of ecological change and to environmental issues. First and foremost, this protocol aims to provide a standardized methodology for plethodontid monitoring across Canada (Zorn et al. 2004). The protocol involves the establishment of permanent forest monitoring plots which contain a series of wooden ACOs (artificial cover objects) spaced evenly across the forest floor. Zorn et al. (2004) suggest that monitoring should ideally occur in both spring and fall of each year to achieve the best results relating to salamander abundance and community structure as an indicator of ecosystem health.

The salamander monitoring program at *rare* is conducted exclusively in the fall due to monetary and time constraints. It was established in 2006 with the installation of twenty-nine ACOs in the Indian Woods. Following a pause in 2007, the monitoring resumed in 2008 and was expanded to include a second monitoring plot in the Hogsback consisting of twenty ACOs. In 2009, the program was once again expanded with the addition of three ACOs to the already established monitoring plot in the Indian Woods, bringing the total of ACOs in that plot to thirty-two. Monitoring has therefore been ongoing each fall since 2008 in both sites, making this fall the fifth consecutive year of data collection.

Salamanders successfully began using the ACOs within weeks of establishment and continue to use them despite resultant disturbances from the monitoring process. The initial years of this monitoring have resulted in the collection of valuable baseline data regarding salamander populations at *rare* to which data from future years can be compared in order to determine how *rare*'s salamander populations are changing over time. Additionally, McCarter (2009) identified specific research questions regarding the goals and mandates of this monitoring initiative at *rare*:

1. What is the current state (species diversity, abundance, age structure) of the salamander populations in *rare* forests, and how do they compare to one another?

2. What are the long-term trends in Eastern Red-backed Salamander abundance and population structure taking place within Indian Woods and the Hogsback?
3. Is the ecosystem integrity of Indian Woods and the Hogsback being maintained or improved under *rare* management?
  - Ecosystem integrity is defined as an ecosystem that has its native abiotic and biotic components intact and likely to persist (Parks Canada 2009)
4. Is either the ecological health or integrity of Indian Woods and the Hogsback being affected by on-site and nearby changes in land use (i.e. restoration, agriculture, residential development and aggregate extraction)?
  - Ecosystem health is defined as an ecosystem that has the capacity to resist and recover from a range of disturbances, while maintaining its functions and processes (Styers et al. 2010; Twery and Gottschalk 1996)

## 2.2 Methods

### 2.2.1 Monitoring Locations

Indian Woods (IN) is an old-growth Sugar Maple-American Beech (*Acer saccharum* - *Fagus grandifolia*) dominated forest located on the western side of the *rare* property, south of Blair Road and north of Whistle Bare Road. The forest expands approximately 20 acres and contains trees as old as 240 years. The Indian Woods salamander monitoring plot is located on the east side of the ephemeral pond near the south edge of the forest (Figure A.2; Table A.1). The plot is accessed by parking at the South Gate on Whistle Bared Road, and walking north along the Grand Allée trail until a second path merges from the west (left) side. This second trail is marked by a blue square sign with a white arrow. From the point of the trail junction, walk east (right) into the forest towards a large ephemeral pond (approximately 100m). The thirty-two ACOs are distributed in a large square made up of four lines of eight ACOs each (Figure A.4). Boards five, six, and seven were missing prior to 2009.

The Hogsback (HO) is a 57-acre forest located approximately 700m southeast of Indian Woods, south of Blair Road and just west of the Newman Drive subdivision. It is comprised of mixed swamp interspersed with ridges of upland forest characterized by Red Maple (*Acer rubra*) and White Pine (*Pinus stroba*). The Hogsback salamander plot is accessed through South Gate, off of Whistle Bare Road, and heading east along the lane to where it turns at the edge of the Hogsback. On foot, keep left and walk north and then east along the edge of the forest, finally heading south into the stand at the area of downed fence marked by pink flagging tape on a fallen log. Continue south into the stand for approximately 50m to the monitoring plots (Figure A.2; Table A.1). Twenty ACOs are distributed in a large rectangle with seven ACOs on the north and south sides and three ACOs on the east and west sides (Figure A.4). Each board is identified with a writeable aluminum tag marked as follows: SITE-YEAR - NUMBER (e.g., HO-08-01) and is flagged with pink or orange flagging tape on an adjacent shrub or tree.

### 2.2.2 Monitoring Protocol

One month prior to the start of monitoring, all ACOs in both Indian Woods and the Hogsback were visited to ensure proper positioning and clear labelling. If necessary, boards were repositioned so

that they were flush against the soil and reoriented into their original location. As the boards have been in place for multiple years, the proper positioning is generally noticeable as an area of bare soil. Labels and flagging tape were replaced as needed, and any holes in the boards were packed with soil to prevent salamanders from hiding during monitoring. Boards that were missing or too damaged or decomposed to be viable were replaced by newly cut boards, and relabeled with the current year.

Each plot was monitored once a week for nine successive weeks from the end of August to the end of October. Indian Woods and the Hogsback were monitored for only five weeks in their pilot years, 2006 and 2008 respectively. Sample datasheets for salamander monitoring can be found on the *rare* server and in Figure C.1 and Figure C.2.

At the beginning of each monitoring session, water was collected into a squeeze bottle from the education pond behind Lamb's Inn. This water was used to calibrate the soil moisture meter (Lincoln Irrigation Corporation, Lincoln, Nebraska, USA) by adjusting the meter with a screw driver so that it read a moisture rating of "10: saturated" when the probe was completely immersed in the water. The start time for the entire monitoring plot and Beaufort's wind and sky codes were recorded on the data sheet at the start of monitoring (Table C.1; Table C.2). Additionally, the precipitation from the 24hrs prior to monitoring was recorded using the data collected by the Environment Canada Weather Office for the Region of Waterloo Airport. In the Indian Woods, the depth of the ephemeral pond was recorded using the measuring stick permanently in place. The first 3cm of measuring stick was submerged in mud in 2012, differing from the first 5cm submerged in other years, so 3cm were subtracted from the measured depth to get the true water level. In 2012, water levels were extremely low so it was possible to see the level of submersion of the measuring stick.

Boards were always visited in sequential order starting with one. Soil temperature (°C) and moisture measurements were collected at each ACO by inserting the probes of the soil thermometer (Ashcroft® Thermometers, USA) and soil moisture meter to a depth of 10cm, as marked with tape on the probes, in the soil beside the board. The ACO was then gently turned over and any salamanders underneath were collected by the observers wearing nitrile gloves and placed into a plastic container with a sponge dampened with pond water previously collected in squeeze bottle. Each salamander was identified to species (colour phase was indicated for Eastern Red-backed Salamanders) and any noticeable physical defects were recorded. A list of common and scientific names for all salamanders observed at *rare* and their abbreviated codes is available in Table D.1. Salamanders were weighed on a digital scale (Equal Digital Scale, model #23-D-50, capacity 50g) in grams to two decimal places. Snout-vent length (SVL) and vent-tail length (VTL) were recorded for each individual using a set of digital calipers (TuffGrade IDI, Commercial Solutions, Alberta, Canada). To ensure measurements were recorded accurately from the vent, individuals were measured through a clear lid while pressed up against moist sponges in the base of the container to secure the salamander and view the ventral side. Following measurements, salamanders were released next to the board. Disturbances under or near the ACOs (e.g. snakes, ant nests, turkey scratches, fungus/mold, ACO movement) were also recorded.

In each monitoring plot, specific ACOs were assigned the status of weather station and each weather station represents a specific subset of ACOs. Table 2.1 and 2.2 show which ACOs are associated with each weather station in Indian Woods and the Hogsback respectively. When each weather station is reached during the monitoring of boards in sequential order, weather variables including average wind speed (taken as the average after ten seconds), air temperature (°C) and percent relative humidity

were collected using the Kestrel 3000 (Nielson-Kellerman, Boothwyn, PA, USA). Following week six, the Kestrel was unable to measure wind speed due to equipment malfunction and wind speed was subsequently estimated according to the Beaufort scale for the remaining weeks of monitoring. A complete list of required equipment is available in List B.1.

**Table 2.1:** Weather stations and the artificial cover objects (ACOs) associated with them in the Indian Woods salamander monitoring plot.

| <b>Weather Station ACO Number</b> | <b>Associated ACOs</b> |
|-----------------------------------|------------------------|
| 3                                 | 1,2,3,4                |
| 7                                 | 5,6,7,8                |
| 11                                | 9,10,11,12             |
| 15                                | 13,14,15,16            |
| 18                                | 17,18,19,20            |
| 23                                | 21,22,23,24            |
| 27                                | 25,26,27,28            |
| 31                                | 29,30,31,32            |

**Table 2.2:** Weather stations and the artificial cover objects (ACOs) associated with them in the Hogsback salamander monitoring plot.

| <b>Weather Station ACO Number</b> | <b>Associated ACOs</b> |
|-----------------------------------|------------------------|
| 2                                 | 1,2,3,4,5              |
| 7                                 | 6,7,8,9,10             |
| 12                                | 11,12,13,14,15         |
| 17                                | 16,17,18,19,20         |

Additionally, soil samples for pH testing were collected from both Indian Woods and the Hogsback at each weather station one month after monitoring had been completed. Three samples were collected from a depth of 10cm from the ground adjacent to the ACO weather station. Samples were brought back to the office and left open to dry for one week prior to pH testing. A Hellige-Truog Soil pH Tester Kit (Forestry Supplies Inc., Jackson, MS, USA) was used to determine the pH for each sample, and the three samples from each weather station were averaged to give a mean pH per weather station. A complete list of required equipment is available in List B.2.

### 2.2.3 Data Analysis

Data were analysed using Microsoft Excel 14.0.6 (Microsoft 2010) and PASW Statistics 17.0 (SPSS Inc.) for Windows. Prior to analysis, assumptions of parametric testing were examined. When transformation was required, the appropriate transformation to decouple variance and mean was determined using Taylor’s Power Law (Perry 1981). Otherwise, the best transformation was applied and the most robust tests were used, followed by cautious interpretation of results. Each salamander

monitoring plot (Indian Woods and the Hogsback) was interpreted as representing a unique population, and each ACO within that plot was interpreted as representing a sample of that population.

Since each monitoring plot had a differing number of ACOs and since in 2006 and 2008 the Indian Woods monitoring plot had three less ACOs than in later years, data had to be standardized to allow for comparisons. Abundance was therefore transformed into catch per unit effort (CPUE) for each monitoring session, as is commonly used in fisheries science (Krebs 2001). To calculate CPUE, the total salamander count for each monitoring session was divided by the number of ACOs in that plot to get the mean weekly catch per ACO. The CPUE calculation included only Eastern Red-backed Salamanders.

A univariate analysis of variance (ANOVA) with two fixed factors (plot and year) was used to look for differences in salamander abundance represented by CPUE. A two-way ANOVA split by plot was used to investigate weekly differences in salamander abundance, with week and year as independent variables. A two-way ANOVA split by plot was used to examine differences in species composition across all years. When interactions occurred data were either split (Zar 1999) or variables were combined and recoded into plot/year combination variables (Leech et al. 2008) depending on the question of interest. This was followed by Bonferroni post hoc testing to determine where the differences between the levels occurred.

Only Eastern Red-backed Salamanders (both colour phases) were considered in a size class comparison. Individuals were classified as either an adult, intermediate, or juvenile based on their snout-vent length as outlined in Zorn et al. (2004). Age classes were defined as follows: juveniles <25mm; intermediates 25mm-35mm; adults >35mm. Eastern Red-backed Salamanders are capable of tail autonomy (Wise and Jaeger 1998), and so while vent-tail length was also measured it is not a reliable indicator of size class. An ANOVA with three fixed factors (plot, year, and size class) was used to look for differences in salamander size class. Interactions between factors would represent that a size class varies among plots or years. Bonferroni post hoc testing followed to determine where differences occurred.

Each plot was analysed separately for their relationship with environmental parameters, as sampling effort varied with plot. 2006 and 2008 (Indian Woods) were eliminated from this analysis since its sampling effort varied from other years. To determine which environmental factors (soil temperature, soil moisture, soil pH, pond depth, precipitation, sky and wind codes, wind speed, relative humidity, and air temperature) affected total salamander abundance, multiple linear regressions were used. Preliminary assessments indicate that no parameters affected abundance in the Hogsback, and in the Indian Woods soil pH, pond depth, precipitation, sky and wind codes, wind speed, and relative humidity did not affect abundance. Further analysis therefore focused only on the Indian Woods, considering soil temperature, soil moisture, air temperature, and year. Hierarchical multiple regressions followed with total abundance as the dependent variable and related parameters as the independent variables. Variables were entered into models based on their inherent relationship with salamanders (i.e. since salamanders live in the soil, soil factors were likely important). How well each model predicted the dependent variable- the goodness of fit of each model- was tested using the Akaike's Information Criterion (AIC) model selection technique.

## 2.3 Results

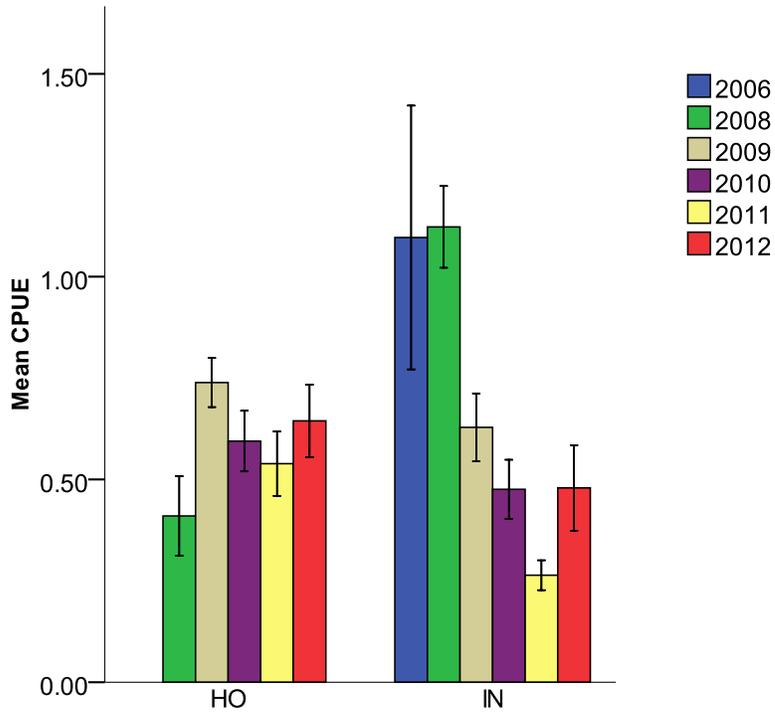
### 2.3.1 Total Abundance

A total of 256 salamanders were observed between September 4 and October 30 at the *rare* Charitable Research Reserve in 2012. In Indian Woods, 138 salamanders were observed, starting with 1 during the first week of monitoring and increasing to a peak number of 31 observations on October 16, before gradually falling to 3 on October 30. In the Hogsback, 118 salamanders were observed, starting with 14 during the first week of monitoring and increasing to 21 observations on October 16th, before falling to 5 on October 30.

Eastern Red-backed Salamanders represented 99.2% of detections; 87.5% were the red-backed form, 11.7% were the lead-backed form of the same species. The remaining 0.8% of salamanders found under ACOs were comprised of one Yellow-spotted Salamander and one Blue-spotted Salamander. Using age classes outlined in Zorn et al. (2004), 66.5% of the total detections of the Red-backed Salamanders were adults. There are 46 instances with two salamanders under one board, and 13 instances of three or more.

### 2.3.2 Eastern Red-backed Salamander Abundance

Plot differences varied with years (interaction  $F_{4,80}=0.468$ ,  $p=0.001$ ), so both factors were considered simultaneously in an eleven-level combination variable of plots and years (Leech *et al.* 2008), and significant differences occurred between these levels (ANOVA  $F_{10,80}=5.586$ ,  $p<0.001$ ). Within years, CPUE at each plot generally did not significantly differ, although largely more observations were documented in the Hogsback. The exception is 2008, the pilot year for monitoring in the Hogsback, where there was a significantly higher CPUE in Indian Woods (post hoc  $p=0.004$ ). CPUE in 2008 at Indian Woods is the highest on record, and significantly differs from several subsequent years at both sites (post hoc  $p<0.017$ ). CPUE in 2011 at Indian Woods is the lowest on record, and significantly differs from the first two years of Indian Woods sampling (post hoc  $p<0.001$ ) as well as CPUE in the Hogsback in 2009 (post hoc  $p=0.01$ ). Within the Hogsback, no years significantly differed (Figure 2.1).



**Figure 2.1:** Average weekly salamander observation per ACO (Catch per Unit Effort (CPUE)) for both Indian Woods (IN) and Hogsback (HO) throughout monitoring, 2006, 2008-2012. Error bars represent +/- one standard error.

Differences in salamander abundance were examined across weeks (Figure 2.2). This analysis used total weekly salamander abundance as the dependent variable as opposed to CPUE, and excluded years 2006 and 2008 when sampling efforts differed. Since number of ACOs in each plot differed, Indian Woods and Hogsback were examined independent of one another. No significant differences occurred between weeks at either plot (IN:  $F_{8,351}=1.086$ ,  $p=0.372$ ; HO:  $F_{8,286}=1.366$ ,  $p=0.211$ ).

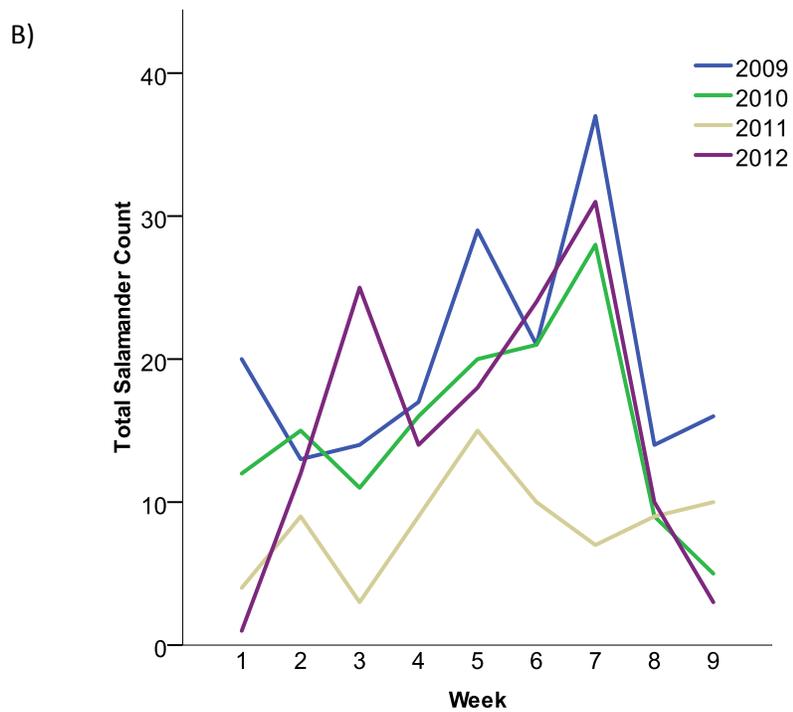
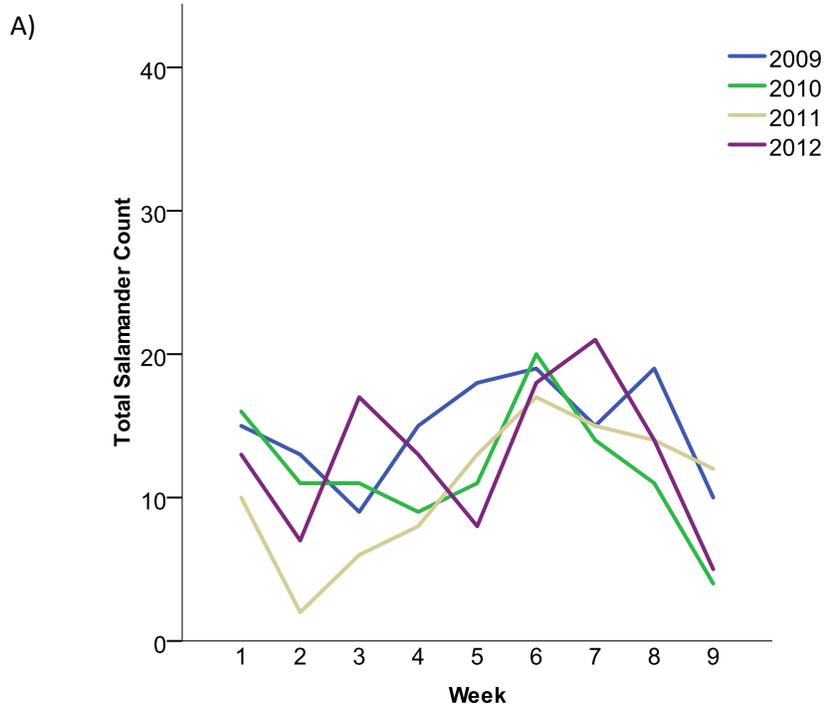
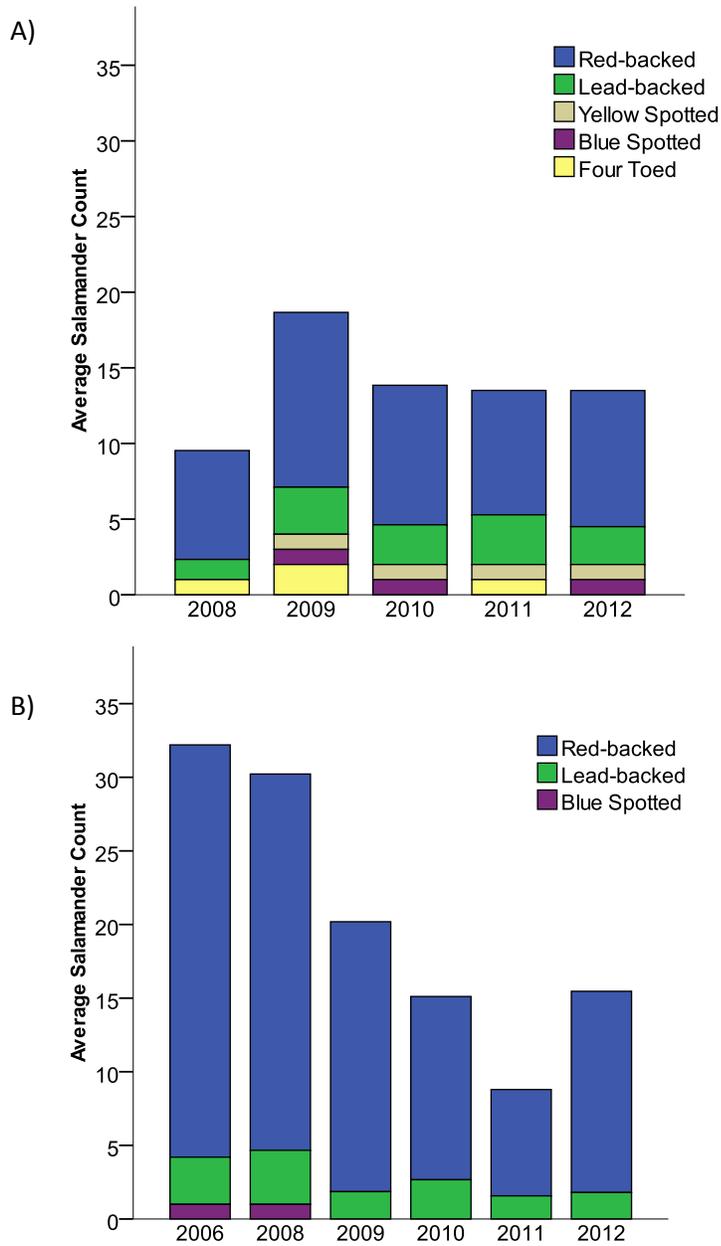


Figure 2.2: Total weekly salamander counts in A) the Hogsback and B) Indian Woods from 2009 to 2012.

### 2.3.3 Salamander Species Composition

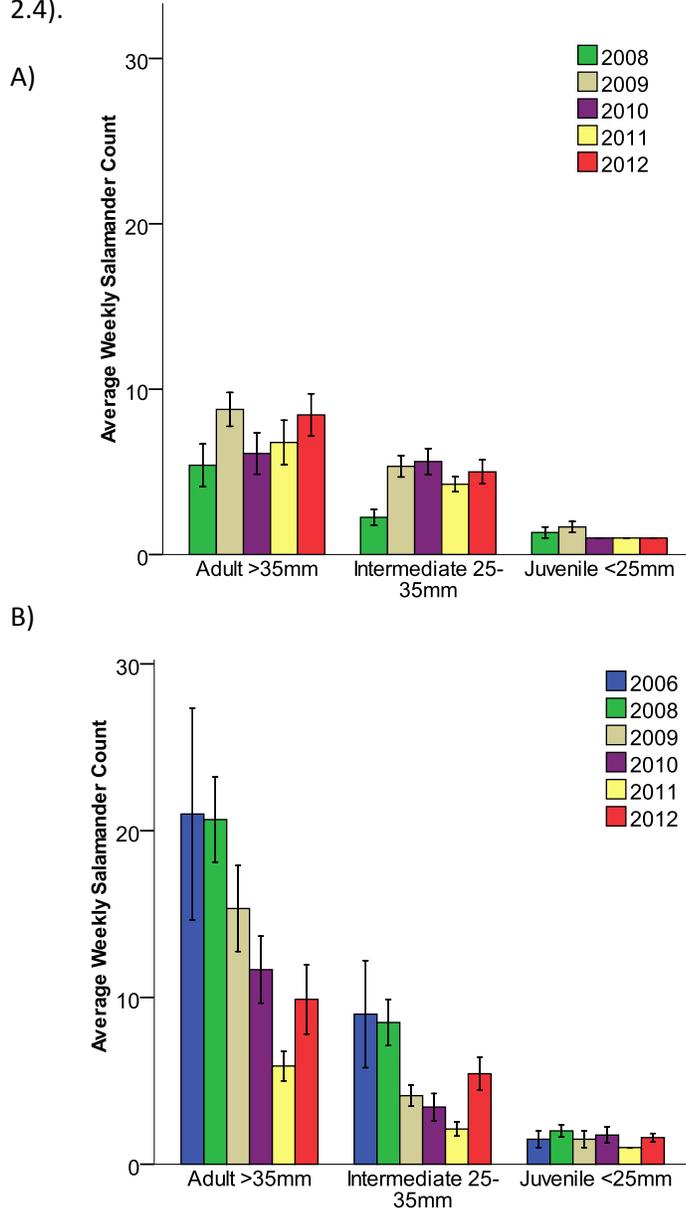
Plot differences varied with species (interaction  $F_{1,153}=7.385$ ,  $p=0.007$ ) and year (interaction  $F_{4,153}=3.252$ ,  $p=0.014$ ), so data were split by plot (Zar 1999) and Indian Woods and Hogsback were each considered independently of one another. In both plots, significant differences occurred between species ( $p<0.001$ ), with significantly more Eastern Red-backed Salamanders occurring than any other species regardless of year (post hoc  $p<0.001$ ). Five species have been observed in the Hogsback since 2008, and only three species have been observed in Indian Woods since 2006 (Figure 2.3).



**Figure 2.3:** Average salamander abundance by species for each monitoring year in A) the Hogsback and B) Indian Woods.

### 2.3.4 Eastern Red-backed Salamander Size Class Distribution

Size class interacted with plot ( $F_{2,180}=5.833$ ,  $p=0.004$ ) so data were split by plot and Indian Woods and Hogsback were investigated separately. In both plots total weekly salamander observations significantly differed by size class (Indian Woods:  $F_{2,104}=80.037$ ,  $p<0.001$ ; Hogsback:  $F_{2,76}=41.554$ ,  $p<0.001$ ), regardless of year (no interaction Indian Woods:  $F_{2,104}=1.173$ ,  $p=0.317$ ; Hogsback:  $F_{2,76}=0.820$ ,  $p=0.587$ ). All size classes significantly differed from one another in both plots (post hoc  $p>0.005$ ; Figure 2.4).



**Figure 2.4:** Average size distribution of salamanders observed weekly during monitoring in both A) Hogsback and B) Indian Woods from 2006 to 2012. Error bars represent +/- one standard error.

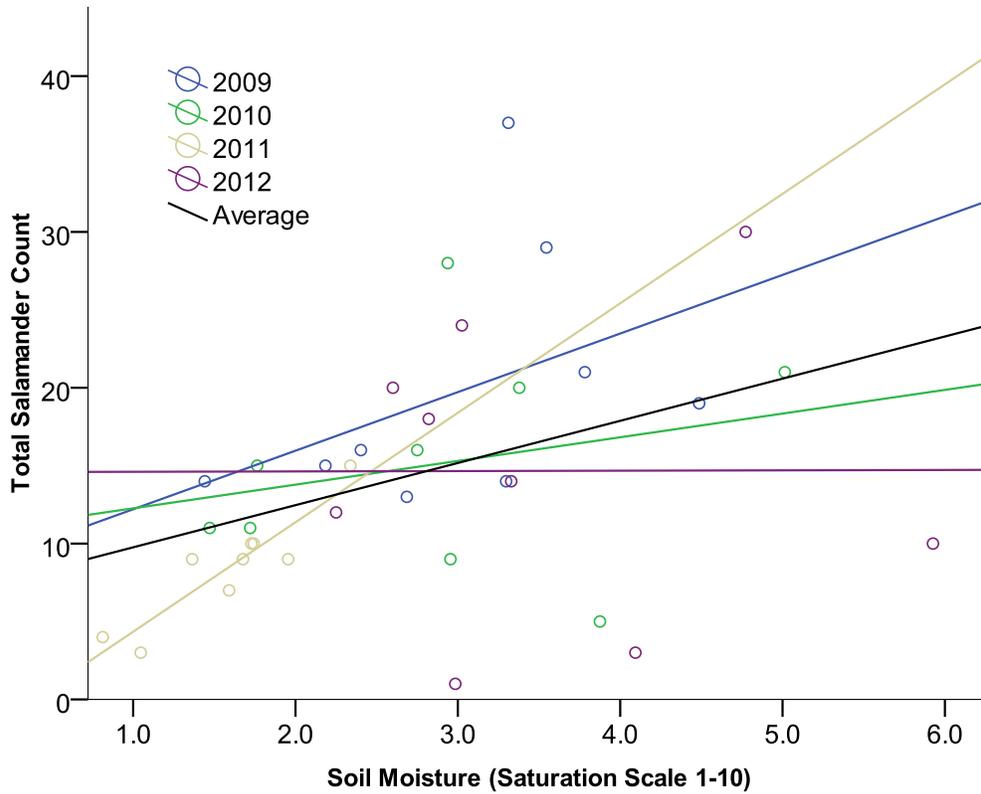
### 2.3.5 Environmental Parameters

Since air temperature and soil temperature were highly correlated ( $r=0.862$ ;  $p<0.001$ ), only one could be included in the analysis and air temperature was therefore removed. The best models predicting salamander abundance in the Indian Woods included soil moisture, soil temperature, and year ( $F_{2,33}=6.631$ ,  $p=0.004$ ,  $r^2=0.243$ )(Table 2.3). Salamander abundance had an overall positive relationship with soil moisture ( $r^2=0.153$ ), particularly high in 2009 and 2011 and low 2012 (Figure 2.5). In the Hogsback, no factors significantly affected salamander abundance and no models were significant predictors.

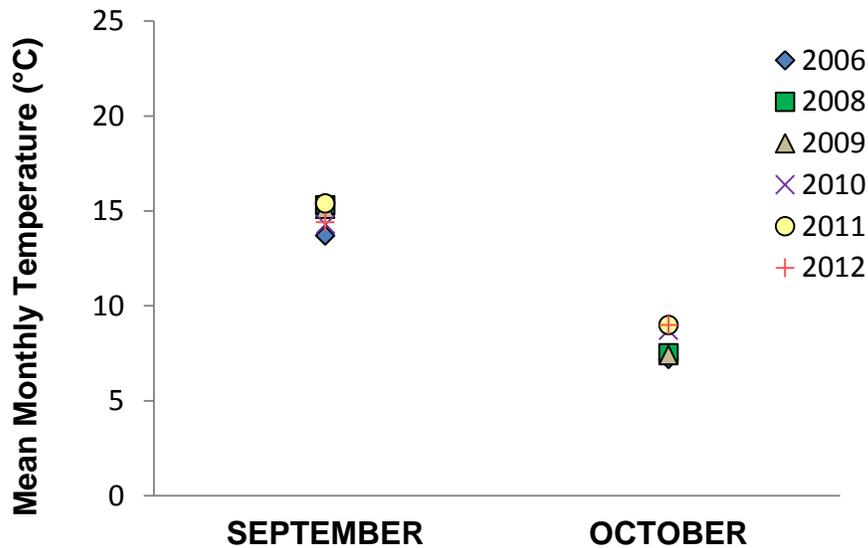
Monthly temperatures during the 2012 monitoring seasons were similar to previous years (Figure 2.6). Precipitation levels were higher than previous years, particularly in October which saw an abundance of rain (Figure 2.7).

**Table 2.3:** Results of hierarchical multiple regressions and AIC model selection using year, soil moisture, and soil temperature to predict salamander abundance in Indian Woods from 2009 to 2012. Plots were examined separately due to differing sample effort. Independent variables were log-transformed to meet parametric assumptions. The magnitude and direction of each independent variable's influence is represented by the standardized beta coefficient ( $\beta$ ).  $\Delta$ AIC is the difference in Akaike's Information Criterion from the above model and  $AIC_w$  is the likelihood of a model being the best model, with the best model indicated in bold.

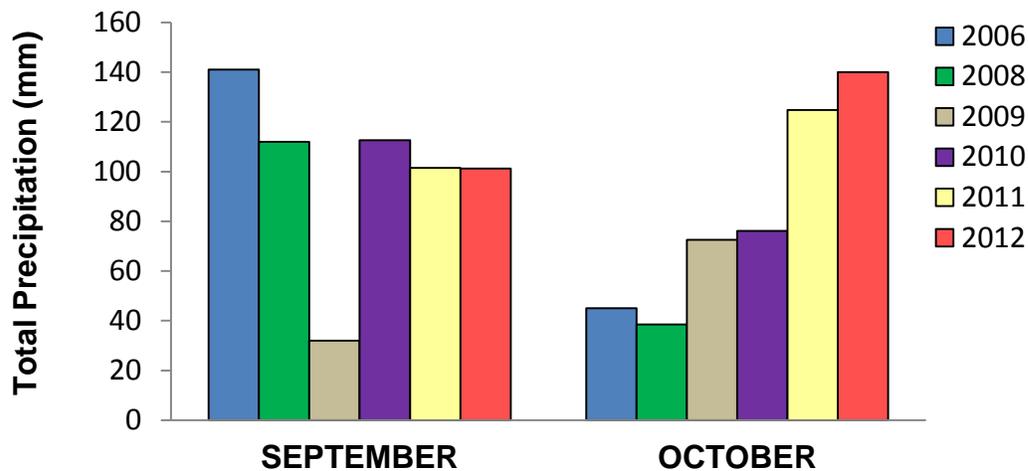
| Model                                   | F            | R <sup>2</sup> | P-value      | $\beta$ Soil Moisture | $\beta$ Soil Temperature | $\beta$ Year  | $\Delta$ AIC | $AIC_w$      |
|---|--------------|----------------|--------------|-----------------------|--------------------------|---------------|--------------|--------------|
| Soil Moisture                           | 8.260        | 0.195          | 0.007        | 0.442                 |                          |               | 2.332        | 0.146        |
| Soil Moisture + Soil Temperature        | 4.417        | 0.211          | 0.020        | 0.396                 | -0.134                   |               | 3.623        | 0.077        |
| Soil Moisture + Soil Temperature + Year | 4.727        | 0.307          | 0.008        | 0.387                 | -0.153                   | -0.310        | 0.955        | 0.291        |
| <b>Soil Moisture + Year</b>             | <b>6.631</b> | <b>0.287</b>   | <b>0.004</b> | <b>0.440</b>          |                          | <b>-0.302</b> | <b>0</b>     | <b>0.469</b> |
| Year                                    | 3.504        | 0.093          | 0.070        |                       |                          | -0.306        | 6.630        | 0.017        |



**Figure 2.5:** Relationship between total salamander abundance at Indian Woods and measured soil moisture for 2009 to 2012.



**Figure 2.6:** Mean monthly temperatures for Waterloo Region during the salamander monitoring season in 2006, 2008-2012 (Environment Canada- 2006, 2008-2009 data from Waterloo International Airport Weather Station, and 2010-2012 data from Kitchener-Waterloo Weather Station).



**Figure 2.7:** Total monthly rainfall for Waterloo Region during the salamander monitoring season in 2006, 2008-2012 (Environment Canada- 2006/2008-2009 data from Waterloo International Airport Weather Station, and 2010-2012 data from Kitchener-Waterloo Weather Station).

## 2.4 Discussion

### 2.4.1 Eastern Red-backed Salamander Abundance

Given their importance in food web dynamics and sensitivity to changes in forest floor conditions, significant changes in plethodontid salamander populations over time may be an early warning of ecosystem stress. Recognizing a population change that could be acting as an early warning sign as opposed to natural population fluctuations requires a monitoring target or threshold to be set (Zorn et al. 2004). Zorn et al. (2004) recommends a monitoring threshold set at “a statistically significant change in plethodontid counts at a plot level over 5 or more years”. With variable sampling effort in the first years of data collection, five consecutive and consistent years of data collection will be completed in 2013. Information gathered on salamander populations in the inaugural years does not contribute to the EMAN protocol for testing monitoring thresholds as it is suggested that the ACOs weather in situ for a winter prior to monitoring to avoid skewing abundance estimates due to the disturbance of plot establishment. Regardless, all data surrounding salamander abundance and diversity is of great value to *rare*, and we can compare the yearly salamander abundance data collected to date.

In Indian Woods, the first two years of monitoring had the highest abundances on record, followed by a steady decline culminating at the lowest abundance recorded in 2011 (Figure 2.1). 2012 abundances rebounded to levels similar to 2009 and 2010. Original establishment of ACOs in the Indian Woods may have impacted the observed abundances by providing additional cover, acting as an artefact in attracting salamanders in early years and leveling out as ACOs became weathered and established over time (Van Wieren 2003). Studies on this topic are varied, with some reporting salamanders almost immediately making use of cover boards (Ballantyne 2004; Bennett et al. 2003; Monti et al. 2000) and others suggesting boards must be left for a year to weather before data collected is valid (Zorn et al. 2004; Droege et al. 1997). It may be dependent on other factors, as in Ballantyne (2004), where excess

precipitation just prior to and at the start of monitoring may have sped the weathering process, making the boards more appealing to salamanders. The low abundance observed in 2011 may be attributable to the high precipitation levels. Jaeger (1972, 1980) reports that cover objects become more important during dry periods, acting as a moisture refuge for salamanders. Given this, salamanders may be less dependent on cover boards in wetter years, having more moist spaces to use for foraging, and thus lower abundances may be observed under ACOs (Van Wieren 2003). However Fall precipitation in 2012 was similar to 2011 and salamander abundances observed were higher, so again many factors including temperature, moisture, and available cover can be having an impact on abundances (Heatwole 1962; Spotila 1972; Feder and Pough 1975; Jaeger 1972, 1979, 1980; Feder 1983; Feder and Londos 1984; DeMaynadier and Hunter 1998; Herbeck and Larsen 1999). While no significant differences were found between weekly abundances, there was variation between weeks with generally a peak at the seventh week and a rapid decline in the two weeks following (Figure 2.2).

In the Hogsback, no yearly differences in abundance have occurred (Figure 2.1). Contrary to the establishment years in the Indian Woods, the original monitoring session in the Hogsback is the lowest of all years, suggesting perhaps acclimatization of the newly placed ACOs was taking place (Zorn et al. 2004; Droege et al. 1997). The relatively consistent observed abundances in the Hogsback are an encouraging trend, reflecting a likely stable population. Following the collection of five consecutive years of data as per the suggestion of EMAN, *rare* will have a better understanding of the significance of these trends in Indian Woods and the Hogsback.

#### 2.4.2 Salamander Species Composition

While the monitoring program at *rare* is primarily designed for plethodontid salamanders (Zorn et al. 2004), other species have also been observed on the property. In the last four years in Indian Woods, only Red-backed Salamanders have been observed with the red-backed colour phase being dominant (Figure 2.3B). Similarly in the Hogsback, while there is a greater species diversity, Red-backed Salamanders are again dominant with the red-back phase more abundant than the lead-back phase (Figure 2.3A). This is unsurprising, as the lead-backed phase experience preferential predation pressures (Moreno 1989; Venesky and Anthony 2007) and the red-backed phase is known to be proportionately higher in more areas and at higher latitudes (Lamond 1994; Harding 1997).

Salamander species diversity has been low in the Indian Woods, where there have only been two individuals observed that were not Eastern Red-backed Salamanders. In both 2006 and 2008, a Blue-spotted Salamander was recorded during October. Mole salamanders are more easily found in the spring (Whitford and Vinegar 1966) and therefore their presence in these early years may have been an abnormality as opposed to their absence in later years. In the future, expanding monitoring to include the spring season may allow for a more complete representation of the salamander species diversity at *rare*.

Species diversity is higher in the Hogsback than Indian Woods (Figure 2.3). Four-toed Salamanders, another member of the plethodontid family, have been observed in 2008, 2009, and 2011. It is typically found in sphagnum moss or boggy woodlands (Conant and Collins 1998), the latter of which is found in the Hogsback forest stand. Multiple mole salamanders have been observed; Blue-spotted Salamanders in 2009, 2010, and 2012 and consecutive observations of Yellow-spotted Salamanders from

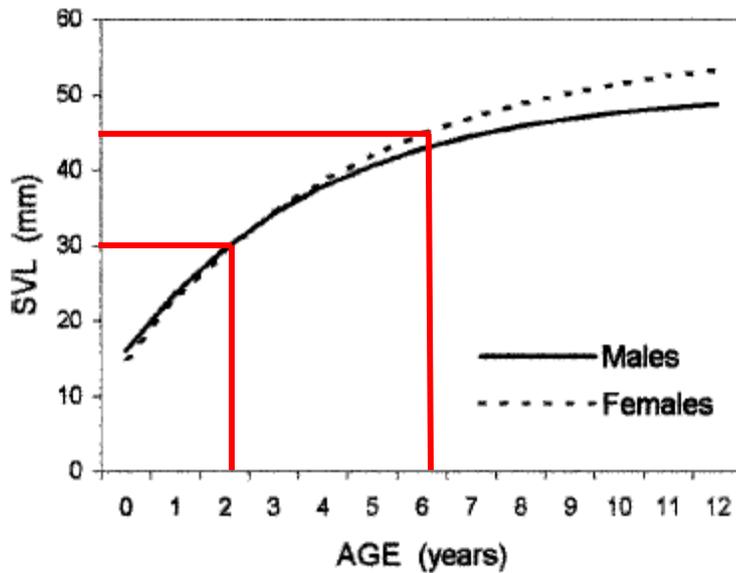
2009 to 2012. Likely this is a repeat observation of the same individual, which has always been observed under the same board. This suggests salamanders may exhibit fidelity to ACOs. Expanding monitoring efforts at *rare* to include gender and individual identification may be of benefit.

#### 2.4.3 Eastern Red-backed Salamander Size Class Distribution

In both Indian Woods and the Hogsback, the greatest proportion of Eastern Red-backed Salamanders in 2012 fell within the snout-vent length range of 40mm-45mm. Salamanders measured in the Hogsback were on average slightly longer (mean SVL: 36.62+/-6.93) and heavier (mean weight: 0.930+/-0.394) than those in the Indian Woods (mean SVL: 38.20+/-5.91; mean weight: 0.975+/-0.446). Based on size class categories outlined in Zorn et al. (2004), significantly more adults were found in both plots than intermediates and juveniles, and further there are significantly more intermediates observed than juveniles (Figure 2.4). A significant positive correlation between unsexed salamander size and age in their first four years has been documented (LeClair et al. 2006). Based on their results, the majority of salamanders found under ACOs at *rare* are between the approximate ages of two and six (Figure 2.8). If other size class distinctions had been used to categorize salamanders at *rare*, such as those outlined in Saylor (1966) and subsequently used in additional studies (Brooks 1999; Ballantyne 2004), data would have been shifted toward more intermediate sized salamanders. In either case, few juveniles (or first year young) have been found under the ACOs at *rare* in either forest stand over monitoring years.

Juvenile populations may be underrepresented by ACO sampling. Adults may be exhibiting territorial behaviours that outcompete juveniles for space (Marsh and Goicochea 2003), or, in the fall, this behaviour could be in connection to mating (Van Wieren 2003). Of twenty-seven occasions in the Indian Woods where multiple Red-backed Salamanders were found under the same ACO, only three occasions involved juveniles. Similarly in the Hogsback, of twenty-six occasions with multiple salamanders located under a single ACO, only two involved juveniles. Red-backed Salamanders have been shown to exhibit kin selection, allowing related juveniles into their territories in stressful conditions (Horne and Jaeger 1988; Jaeger et al. 1995; Simons et al. 1997) however this seems to be occurring minimally, if at all, during the fall months at *rare*. Territoriality of boards in connection to mating may be part of the cause for the underrepresentation of juveniles in this study.

Another likely hypothesis is that larger salamanders prefer the wider cover provided by ACOs. Mathis (1990) and Moore et al. (2001) found significant positive correlations between salamander size and cover object size. ACOs used in this study may therefore be more attractive to larger adults. Gabor (1995) found this relationship with cover object size and salamander size existed only where direct sunlight reached the board. In cases where direct sunlight does not heat the boards, cover objects were chosen in relation to food quality and quantity in surrounding areas. We do not currently record canopy cover during monitoring, but based on its potential impact it may be an important factor to add in the future.



**Figure 2.8:** Growth in length (SVL) of Red-Backed Salamanders modified from LeClair et al. 2006. Red lines bound the dominant size range observed at *rare* plots.

Adult and intermediate sized salamanders seem to have been steadily declining in the Indian Woods up until 2012. It could be that 2011 was an anomaly year with lower than usual abundances and what we see in 2012 is a general norm. Alternatively, and more troublingly, the steady decline in adults, intermediates (Figure 2.4), and total salamander observations in general (Figure 2.1) could be an ongoing trend interrupted by 2012, which was undoubtedly an anomaly year for weather- an extremely dry summer punctuated by a fairly wet fall. Only the continuation of monitoring can help to answer these questions. The same declining trend is not observed in the Hogsback. Adult and intermediate sized salamanders exhibit a general steadiness over monitoring years. The Hogsback, a forest-wetland complex, appears to be less disrupted by annual climactic fluctuations that the remnant old growth Indian Woods.

#### 2.4.4 Environmental Parameters

Many factors have been shown to impact plethodontid salamanders including temperature (Spotila 1972; Feder & Pough 1975), moisture (Grover 1998; Feder & Londos 1984), soil pH (Wyman and Hawksley-Lescault 1987; Sugalski and Claussen 1997; Moore and Wyman 2010) and others (Heatwole 1962; Feder 1983; DeMaynadier and Hunter 1998; Jaeger 1972, 1980). This study found that the location of the plot had a large effect on whether or not environmental or temporal variables had an impact on abundance. In the Hogsback, a forest-wetland complex with a thick canopy, no relationship was found between salamander abundance and any tested variables (soil temperature, soil moisture, soil pH, precipitation, sky and wind codes, wind speed, relative humidity, air temperature, and year). In the Indian Woods, a remnant old-growth forest with a thin, sparse canopy in areas, total abundance was related to temperature of both the air and soil, moisture level in the soil, and year of monitoring.

The significance of the temporal variable year is interesting (Table 2.3), since plethodontid salamander populations typically have high stability (Welsh and Droege 2001; Zorn et al. 2004). Some form of population cycling could possibly be accountable for this observed effect, or perhaps this can be attributed to predator-prey cycling, since plethodontids are known to be aggressive predators of soil invertebrates (Wyman 1988) and can significantly reduce soil detritivore numbers (Wyman 1998). Alternatively, this could be a reflection of extremely variable yearly conditions which may be more influential in the more exposed Indian Woods.

The significance of soil moisture on salamander abundance is not surprising (Table 2.3). Plethodontid salamanders require moist skin to facilitate gas exchange across their cutaneous membrane for respiration (Behler and King 1979; Welsh and Droege 2001). These salamanders are therefore highly dependent on receiving moisture from their micro-environment and are most likely to reside in damp wooded areas (Froom 1982). Plethodontid behaviours, such as foraging and reproduction, can be altered depending on the moisture available in their microhabitats. During cool, moist weather they can disperse across the forest floor, while in drier conditions they would be confined to moist microhabitats or spend very little time in dry exposed areas (Jaeger 1972, 1980; Feder 1983; Droege et al 1997). The relationship between soil moisture and total abundance in the Indian Woods is overall a strong positive one, where more salamanders are observed where the soil moisture is higher (Figure 2.5). This relationship was particularly strong in 2011 ( $r^2=0.834$ ), a year where soil moisture measurements were lower than any other year (Table 2.4). Average air temperature was higher than other years, and humidity was lower so the boards may have been providing crucial moisture refuges for salamanders when moist microhabitat was limited (Jaeger 1972; Van Wieren 2003). This relationship was additionally strong in 2009 ( $r^2=0.182$ ), a year when precipitation in the fall, particularly September, was especially low (Figure 2.7). Likely a similar moisture refuge was occurring. The relationship is weakest in 2012 ( $r^2=8.498 \times 10^{-6}$ ) when an abundance of rainfall (Figure 2.7) and high average soil moisture (Table 2.4) may have made a ubiquitously wet environment, reducing dependency on cover boards to provide a moist microhabitat.

**Table 2.4:** Average soil moisture levels during the salamander monitoring season in 2009-2012 at Indian Woods and the Hogsback.

| Plot         | Year        | Mean Soil Moisture Level |
|--------------|-------------|--------------------------|
| Indian Woods | 2009        | 3.02+/-0.93              |
|              | 2010        | 2.87+/-1.14              |
|              | <b>2011</b> | <b>1.58+/-0.460</b>      |
|              | 2012        | 3.53+/-1.18              |
| Hogsback     | 2009        | 4.87+/-11.07             |
|              | 2010        | 5.47+/-1.42              |
|              | 2011        | 3.65+/-0.912             |
|              | 2012        | 4.63+/-1.37              |

Sugalski and Claussen (1997) found soil pH to be the more influential factor on salamander distribution, more so than soil moisture. This is not the case in this study, likely because soil conditions in both forest stands fall within or close to their preferred pH range of 6.0 to 6.8 (Heatwole 1962), and it is suggested that plethodontid salamanders avoid soil with a pH of 3.8 or less (Wyman and Hawksley-Lescault 1987; Wyman 1988). In future monitoring years, if pH becomes a more accurate predictor of salamander abundance, it may be an early warning sign of soil acidification.

## 2.5 Conclusions and recommendations

With the addition of one more monitoring year, both plots will have met the minimum requirements for a monitoring threshold and *rare* can begin to look for long-term changes in salamander populations. This program acts as an early warning sign for environmental change in two of *rare*'s forest stands. It is therefore recommended that a full 9 week monitoring program continue at both plots. In the future, the addition of a spring monitoring session would be an asset to *rare*, when it is feasible to run long-term. This will allow *rare* to gather a better understanding of the true biodiversity of species on the property, and may tease apart some of trends relating to the monitoring season.

One definite gap in the current data collection is the lack of information on canopy cover at ACOs. Since we know that most salamanders react negatively to bright light (Test 1946; Ray 1970) and that salamanders appear to prefer closed canopy interior conditions (DeMaynadier and Hunter 1998), it would be beneficial to measure percent canopy cover at each board in addition to the other environmental parameters that are currently measured. Direct sunlight on boards may increase temperature and decrease moisture (Heatwole 1962; Fite 1976; Roth 1987) and could impact the size distribution of salamanders found under boards (Gabor 1995). It is recommended that this measurement be added to future salamander monitoring at *rare*.

The planned addition of Mirrored Research salamander boards into the Cliffs and Alvars forest will continue to expand the program to include all three forest stands on *rare* property, and allow for a more complete analysis of ecological health.

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## 3.0 Forest Canopy and Tree Biodiversity Monitoring

### 3.1 Introduction

#### 3.1.1 Forest Monitoring

Forests are critical to environmental health and stability (Environment Canada and Canadian Forest Service 2004). They house a significant amount of the world's biodiversity of flora and fauna, providing habitats for numerous ecosystems (Butt 2011). They are also an integral part of soil conservation, water cycling, and air quality mediation (Butt 2011). Globally, initiatives establishing policy and protocol related to the safeguarding of forests are a high priority. In southern Ontario, forests have experienced a great deal of change in the past 200 years. Prior to European settlement, southern Ontario was largely covered by a patchwork of deciduous and mixed hardwood forests (Ontario Ministry Natural Resources 1999). Due to rapid development and a change in land use, forest species have been removed and land cover has been significantly altered. What remains are forests that are highly fragmented and smaller in size than what were historically present (Waldron 2003). These forests face significant pressures from both biotic and abiotic factors.

Establishing long-term monitoring across a network of sites can aid in developing an improved understanding of baseline levels of variability and health in natural systems (Gardner 2011). Monitoring crown conditions and stem defects is essential in providing an early warning system to recognise changes in tree health of Canadian forests and urban areas (Environment Canada and Canadian Forest Service 2004). Records of tree damage will help to identify the cause and effect of tree and forest decline. Information on population or species decline can be used as a platform to launch conservation initiatives (Gardner 2011), and may influence management objectives when considering human-impact on forest tracts.

#### 3.1.2 EMAN Forest Monitoring at *rare*

With the rapid development of southern Ontario, there are very few undisturbed remnant old-growth forests remaining (Ontario Ministry Natural Resources 1999). At the *rare* Charitable Research Reserve, one such remnant old growth exists, a Sugar-Maple-American Beech dominated forest termed Indian Woods harbouring trees more than 240 years old. Additional forest stands at *rare* include the Cliffs and Alvars, a mixed deciduous forest that was partially grazed by cattle within the last century, and the Hogsback, a relatively undisturbed mixed swamp forest. All of these forest ecosystems contribute invaluable services to the region by sequestering carbon dioxide and improving air and water quality (Führer 2000), as well as providing increasingly rare habitat to countless plants and animals that require mature forest interior (Ontario Ministry of Natural Resources 1999).

These forests face diverse challenges in the landscape of Waterloo Region; *rare* is bordered by conventional farm fields, aggregate mining operations, subdivisions, and busy roads. Many of these neighbouring lands are scheduled for drastic changes and development within the next few years. By acquiring baseline records of conditions of the *rare* forests and continuing long-term monitoring, we may be able to track changes in the forest ecosystems, and use those changes to develop an effective management plan to protect *rare* forest ecosystems.

The research questions that we hope to address with long-term forest canopy tree biodiversity monitoring were identified at the establishment of the program (McCarter 2009):

1. What is the current state (biodiversity, composition, health) of *rare*'s forests, and how do they compare to one another?
2. What are the long-term trends in tree mortality, recruitment and replacement taking place within the forests at *rare*?
3. Is the ecosystem integrity of the forests being maintained or improved under *rare* management?
4. Is either the ecological health or integrity of *rare* forests being affected by on-site and nearby changes in land use (i.e. restoration, agriculture, residential development, and aggregate extraction)?

The forest canopy tree biodiversity monitoring program at the *rare* Charitable Research Reserve began in 2009 with the establishment of three plots in the Cliffs and Alvars forests and three plots in the Indian Woods. Preliminary monitoring data, such as trees species, location, and diameter at breast height (dbh), were collected in this first year. In the 2010 monitoring year, three plots were established in the Hogsback forest so that all three major wooded areas on the *rare* property would be represented in the monitoring program. All nine forest plots were completely monitored in 2010 and an Ecological Monitoring and Assessment Network (EMAN) Tree Health Protocol was added to the monitoring program.

## 3.2 Methods

### 3.2.1 Forest Plot Locations

Forest monitoring plots are established in three forest stands on *rare* property. Each of these stands houses three monitoring plots, which together are used to describe their respective stands.

**Cliffs and Alvars:** A mature Sugar Maple-American Beech dominated forest located on the north side of Blair Road, bordered by Cruickston Creek on the west, Newman Creek on the east and the Grand River to the north. The three plots in the Cliffs and Alvars forest are located approximately 50m north of the Grand Trunk Trail, arranged parallel to the trail (Figure A.3; Table A.2). To access these plots, walk from the ECO Centre to the Grand Trunk Trail. Follow the trail to the east (right) until completely under the canopy (approximately 200m). Shortly after, the forest opens up and a small seasonal trail heads north. The plots are located to the left and right of this trail past the large fallen trees. Plot corners are clearly marked with pigtail stakes and orange or pink flagging tape.

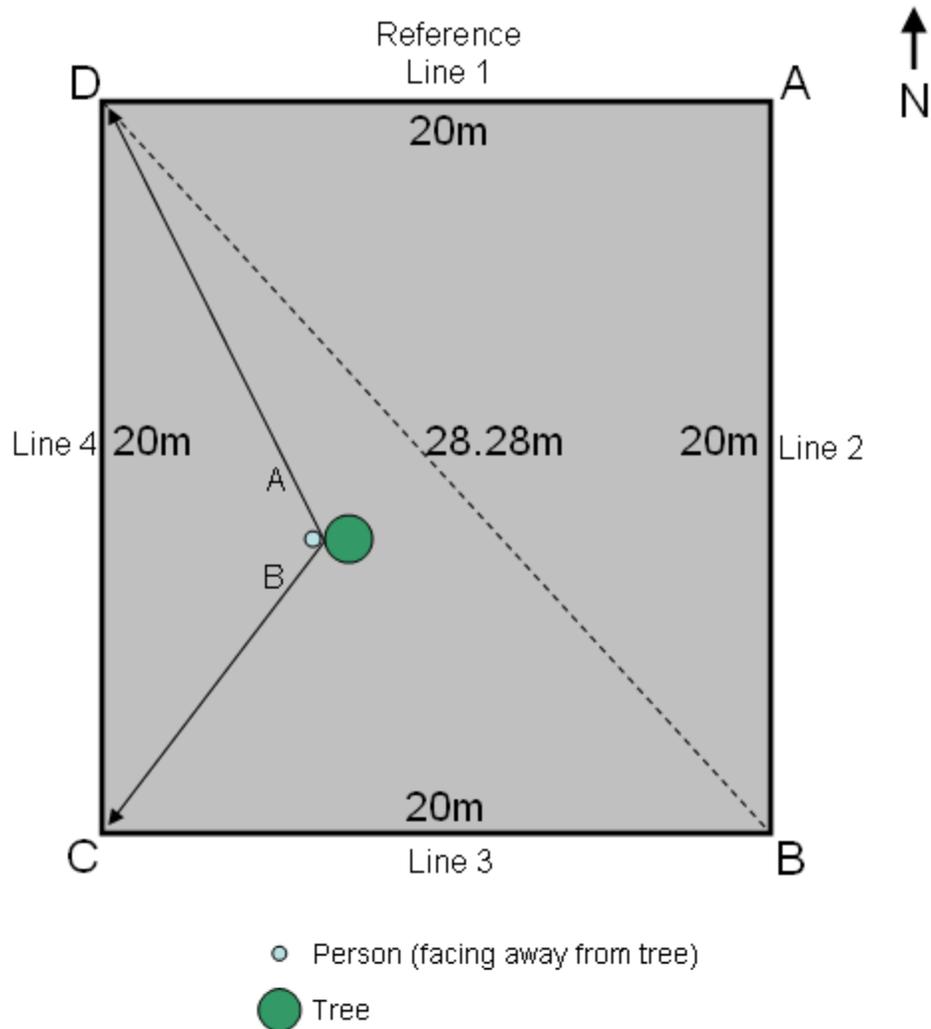
**Indian Woods:** A remnant old-growth forest located south of Blair Road and north of Whistle Bare Road, on the west side of the property. The three forest plots in Indian Woods are oriented in a north-south line in the centre of the forest, approximately 100m east of the Grand Allée. The third plot can be accessed by turning east into the forest off the Grand Allée towards the salamander monitoring plot and continuing to the top of the hill overlooking the pond. The first and second plots can be found by heading north from the third plot (Figure A.3; Table A.2). The plots are approximately 30m apart and the flagging tape on the corners of each plot should be visible from the adjacent plot.

**Hogsback:** Located at the south-east corner of the property, the Hogsback is bisected by Cruickston Creek and bordered by the Newman Drive subdivision to the east. The Hogsback is a mixed swamp forest with upland ridges dominated by White Pine, Red Maple, American Beech, and Sugar Maple. The three forest plots were established on these elevated ridges as the lower areas will likely be too swampy to access in wetter years. The second forest plot overlaps with the Hogsback salamander plot and can be reached by driving east down South Gate Road to the edge of the forest stand, and following the hedgerow around the forest (north, east, north, east), until heading south into the forest at the part of fence lowered with a fallen log, marked by pink flagging tape. This entry point is at the southern edge of Hogsback Field (303). The first plot is found approximately 30m north of the second plot on the same elevated ridge, and the third plot is located 30m southeast of the second plot, separated by a small boggy area (Figure A.3; Table A.2).

### *3.2.2 Monitoring Protocol: Plot Establishment*

Following the EMAN Forest Canopy Tree Biodiversity Monitoring Protocol (Environment Canada and Canadian Forest Service 2004), the plots established in 2009 and 2010 at *rare* are 20m x 20m permanent plots location in the forest interior. According to EMAN, plots should not be closer than three times the average tree height to any forest edge (estimated at 90m-100m for our forests); however this was not always possible due to the small size of Indian Woods and swampy topography of the Hogsback so in these cases plots were established as far from any edge as possible. The plots were oriented along the cardinal directions and the corners were marked with galvanized steel pigtail stakes with labelled flagging tape (Figure 3.1). All trees within the plot with a diameter equal to or greater than 10cm at breast height (dbh) were included in the monitoring. Trees in Indian Woods and Hogsback were labelled with pigtail stakes inserted in the ground at the base of the tree with pre-printed aluminum tags attached. Cliffs and Alvars was labelled using forestry tags marked with unique identification codes (ex. CA-02-08, Cliffs and Alvars-Plot 2-Tree 8) fixed to the trail with a downward angled nail.

The trees were tagged in a clockwise spiral inward from the northwest corner of the plot. The species of each tree was recorded at the time of plot establishment (Table E.2) and its distance to two plot corners was recorded for plot map generation. In this plotting technique, one observer stands with their back to the tree, facing the nearest line of the plot. The line number was recorded, and the “A” distance was measured from the tree to the corner to the right-hand side of the observer facing the line, while the “B” distance was measured from the tree to the corner to the left-hand side of the observer (Figure 3.1). Trees that split into multiple stems under breast height had each stem measured independently. Sample datasheets for tree health monitoring can be found on the *rare* server and in Figure C.3 and Figure C.4.



**Figure 3.1:** Diagram of an EMAN forest canopy tree biodiversity plots from McCarter 2009. The A and B distances are used to map the position of the tree within the plot. The A distance is measured from the tree to the corner to the right of the observer standing facing the reference line. The B distance is measured to the corner on the left side of the observer.

### 3.2.3 Monitoring Protocol: Procedure

Each plot should be visited once annually, ideally in the summer or when leaves are still present on trees for ease of identification. In 2012, plots were visited on August 29<sup>th</sup> and 30<sup>th</sup> (Hogsback), September 28<sup>th</sup> and October 1<sup>st</sup> (Cliffs and Alvares), and September 29<sup>th</sup> and October 4<sup>th</sup> (Indian Woods). The following variables were recorded for each tree in the monitoring plots: diameter at breast height (Woven Fibre Glass 5m Diameter Tape, Richter Measuring Tools), tree height (Haglöf Electronic Clinometer & Mastercraft© Fibre glass measuring tape), and tree condition based on Environment Canada and Canada Forestry Services EMAN codes (Table 3.1). Tree health was monitored by recording stem defects, crown class, crown rating (Table 3.2), and any other health notes, again based on Environment Canada’s EMAN protocol. Marginal trees in each plot were checked to see if they had

graduated into the 10cm dbh size class (minimum for inclusion). Trees that had newly met minimum requirements were tagged in a manner consistent with their plot and measured into the plot using distance from adjacent corners as described above. A complete list of equipment required can be found in List B.3. All trees were plotted into BioMon (BioMon for Windows Suite Version 2), a biodiversity monitoring software package, to generate tree species maps for each forest plot (Figure A.5-Figure A.13).

**Table 3.1:** Tree condition codes from EMAN protocol (Environment Canada and Canada Forestry Service 2004)

| Condition | Code                         |
|-----------|------------------------------|
| AS        | Alive Standing               |
| AB        | Alive Broken                 |
| AL        | Alive Leaning                |
| AF        | Alive Fallen/Prone           |
| AD        | Alive Standing with Dead Top |
| DS        | Dead Standing                |
| DB        | Dead Broken                  |
| DL        | Dead Leaning                 |
| DF        | Dead Fallen/Prone            |

**Table 3.2:** Crown class and rating codes from EMAN protocol (Environment Canada and Canada Forestry Service 2004)

| Crown Class   | Code | Crown Rating   |
|---|------|--|
| <b>Dominant:</b> Crown extends above the general canopy level and receives full sunlight from above and partly from the sides; larger than the average trees in the stand   | 1    | <b>Healthy:</b> Appears in good health, no major branch mortality, <10% branch/twig mortality          |
| <b>Codominant:</b> Crown forms the general canopy level and receives full sunlight from directly above and comparatively little from the sides  | 2    | <b>Light-Moderate Decline:</b> Branch and twig mortality <50% of the crown, <50% branch/twig mortality |
| <b>Intermediate:</b> Shorter than the two preceding classes, and receiving little direct sunlight from above and from the sides; their crowns extend into the base of the canopy of the dominant and codominant trees | 3    | <b>Severe Decline:</b> Branch and twig mortality >50% of the crown, >50% branch/twig mortality         |
| <b>Suppressed:</b> Receives no direct sunlight from above or the sides, their crowns are entirely below the general level of the crown cover.   | 4    | <b>Dead, Natural:</b> Tree is dead; either standing or downed  |
| <b>Open:</b> Exposed to full sunlight from directly above and on all sides; typically growing in a field or along a boulevard.  | 5    | <b>Dead, Human:</b> Tree cut down, removed, or girdled   |

### 3.2.4 Data Analysis

Data were analysed using Microsoft Excel 14.0.6 (Microsoft 2010) and PASW Statistics 17.0 (SPSS Inc.) for Windows. Prior to analysis, assumptions of parametric testing were examined. When transformation was required, the appropriate transformation to decouple variance and mean was determined using Taylor's Power Law (Perry 1981). Otherwise, the best transformation was applied and the most robust tests were used, followed by cautious interpretation of results.

For each forest stand, summary statistics were calculated by combining the data from the three plots which represent the same stand. Within each stand, the number of species present, the number of trees present, the mean diameter at breast height for included trees, and the total basal area were all recorded from the three tree plots combined. Basal area was calculated as the cross sectional area of all tagged tree stems in the plot and was determined using the dbh data.

A univariate analysis of variance (ANOVA) with two fixed factors, year and a recoded size class and location combination variable, was used to investigate differences between size classes at each location, using the abundance of trees in each of eight size classes from 0.1m at dbh to 0.8+m at dbh. Due to an interaction, variables were combined and recoded into size class/year combination variables (Leech et al. 2008) in order to effectively answer the question of interest. Only relevant comparisons were considered in the subsequent Bonferroni post hoc testing. Only living trees were included in this analysis.

Mean stem dbh and standard deviation were calculated for each forest stand, and the species diversity and evenness were calculated using the Shannon-Wiener Diversity Index (Figure 3.2). Additionally, the relative density (Figure 3.3), relative frequency (Figure 3.4), relative dominance (Figure 3.5), and importance value (Figure 3.6) were calculated for each species (Roberts-Pichette and Gillespie 1999). Only living trees were included in these calculations.

|   |
|---|
| <p><b>Shannon index:</b></p> $H = -\sum_{i=1}^S p_i \ln p_i$ <p>Where <math>p_i</math> is the proportion of individuals belonging to the <math>i</math>th species</p> <p><b>Evenness:</b></p> $E_H = H/\ln(S)$ <p>Where H is the Shannon index and S is the number of species</p> |
|---|

**Figure 3.2:** Shannon-Wiener Diversity Index and Evenness formulas.

$$\text{Relative Density} = \frac{\text{Number of tree species A in the plots}}{\text{Total number of trees in the plots}} \times 100$$

**Figure 3.3:** Formula for calculating the relative density of tree species in a forest stand, with all three plots per stand combined.

$$\text{Relative Frequency} = \frac{\text{Frequency of species A in the plots}}{\text{Total frequency of all trees in the plots}} \times 100$$

$$\text{Where Frequency} = \frac{\text{Number of plots with species A}}{\text{Total number of plots in the stand}}$$

**Figure 3.4:** Formula for calculating the relative frequency of tree species in a forest stand, with all three plots per stand combined.

$$\text{Relative Dominance} = \frac{\text{Basal area of species A (m}^2\text{)}}{\text{Total basal area of all species in the plots (m}^2\text{)}} \times 100$$

**Figure 3.5:** Formula for calculating the relative dominance of tree species in a forest stand, with all three plots per stand combined.

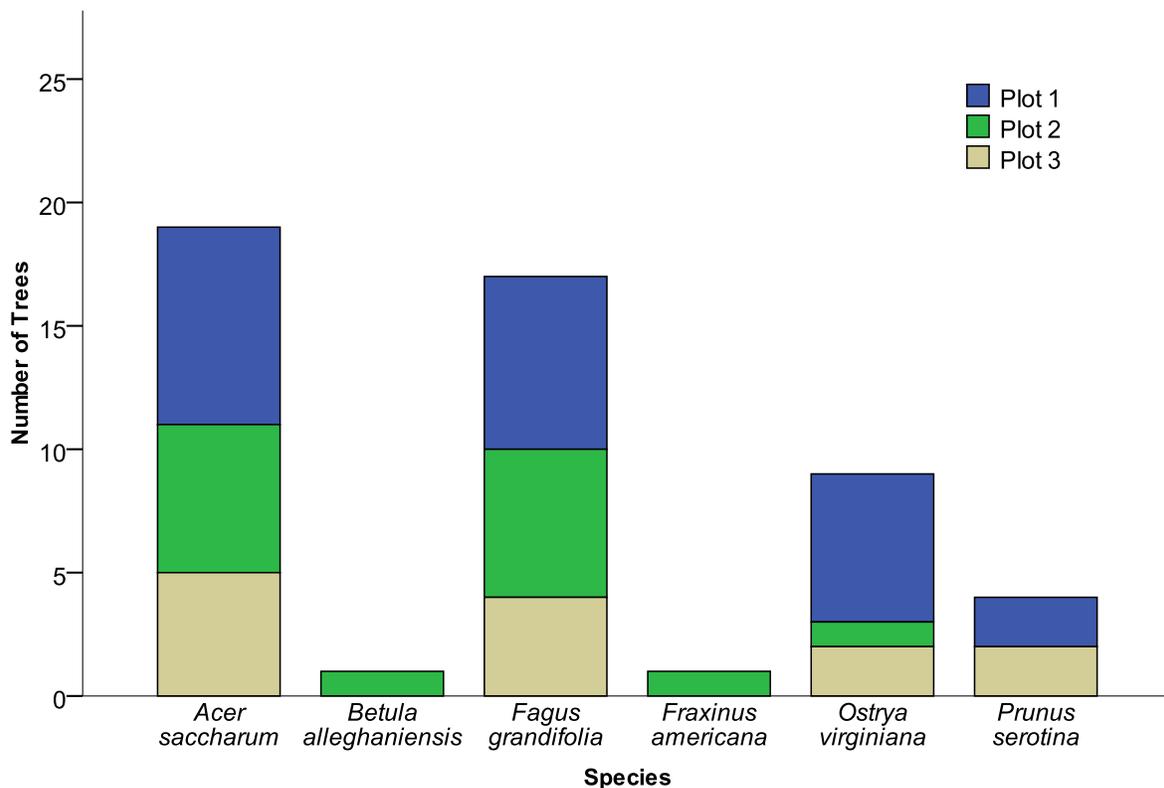
$$\text{Importance Value} = \text{Relative Density} + \text{Relative Frequency} + \text{Relative Dominance}$$

**Figure 3.6:** Formula for calculating the importance value of each tree species in a forest stand.

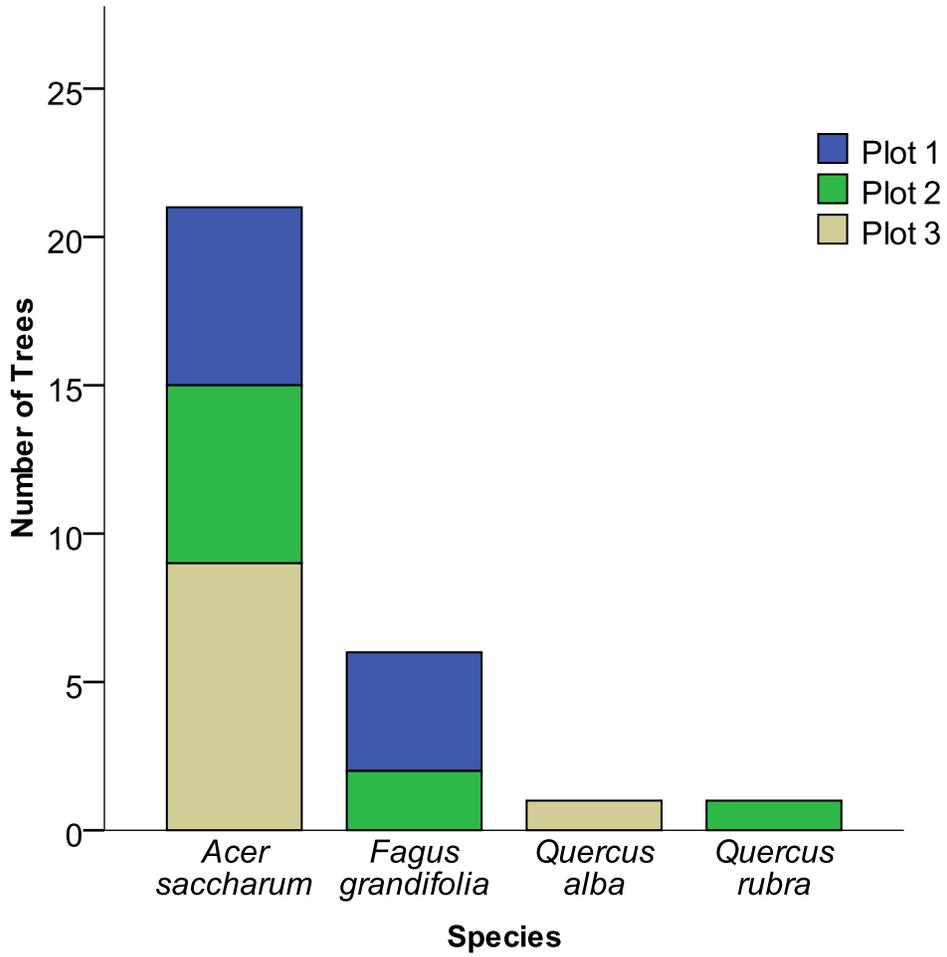
### 3.3 Results

#### 3.3.1 Tree Species Diversity

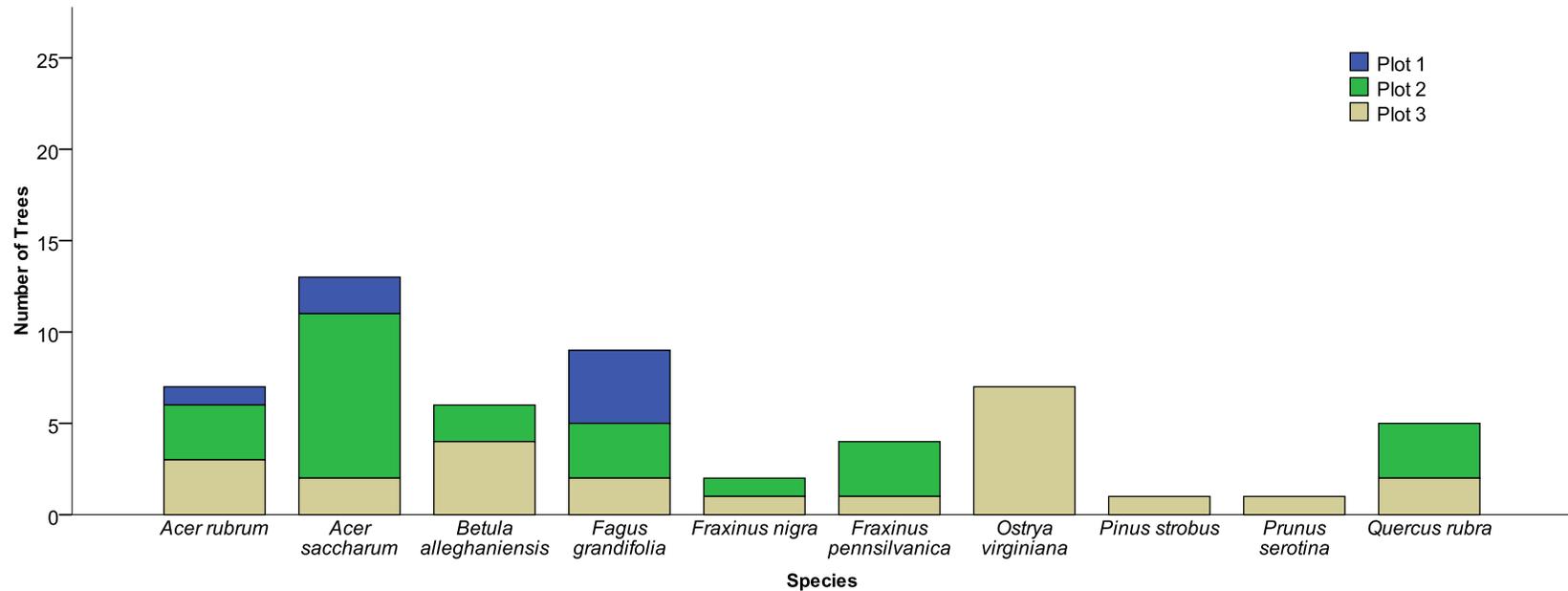
In 2012, the Cliffs and Alvars forest monitoring plots contain six living species (Figure 3.7) which is a decrease of one tree species from previous years, Butternut (*Juglans cinerea*). The stand is co-dominated by Sugar Maple (*Acer saccharum*) and American Beech (*Fagus grandifolia*). The Shannon-Wiener Diversity Index is lower than in all previous monitoring years, at 1.39 and an evenness value of 0.778 (Table 3.3). The Indian Woods has the lowest diversity in 2012 with four living species (Figure 3.8). The stand is largely dominated by Sugar Maple, and has a Shannon-Wiener Diversity Index of 0.792, a slight increase from two previous years, and an evenness value of 0.571 (Table 3.3). The Hogsback is the most diverse forest stand monitored at *rare* with a Shannon-Wiener Diversity Index of 2.08, remaining constant over the course of monitoring, and an evenness value of 0.903 (Table 3.3). The Hogsback monitoring plots contain ten living tree species, dominated by Sugar Maple, American Beech, and in plot three, Hophornbeam (*Ostrya virginiana*) (Figure 3.9).



**Figure 3.7:** Tree species composition and abundance from three forest plots in the Cliffs and Alvars.



**Figure 3.8:** Tree species composition and abundance from three forest plots in the Indian Woods.



**Figure 3.9:** Tree species composition and abundance from the three forest plots in the Hogsback.

**Table 3.3:** Summary of forest monitoring plot observations with Shannon-Wiener Diversity Index and evenness value for each forest stand, with three plots per stand pooled to calculate values.

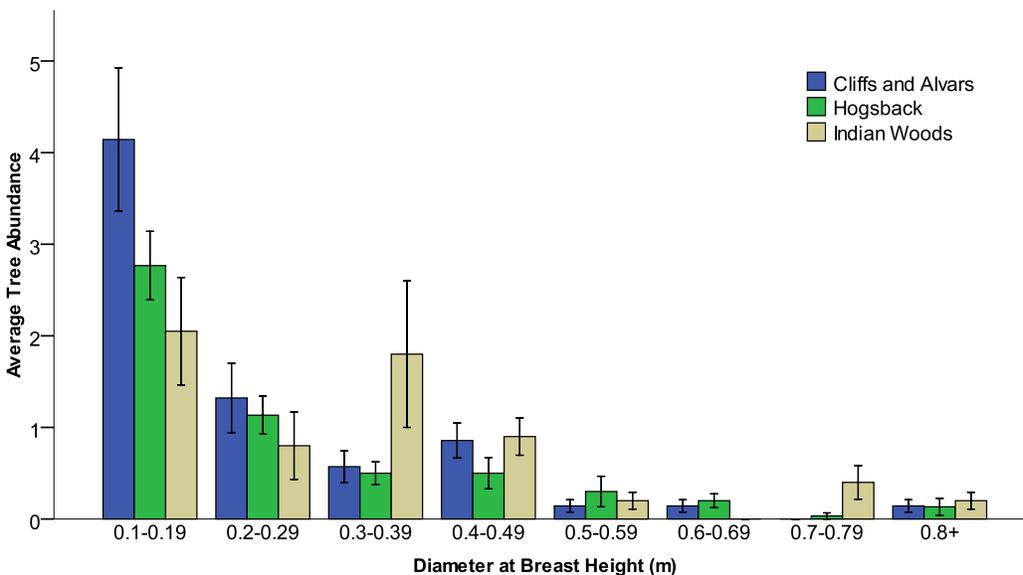
| Measures                       | Indian Woods |       |       |       | Cliffs and Alvars |       |       |       | Hogsback |       |       |
|--------------------------------|--------------|-------|-------|-------|-------------------|-------|-------|-------|----------|-------|-------|
|                                | 2009         | 2010  | 2011  | 2012  | 2009              | 2010  | 2011  | 2012  | 2010     | 2011  | 2012  |
| Number of Live Trees           | 34           | 32    | 32    | 29    | 49                | 52    | 51    | 51    | 55       | 55    | 55    |
| Number of Dead Trees           | 4            | 7     | 7     | 10    | 8                 | 8     | 9     | 10    | 6        | 6     | 6     |
| Number of Species              | 5            | 4     | 4     | 4     | 7                 | 7     | 7     | 6     | 10       | 10    | 10    |
| Mean dbh (m)                   | 0.334        | 0.332 | 0.335 | 0.331 | 0.228             | 0.231 | 0.232 | 0.234 | 0.249    | 0.251 | 0.255 |
| Shannon-Weiner Diversity Index | 0.843        | 0.746 | 0.746 | 0.792 | 1.503             | 1.509 | 1.469 | 1.394 | 2.078    | 2.078 | 2.078 |
| Evenness                       | 0.524        | 0.538 | 0.538 | 0.571 | 0.772             | 0.775 | 0.755 | 0.778 | 0.903    | 0.903 | 0.903 |

### 3.3.2 Stand Characteristics and Size Class

Size class differences varied with location (interaction  $F_{14,536}=1.705$ ,  $p=0.051$ ), so both factors were considered simultaneously in a combination variable of size class and location (Leech et al. 2008), and significant differences occurred between these levels (ANOVA  $F_{23,536}=11.40$ ,  $p<0.001$ ) (Figure 4.10). The smallest size class considered in this monitoring program, 0.10-0.19m diameter at breast height, housed significantly more trees than any other size class in both Cliffs and Alvars and the Hogsback (post hoc  $<0.005$ ), and had similar abundances across all three forest stands (post hoc  $>0.306$ ).

In the Cliffs and Alvars, the majority of trees were found to be in the smallest size class, with the second size class, 0.2-0.29m at dbh, additionally being significantly different from the size class with the least abundance of trees, 0.7-0.79m at dbh (Figure 3.10). In 2012, monitoring plots added one new tree which newly reached the minimum diameter size requirements and lost one tree to mortality, a small Butternut (*Juglans cinerea*). In the Hogsback, similarly to Cliffs and Alvars, the most trees belonged to the smallest size class, with the second size class significantly differing from the largest classes, 0.7-0.79m and 0.8+m at dbh (Figure 3.10). The Hogsback monitoring plots have suffered no mortalities since the beginning of monitoring, and have yet to recruit a new tree. The Indian Woods, a remnant old growth forest, had a lower abundance of trees and lacked a distinct peak in abundance in the smallest size class. The trees were more evenly distributed in the first four size classes (Figure 3.10). The smallest size class significantly differed from the 0.6-0.69m at dbh size class, which had the lowest abundance in this stand. In 2012, the Indian Woods lost three trees to mortality, all Sugar Maples of relatively small size.

The yearly growth per forest stand is documented in Table 3.3, indicating the mean dbh of trees within the forest plots at each location. Abundance, basal area, relative density, relative frequency, relative dominance, and importance value for each species are shown in Table 3.4 for each location in 2012.



**Figure 3.10:** Tree trunk size distribution measured at breast height in 2012 for the three forest stands monitored at *rare*, Cliffs and Alvars, Hogsback, Indian Woods.

**Table 3.4:** Tree species composition and summary statistics for the three monitored forest stands at *rare* in 2012.

| Location          | Species name                  | Abundance | Basal Area (m <sup>2</sup> ) | Relative Density | Relative Frequency | Relative Dominance | Importance Value |
|-------------------|-------------------------------|-----------|------------------------------|------------------|--------------------|--------------------|------------------|
| Indian Woods      | <i>Acer saccharum</i>         | 21        | 2.45                         | 72.41            | 42.86              | 72.65              | 187.92           |
|                   | <i>Fagus grandifolia</i>      | 6         | 0.21                         | 20.69            | 28.57              | 6.10               | 55.36            |
|                   | <i>Quercus alba</i>           | 1         | 0.16                         | 3.45             | 14.29              | 4.88               | 22.61            |
|                   | <i>Quercus rubra</i>          | 1         | 0.55                         | 3.45             | 14.29              | 16.38              | 34.11            |
| Cliffs and Alvars | <i>Acer saccharum</i>         | 19        | 1.58                         | 37.25            | 23.08              | 49.56              | 109.89           |
|                   | <i>Betula alleghaniensis</i>  | 1         | 0.15                         | 1.96             | 7.69               | 4.85               | 14.51            |
|                   | <i>Fagus grandifolia</i>      | 17        | 1.09                         | 33.33            | 23.08              | 34.33              | 90.74            |
|                   | <i>Fraxinus americana</i>     | 1         | 0.02                         | 1.96             | 7.69               | 0.48               | 10.13            |
|                   | <i>Ostrya virginiana</i>      | 9         | 0.10                         | 17.65            | 23.08              | 3.23               | 43.96            |
|                   | <i>Prunus serotina</i>        | 4         | 0.24                         | 7.84             | 15.38              | 7.55               | 30.78            |
| Hogsback          | <i>Acer rubrum</i>            | 7         | 1.14                         | 12.73            | 15.00              | 28.91              | 56.63            |
|                   | <i>Acer saccharum</i>         | 13        | 0.86                         | 23.64            | 15.00              | 21.91              | 60.55            |
|                   | <i>Betula alleghaniensis</i>  | 6         | 0.13                         | 10.91            | 10.00              | 3.38               | 24.29            |
|                   | <i>Fagus grandifolia</i>      | 9         | 1.17                         | 16.36            | 15.00              | 29.80              | 61.16            |
|                   | <i>Fraxinus nigra</i>         | 2         | 0.03                         | 3.64             | 10.00              | 0.66               | 14.29            |
|                   | <i>Fraxinus pennsylvanica</i> | 4         | 0.12                         | 7.27             | 10.00              | 3.14               | 20.42            |
|                   | <i>Ostrya virginiana</i>      | 7         | 0.11                         | 12.73            | 5.00               | 2.85               | 20.58            |
|                   | <i>Pinus strobus</i>          | 1         | 0.01                         | 1.82             | 5.00               | 0.26               | 7.08             |
|                   | <i>Prunus serotina</i>        | 1         | 0.06                         | 1.82             | 5.00               | 1.43               | 8.25             |
|                   | <i>Quercus rubra</i>          | 5         | 0.30                         | 9.09             | 10.00              | 7.66               | 26.75            |

## 3.4 Discussion

### 3.4.1 Tree Species Diversity

The Cliffs and Alvars forest is a mature stand co-dominated by Sugar Maple and American Beech, which together make up 70.6% of the trees in the three monitoring plots. The diversity index for this stand fell between that of the other two stands, as did the evenness (Table 3.3), however with the loss of the lone Butternut it was the lowest diversity rating and highest evenness rating for the stand since monitoring began. Most species found here prefer well drained upland habitats and are tolerant of shade (Laird Farrar 1995), performing well in the complete canopy. In exception is the Yellow Birch (*Betula allaghaniensis*), which favours moist soils but commonly occurs in mixed woods with Sugar Maple and Beech, and Black Cherry (*Prunus serotina*) which is intolerant of shade and found in canopy gaps (Laird Farrar 1995).

Indian Woods is an eastern deciduous remnant old-growth forest dominated by Sugar Maple; an ecosystem that is rare in the region and to southwestern Ontario (Robson et al. 2012). The diversity of the Indian Woods forest plots was the lowest of the three forest stands examined and has the smallest evenness value (Table 3.3) indicating that the abundance of trees within each species varies greatly. Old-growth forests are often viewed as a final stage in forest succession, representing a climax community that will persist in a state of dynamic equilibrium in the prevailing environmental conditions (Krebs 2001). As succession progresses and the canopy closes, the composition of canopy trees shifts toward more shade tolerant species such as Sugar Maple and American Beech (the second most abundant species in Indian Woods plots) in eastern deciduous forests (Fox 1977). These species are able to grow suppressed in the understory and exploit canopy gaps when they occur, outcompeting other shade-sensitive species (Weiskittel and Hix 2003).

The Hogsback forest is a forest-wetland complex and as such offers a greater diversity of habitats than the other two forest stands monitored at *rare*. The dominant species here vary with plot, with Sugar Maple, American Beech, and Hophornbeam each being the dominant species in one plot. The diversity of this forest is the highest observed at *rare*, as is the evenness value (Table 3.3). The abundance of trees from different species is similar, without one or two clearly dominant species as seen in other plots. The wet margins of the Hogback plots are likely a source of increased diversity, as Yellow Birch, Black Ash (*Fraxinus nigra*), Green Ash (*Fraxinus pennsylvanica*), and Red Maple (*Acer rubrum*) all thrive in wet soils (Sibley 2009).

### 3.4.2 Stand Characteristics and Size Class

The tree size distributions of the three forests were plotted in Figure 3.10 to give a visual representation of the size-class composition of the stands. This information will be useful baseline data to which monitoring data from future years may be compared to examine recruitment and replacement patterns of each stand (Forrester and Runkle 2000; Parker 2003). The size of trees can be used to estimate the age of a forest stand, and in conjunction with height and species composition, can help characterize a forest structure. Both Cliffs and Alvars and the Hogsback have significantly more trees in the smallest size class. This indicates a forest with many young trees. The Hogsback appears to exhibit the classic distribution of trunk size in a young forest stand with fewer trees in bigger size classes, and

tree sizes progressing in a right skewed manner. The Cliffs and Alvars have a similar distribution with one size class exception, the 0.4-0.49 group. Historically, this forest stand was grazed by cattle in the early twentieth century and this past use of the land could account for this increased number of trees in the 0.4-0.49m dbh size class. Trees that are now in the 0.4-0.49m category could have possibly been large enough at the time of this grazing to not be stripped by cattle. Conversely, those trees in the 0.3-0.39m grouping were more likely to be targets of grazing and thus succumbed to this hazard. Alternative to both Cliffs and Alvars and the Hogsback, the Indian Woods has a more even distribution across size categories, particularly the first four groupings. The low species richness and more even distribution indicate that while there is regeneration occurring in this forest stand, it is settling as a climax community forest where dominant trees are stable in the understory for many years using a series of gaps to reach the canopy (Forrester and Runkle 2000). It is likely that this forest will continue to be dominated by Sugar Maple and American Beech trees, both shade tolerant and able to withstand years under a complete canopy. Continued long-term monitoring of these plots is essential.

The importance value (IV) was calculated for each species in each plot, incorporating the relative density, relative frequency, and relative dominance of each species (Table 3.4). The importance value therefore comparatively looks at species within plots considering how common and abundant that species is as well as the total amount of forest area that species occupies within each plot. Since we are using relative values, the maximum importance value is 300 (Figure 3.6). In the Indian Woods, Sugar Maple is overwhelmingly the most important species. With the low diversity of this plot and the high abundance of Sugar Maples this is not surprising. The American Beech present in the plot are few and generally small in size, while the Red and White Oak (*Quercus rubra* and *alba*) are singular trees but quite large in size, inflating their importance value. In the Cliffs and Alvars, Sugar Maple is again the most important species followed closely here by American Beech. Both species dominate this forest stand and out-compete their less shade tolerant neighbours when canopy gaps emerge. In the Hogsback, American Beech has the highest importance value very near that of the Sugar Maple. Interestingly, this is the only stand where the most abundant species, Sugar Maple, is not associated with the highest importance value. This reflects the larger size of the American Beech trees located here. The same is true for the Red Maple (*Acer rubrum*), which has an importance value closely ranked with Sugar Maple and American Beech, but fewer individual trees. This indicates that a fewer number of Red Maples have a strong influence on the forest community. Red Maple can thrive in a wider range of soil types than any other forest species in North America (Walters and Yawney 1990) and grows on diverse sites including dry upland ridges and slopes and swamps; both habitats located in the Hogsback forest. While generally Red Maple tends to give way to the more shade tolerant Sugar Maple and American Beech in a mature forest, in wet areas that reach an edaphic climax, Red Maple may be able to maintain a dominant status (Walters and Yawney 1990).

### 3.5 Tree Heath and Recommendations

One of twelve Butternut trees of *rare* property falls within the monitoring plots in the Cliffs and Alvars area. It has been misidentified as dead-standing previously, but was living with severe crown dieback and extensive wounds, until it was found dead and broken just below a height of five meters in 2012. Butternut is listed as Endangered by both the Federal Species at Risk Act (SARA) and provincially

on the Species at Risk in Ontario (SARO). The decline of Butternut in North America is attributed to Butternut canker caused by a fungal pathogen (*Sirococcus clavigignenti-juglandacearum*) that evidence suggests is a relatively recent introduction to North America (Broders and Boland 2010). Symptoms of the disease are elongated, sunken cankers, which commonly originate at leaf scars, buds, or wounds (Davis and Meyer 1997). There is currently no prevention, control, or treatment for the disease and most Butternut conservation efforts are focused on the detection of resistant individuals for seed banking and grafting (Forest Gene Conservation Association 2010). As no remaining living Butternut are located in any monitoring plots, continued observation of Butternut on the property should occur outside of this monitoring program, and continued review of new literature and policy should occur to effectively manage this species at risk.

Continued monitoring at all forest plots is recommended. It is suggested that photo evidence of suspected diseased or dying trees be taken to assist in identification and yearly comparisons to protect the health of the forest stands and species therein. It may be beneficial for monitors to undergo more extensive training to better identify tree ailments, particularly for signs of known problems like the Emerald Ash Borer Beetle (*Agilus planipennis*) and Beech Bark Disease (*Nectria coccinea*).

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## 4.0 Soil Humus Decay Rate Monitoring

### 4.1 Introduction

#### 4.1.1 Soil Characteristics and Functions

Decomposition is defined as the physical, chemical, and biological breakdown of organic material into simpler matter, and it is a significant producer of carbon dioxide, as well as methane and nitrogen gases (Berg and McClaugherty 2008). Soil humus, the stable organic material remaining after initial decomposition, acts as the reservoir for the carbon that was not released during decay, as well as storage for the nutrients that support plant growth and the microbial and fungal communities of the soil (Berg and McClaugherty 2008). The rate at which decomposition occurs is dependent on many factors, including the composition of the material being decomposed, the ecology (species composition and abundance) of the decomposer organisms available in the soil, and a suite of environmental variables, including soil temperature, moisture, pH and aeration (Tenney and Waksman 1929).

#### 4.1.2 Soil Humus Decay Rate Monitoring at *rare*

In response to concerns that climate change may affect forest carbon budgets, Natural Resources Canada developed the Canadian Intersite Decomposition Experiment (NRC 2007) to examine the long-term litter decomposition rates and nutrient mineralization of forests across Canada. In Canadian forests, large amount of carbon are stored in trees, soils, and decaying plant litter and any change in the balance between the uptake of carbon through photosynthesis and the release of carbon through decay and other activities could have an impact on levels of atmospheric carbon dioxide, an important greenhouse gas linked to global climate change. Thus, warmer temperatures could increase decay rates, which in turn would release carbon stored in the soils and litter and potentially accelerate rises in atmospheric carbon dioxide. The moderate temperature zone of southwestern Ontario was excluded from the NRC long-term decomposition study. As long-term monitoring of soil decay rates can provide valuable information on the relationship between soil decomposition and environmental factors, it may serve to inform forest management decisions at *rare*. For example, the effects that nearby aggregate mining or pesticide application may have on the health of our forest soils are unknown. Decay rate monitoring, together with the other biological monitoring protocols in place at *rare* such as forest tree biodiversity and plethodontid salamander monitoring, can provide us with a greater understanding of the integrity and stability of our forest ecosystems.

The first EMAN soil humus decay rate monitoring plots at *rare* were established on November 9, 2009 at the Cliffs and Alvars forest canopy tree biodiversity plot one. The success of the first monitoring year resulted in an expansion of the study in 2010 by the establishment of monitoring plots in both the Indian Woods and the Hogsback forest stands, within the first tree plot at each location.

At *rare*, the objective of this monitoring procedure is to contribute to the assessment of forest ecosystem functioning by monitoring yearly mass loss in standardized decay sticks as a representation of soil decomposition rates. As per the EMAN soil humus decay rate monitoring protocol (Parks Canada 2006), Annual Decay Rate (ADR) plots were located at the corners of the permanent forest canopy tree biodiversity plots in each forest stand. The information gain from decay monitoring can then be directly

linked to the forest health and productivity data. Decay rates compared over years are expected to remain relatively stable, and soil inserts positioned on the surface of the soil are expected to experience less mean weight loss than those placed below the surface where they are more accessible to soil microorganism responsible for decomposition. A change in decay rates would reflect a change in the physical or biological soil environments.

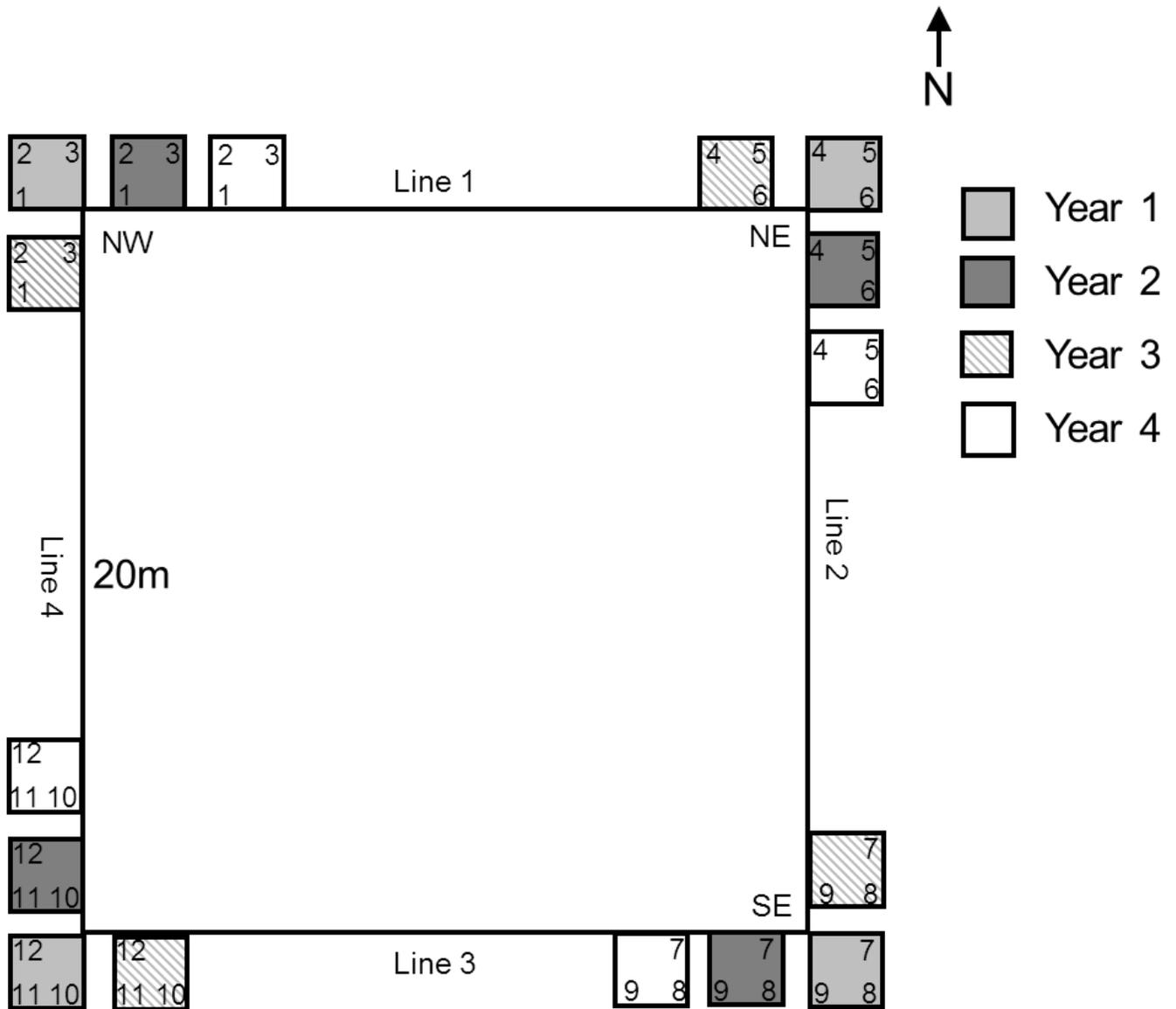
## 4.2 Methods

### *4.2.1 Soil Humus Decay Rate Plot Locations*

ADR plots were established on all four corners of forest canopy biodiversity monitoring plot one in each of the three forest stands (Figure 4.1). Each forest monitoring plot had twelve ADR plots established, with three at each of the four corners. ADR locations must be shifted each year to avoid the use of previously disrupted soil. In 2012, ADRs were located clockwise from 2010 locations (Figure 4.1). Descriptions of forest stands and instructions to access plots can be found in Section 3.2.1 and a map can be found in Figure A.3 with associated GPS coordinates in Table A.2

### *4.2.2 Monitoring Protocol: Decay Stick Installation*

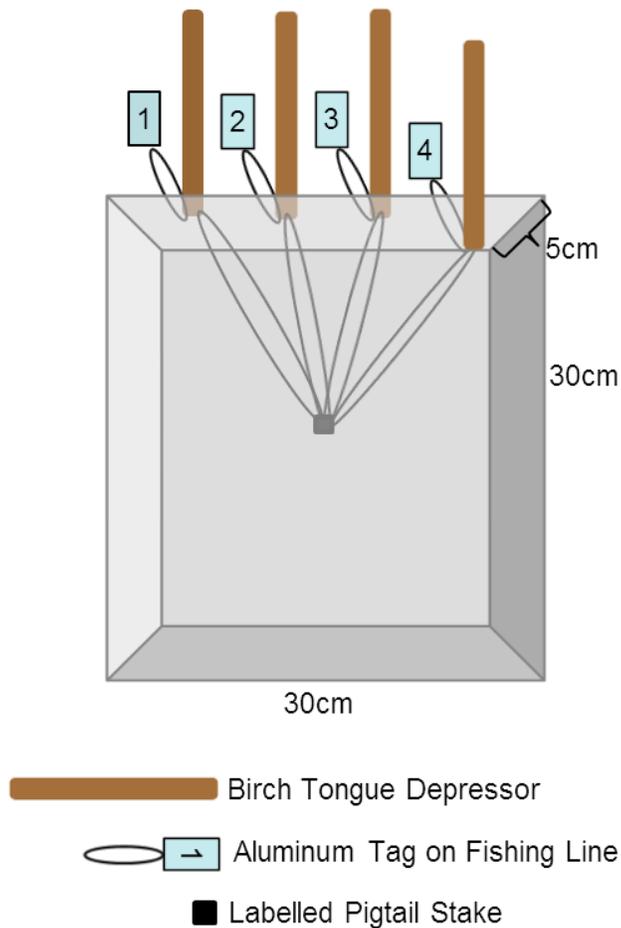
Decay sticks were prepared in-house prior to ground installation. To prepare the tongue depressors (MedPro, 100% natural birch wood, ultra smooth finish) a 2mm hole was drilled at one end of each stick to allow for the attachment of identification tags. While only 144 decay sticks are used during monitoring, it is best to prepare approximately fifteen sticks in excess in case of damage prior to installation. Once drilled, decay sticks were transported to the University of Guelph (Dr. Brian Husband Research Lab) and oven-dried at 70°C for 48 hours. Following this, decay sticks were left for 24 hours at room temperature and then weighed (+/- 0.001g) on a Sartorius 1265MP balance. A sample datasheet to record stick weight pre and post decay can be found in Figure C.5. After recording their mass, decay sticks were tagged with pre-labelled aluminum tags attached with approximately 30cm of extra-strong (40LB) fishing line. With the exception of the initial year of monitoring, decay sticks were placed in 100% vinyl mesh bags (dimension: 17cm x 4cm with an approximate pocket size of 16cm x 3cm; hole size: 3mm x 2mm). Vinyl mesh bags were prepared in advance of decay stick placement, with an excess created in case of damage during installation. These bags were an amendment to the monitoring protocol added in 2010 in an attempt to keep all the decay stick's pieces together and increase the number of decay sticks excavated intact. Mesh bags are often used in studies of leaf litter decay rate (Moore et al. 2005; Albers et al. 2004; Gallardo et al. 1995). A complete list of equipment required for installation can be found in List B.4.



**Figure 4.1:** Distribution of annual soil humus decay rate (ADR) plots (numbered 1-12) around a forest canopy tree biodiversity plot. Twelve ADR plots are arranged around the corners of each plot; three located in the originally recommended location of the corner and moved counter-clockwise and clock-wise in alternating years from the original location to avoid previously sampled soil areas. Plots are colour coded by monitoring year.

A 1m<sup>2</sup> quadrat was marked on each corner of the forest plots and three ADR plots were positioned within each quadrat on the corners radiating out from the corner of the forest plot (Figure 4.1). At each ADR plot, a 30cm x 30cm hole was excavated with the soil plug removed intact if possible and placed to the side. Using a knife or chisel, three slots were made parallel to the forest floor on the north wall of the excavated hole. The slots were of large enough size to accommodate the bagged decay

sticks snugly. Slots were measured 5cm below the soil surface and were re-measured upon completion with the accurate depth below the surface recorded. The three slots were measured to be approximately 10cm apart. The bagged decay sticks were inserted into the slots, with the pre-labelled aluminum tags previously attached via fishing line left on the soil surface. A pigtail stake marked with flagging tape labelled with the forest stand and ADR plot number (i.e. CA-ADR 2) was inserted into the centre of the excavated hole. Fishing line was used to attach each bagged decay stick to one another and the centre pigtail stake, with enough excess that they would not be shifted. This fishing line is to be used as a guide to locate the sticks upon excavation and therefore should not be so taut as to affect their movement throughout the year. A fourth bagged decay stick was attached to the centre pigtail stake via fishing line and left on the soil surface (Figure 4.2). The excavated hole was then refilled with the displaced soil and soil plug, and the exposed tags were covered with leaf litter to prevent public or wildlife tampering. In 2012, decay sticks were installed on November 1<sup>st</sup> in the Cliffs and Alvars, November 6<sup>th</sup> in the Indian Woods, and November 7<sup>th</sup> in the Hogsback.



**Figure 4.2:** Diagram of annual soil humus decay rate (ADR) monitoring plot set-up as viewed from above. Decay sticks 1-3 are installed parallel to the soil surface at a depth of 5cm, separated 10cm from each other. Stick 4 is placed on the soil surface, and all decay sticks are tied to the central pigtail stake. Figure from Robson (2010).

#### 4.2.3 Monitoring Protocol: Decay Stick Excavation

Decay sticks were excavated one year following their installation. In the event of an early frost and ground freeze, the date of excavation should be moved forward. Using a trowel, soil surrounding the pigtail stake in each ADR, where decay sticks were suspected to be, was slowly removed. As tags and fishing line were uncovered, they were used to help locate the decay sticks and to gently pull the bagged decay sticks from the ground once a hole has been dug. Each decay stick and its associated tag were placed in an individual re-sealable plastic bag. A complete list of equipment required for excavation can be found in List B.4.

Decay sticks were each removed from their vinyl bags and any dirt that adhered to the stick was removed. Each stick was gently brushed with a dry paintbrush and then gently scrubbed with a second paintbrush in water. Decay sticks were placed in individual paper envelopes following cleaning, and each envelope was labelled with the site and tag number. Decay sticks, inside their envelopes, were then oven-dried at 70°C for 48 hours and subsequently let to sit for 24 hours at room temperature before being weighed (+/- 0.001g). Weights were recorded on a datasheet available on the *rare* server and in Figure C.5).

#### 4.2.4 Data Analysis

Data were analysed using Microsoft Excel 14.0.6 (Microsoft 2010) and PASW Statistics 17.0 (SPSS Inc.) for Windows. Prior to analysis, assumptions of parametric testing were examined. When transformation was required, the appropriate transformation to decouple variance and mean was determined using Taylor's Power Law (Perry 1981). Otherwise, the best transformation was applied and the most robust tests were used, followed by cautious interpretation of results.

Percent dry weight loss for each decay stick was calculated, as changes in dry weight loss can be examined as a proxy for soil decomposition function. Weight loss was compared across years and sites using a univariate analysis of variance (ANOVA) followed by Bonferroni post hoc testing to determine where the differences occurred.

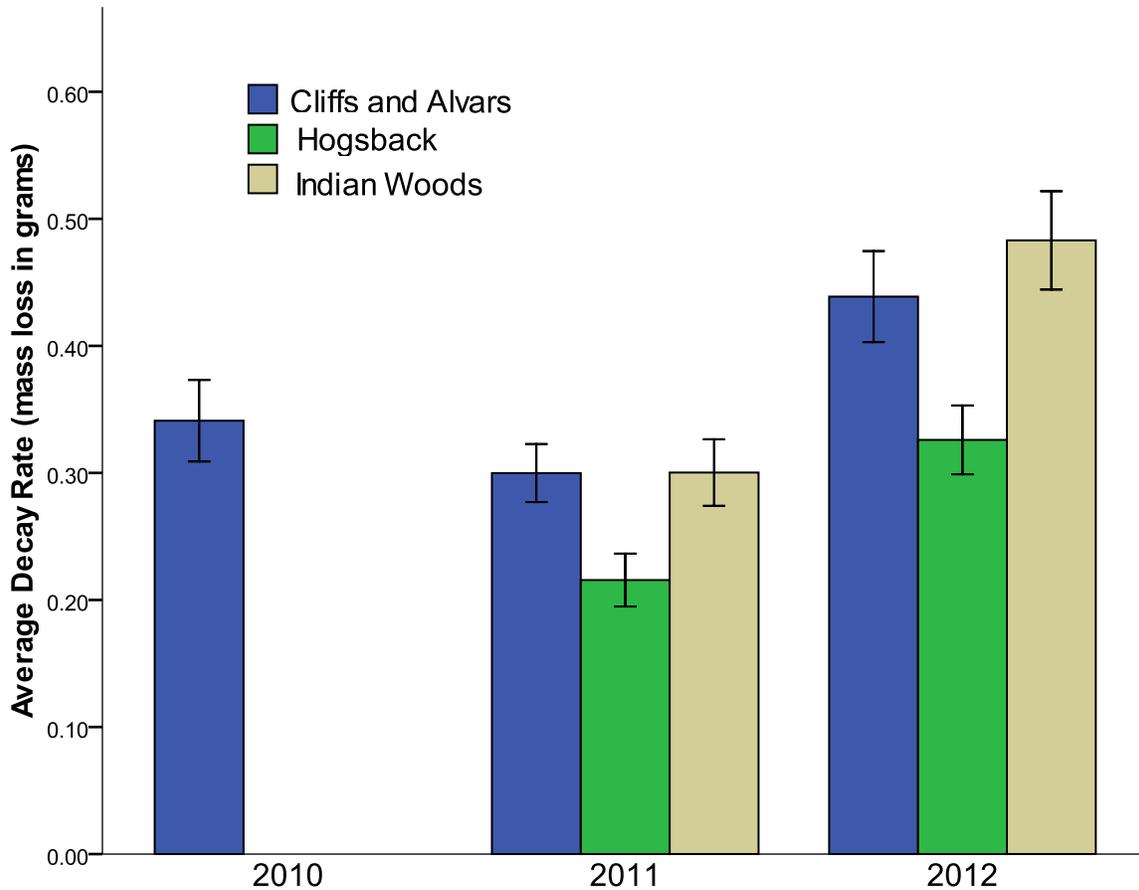
### 4.3 Results

In 2012, a total of 142 decay sticks were recovered from annual decay rate plots. Two decay sticks were lost during the sampling year, one each from the Cliffs and Alvars and Indian Woods forest stands. As expected, decay sticks positioned below ground were found to have lost significantly more mass than those positioned on the soil surface ( $F_{1,330}=119.68$ ,  $p<0.001$ ) (Table 4.1).

Significant differences were found in decay rates across years ( $F_{2,325}=16.44$ ,  $p<0.001$ ) and forest sites ( $F_{2,325}=8.618$ ,  $p<0.001$ ). Post hoc testing revealed that decay rates in 2011 differed from those recorded in 2012 ( $p>0.001$ ) and both Cliffs and Alvars and Indian Woods significantly differed from decay rates in the Hogsback (Figure 4.3).

**Table 4.1:** Annual decay rates measured as percent mass loss of decay sticks from Cliffs and Alvars, Indian Woods, and the Hogsback forest stands in all monitoring years. Decay sticks below and above ground had significantly different mass losses, regardless of site or year. SD= Standard Deviation.

|                     | Cliffs and Alvars |      | Indian Woods |      | Hogsback |      |
|---------------------|-------------------|------|--------------|------|----------|------|
|                     | Mean (%)          | SD   | Mean (%)     | SD   | Mean (%) | SD   |
| All sticks          | 43                | 21.7 | 39.1         | 24.4 | 27.1     | 17.5 |
| Sticks below ground | 44.3              | 17.6 | 44.8         | 22.8 | 29.9     | 17.1 |
| Sticks above ground | 10.4              | 9.60 | 21.1         | 20.2 | 18.3     | 15.9 |



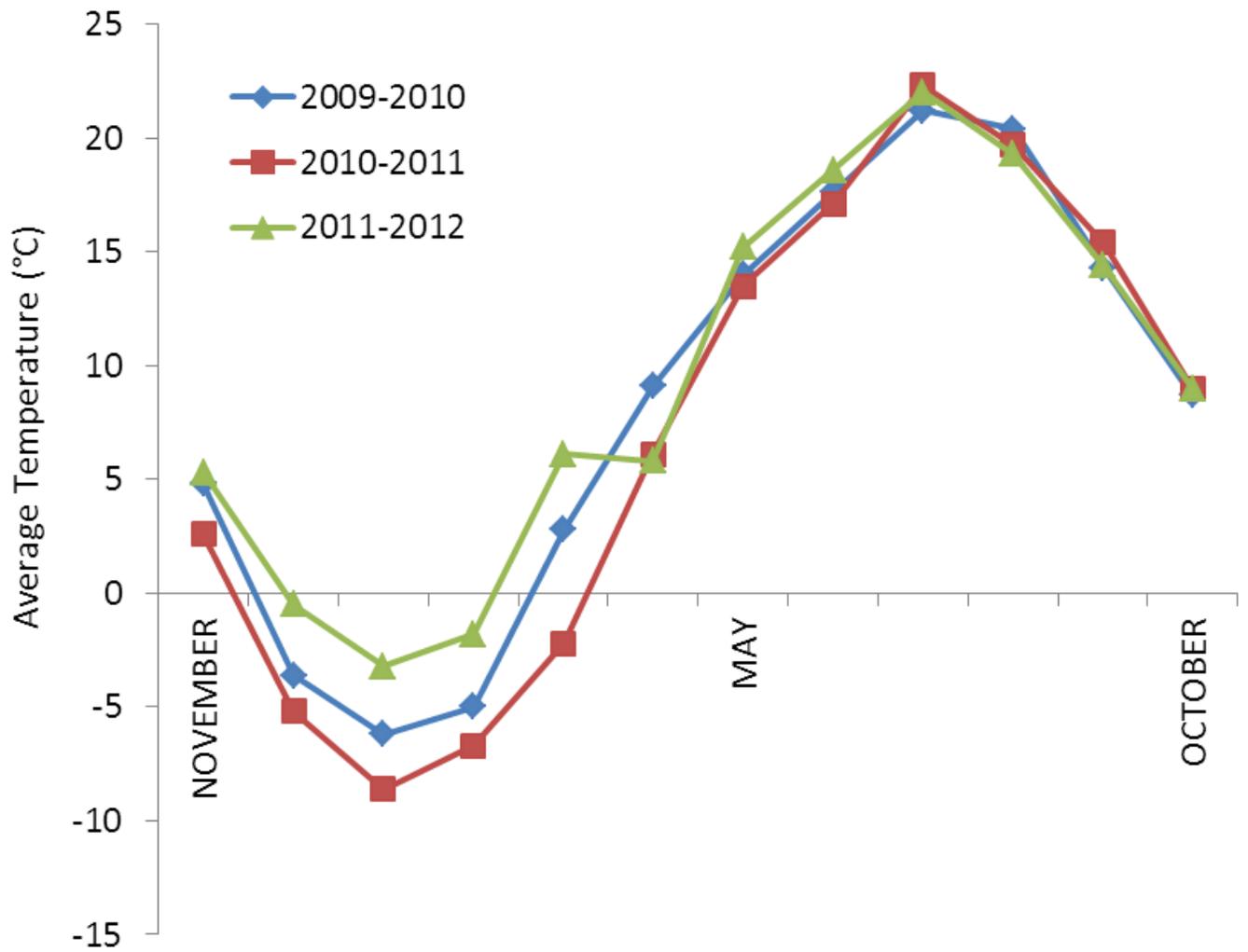
**Figure 4.3:** Average decay rate comparison over monitoring years and sites. Only Cliffs and Alvars was monitored in 2010. Significant differences occur between the 2011 and 2012 monitoring years, and between the Hogsback and all other sites. Error bars represent +/- one standard error.

#### 4.4 Discussion

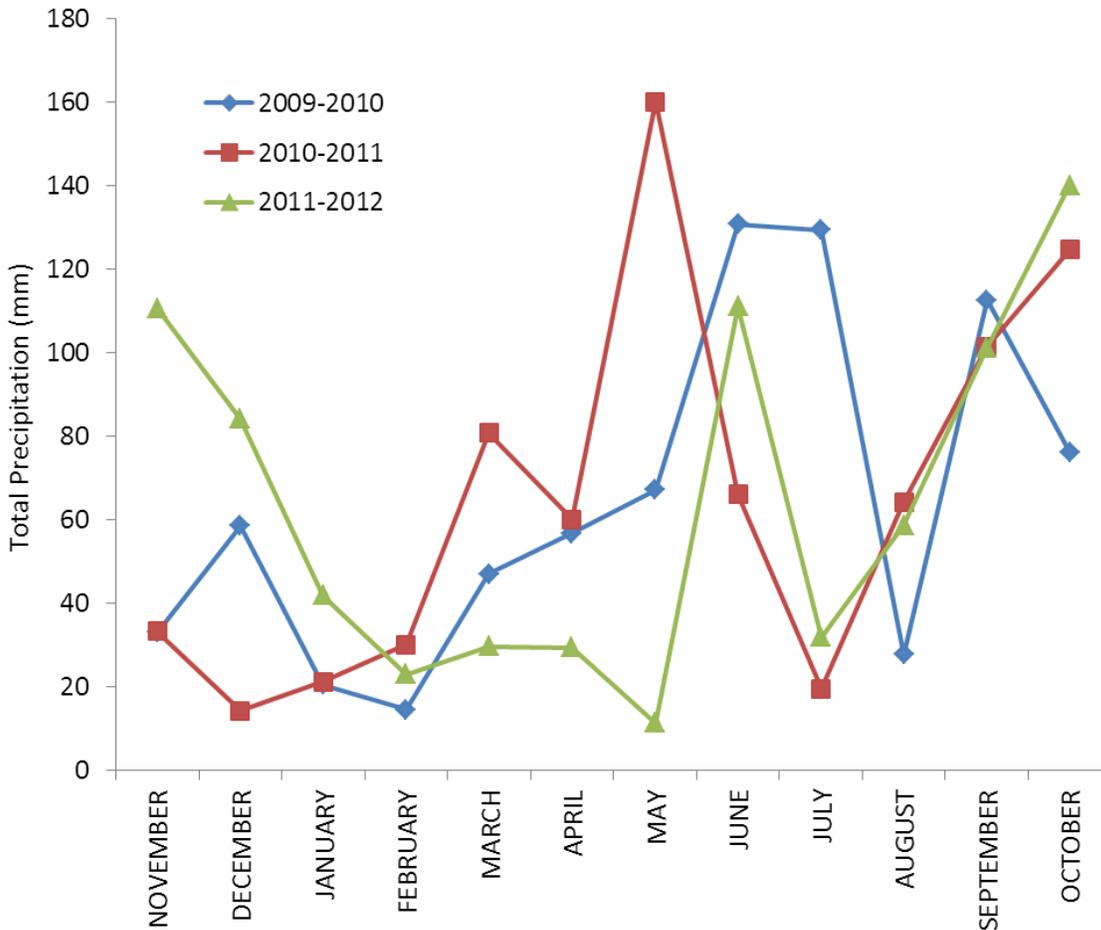
Rates of decay can be influenced by a variety of factors including climate, temperature, substrate type, nutrient concentrations and availability, litter type and size, and soil organisms (Parks Canada 2006). Weight loss associated with decomposition is strongly dependent on aerobic microbial activity (Bunnell et al.1977). Decay sticks that were placed below ground were more accessible to soil microorganisms, fungi, and moisture, which could explain the higher decay rate observed below ground (Table 4.1).

Moisture and temperature, which vary greatly with local conditions, are the principle factors that affect rate of decay (Singh and Gupta 1977), as they strongly influence microbial activity (Bunnell et al. 1977). ). Soil decay rates appear to be more strongly impacted by changes in whichever factor, temperature or moisture, is most limiting. Lower moisture contents result in a limited response to temperature changes and lower temperatures result in a limited response to changes in moisture level (Schlentner and Van Cleve 1984). Significant differences were found in decay rates between 2011 and 2012, which is also where the most extreme weather differences occur. Temperature variation was most extreme between winter 2010 and winter 2011 (which correspond to decay rates from the 2011 and 2012 monitoring years). These warmer temperatures during the winter months likely increased decay rates for those sticks excavated in 2012, as higher temperatures generally result in higher decay rates (Olson 1963; Van Cleve 1971; Singh and Gupta 1977). Additionally, the amount of precipitation may have also played a role as heavy rainfall and a high percentage of rainy days typically speed up decomposition (Singh and Gupta 1977). Based on climate data from the Kitchener-Waterloo Weather Station (Environment Canada), it rained more than 0.5mm on 26% of days in 2011 and 31% of 2012. Once again, the most extreme differences between years can be observed in the winter (Figure 4.5) where very high in 2011 and low in 2010. Thus, lower temperature and moisture levels in the 2011 monitoring year than the 2012 monitoring year are likely factors associated with the significant change in soil decay rates. As 2012 was punctuated by weather extremes, continued monitoring of soil decay rates is important to determine soil health trends in these forest stands.

The Hogsback forest significantly differed from both other stands with lower decay rates. The Hogsback is a forest-wetland complex that has a mixture of upland and lowland areas with swampy features. In particular, one corner of the monitoring plot located here is found within a swamp. If decay sticks are continuously exposed to extremely high moisture levels or are completely submerged in water, decay rates may be slowed by lack of oxygen to support microbial activity (USDA 2007; Schlentner and Van Cleve 1984).



**Figure 4.4:** Temperature data for Waterloo Region by month during soil humus decay monitoring years, where average temperature is the average monthly temperature (Environment Canada- data from Kitchener-Waterloo Weather Station).



**Figure 4.5:** Precipitation data for Waterloo Region by month during soil humus decay monitoring years, where the total precipitation from each month is displayed (Environment Canada- data from Kitchener-Waterloo Weather Station).

#### 4.5 Conclusions and Recommendations

The soil humus decay rate monitoring program at *rare* has undergone valuable improvements and expansions in the last three years. It is recommended that the program continue for a minimum of five consecutive years to ensure the establishment of baseline data that can be a measure of soil change beyond weather extremes. Increasing decay rates could be an impact of increasing global temperatures, or could be a result of an anomaly weather year in 2012. Only continued monitoring can investigate these potential trends. It would be beneficial to additionally monitor soil moisture content in plots each month during monitoring to allow for a closer comparison of decay rates to average soil moisture than simply regional precipitation.

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APPENDIX A: Maps and Coordinates

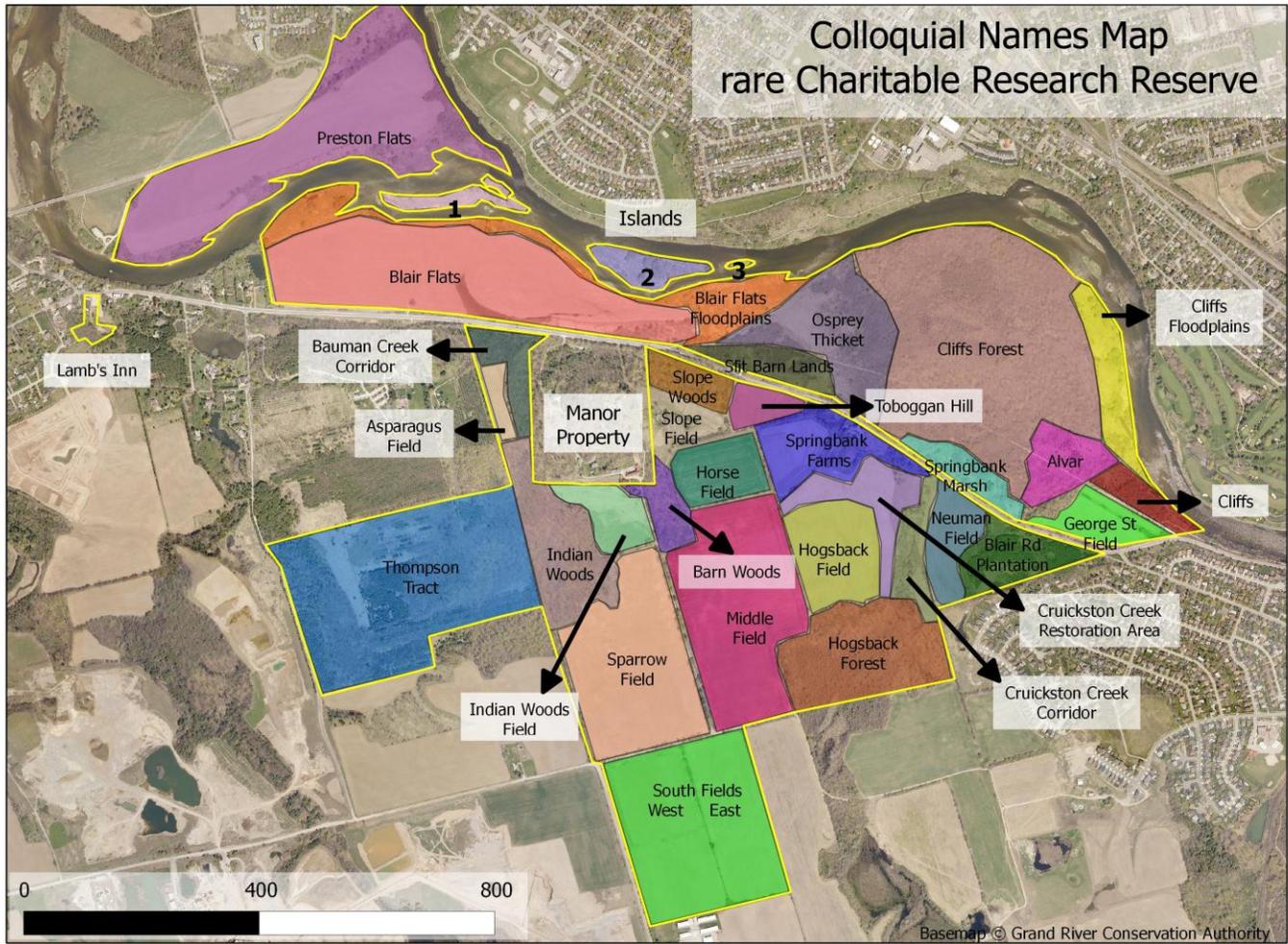
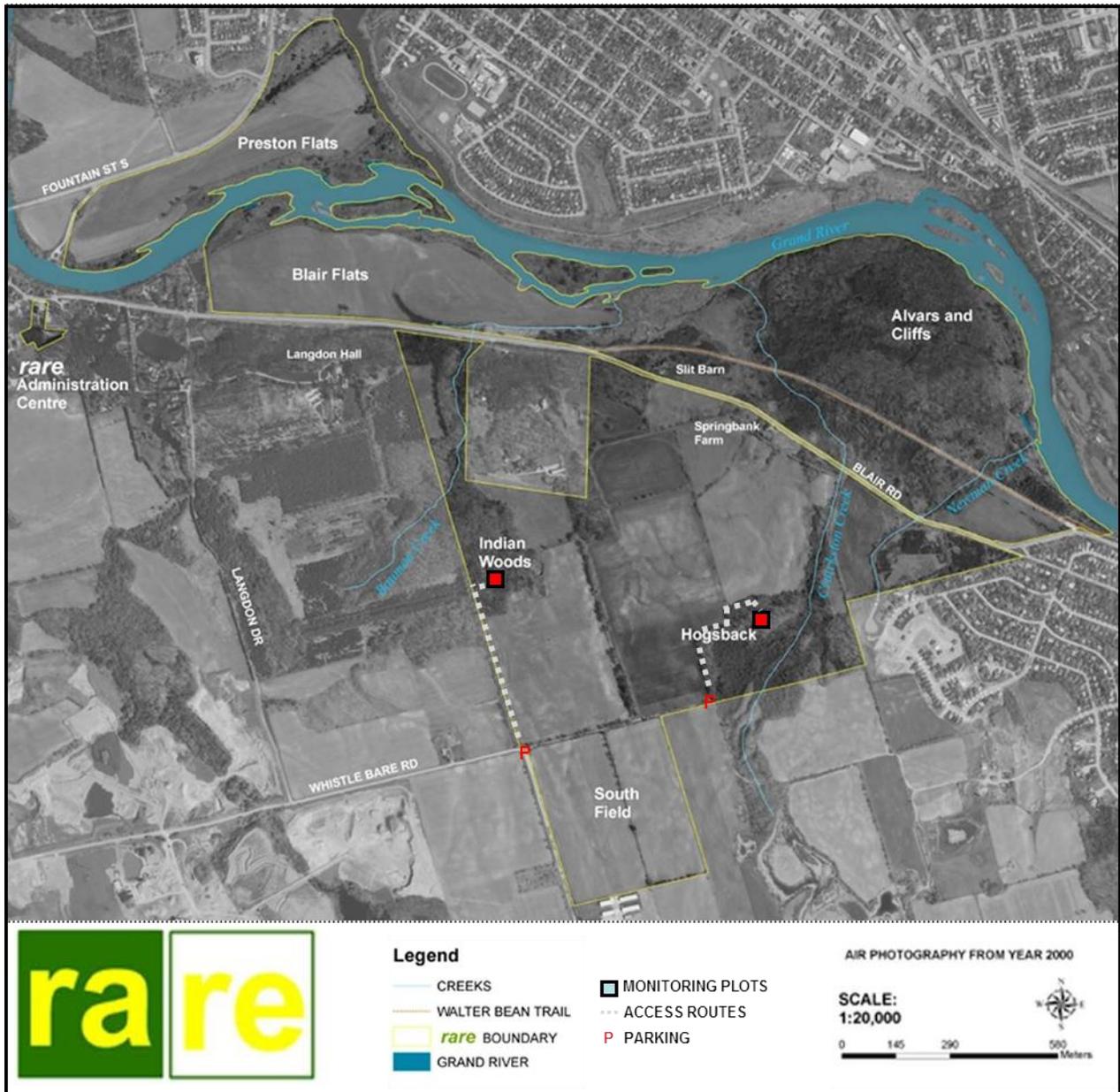
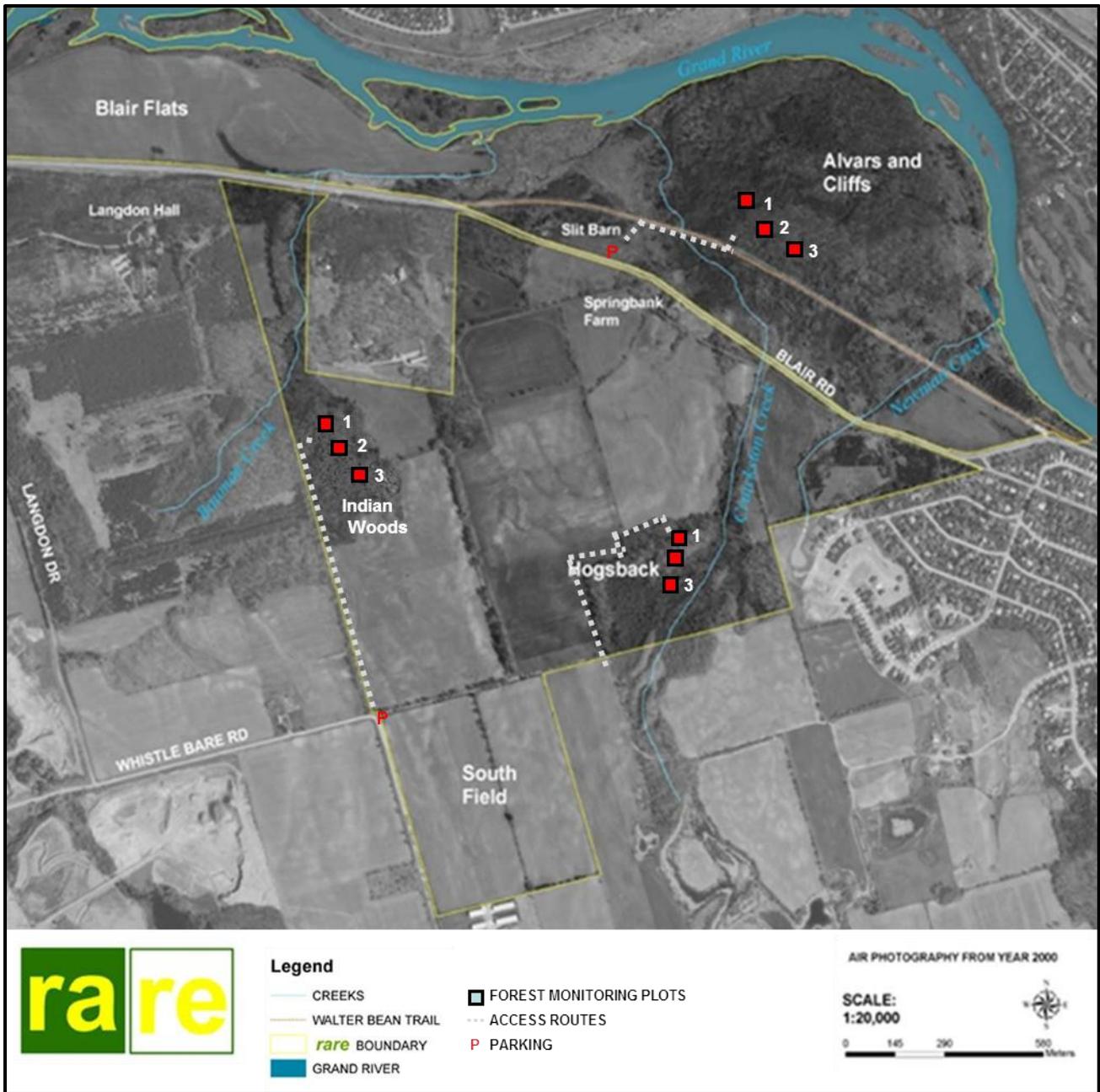


Figure A.1: rare Charitable Research Reserve property map.



**Figure A.2:** Location of salamander monitoring plots in Indian Woods and Hogsback. Dotted lines indicate walking path to sites, with parking location designated by P.

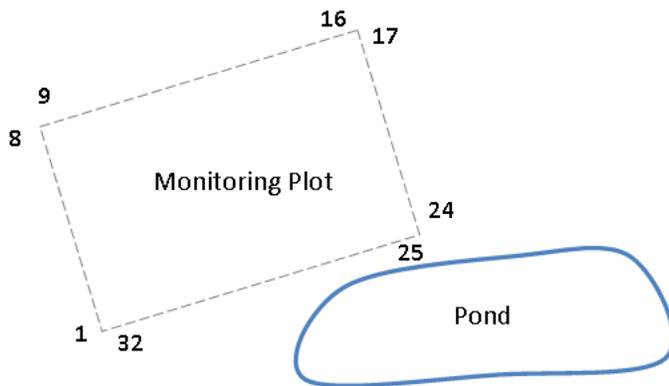


**Figure A.3:** Location of forest health and soil humus decay rate monitoring plots in Indian Woods, Cliffs and Alvars, and the Hogsback. Each forest stand has three plots for forest health assessment and soil humus decay rate monitoring occurs only in plot one at each stand. Dotted lines indicate walking path to sites, with parking location designated by P.

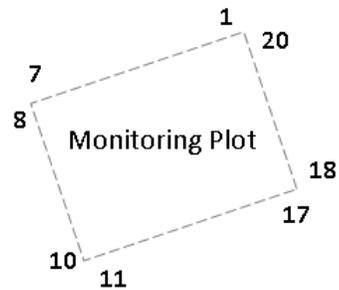
**Table A.1:** GPS coordinates of artificial cover objects (ACO) used for plethodontid salamander monitoring in Indian Woods and the Hogsback (from McCarter 2009).

| Monitoring Plot | ACO | Latitude and Longitude      | UTM (zone 17T)   |
|-----------------|-----|-----------------------------|------------------|
| Indian Woods    | 1   | N43°22'32.05" W80°21'55.49" | 551408E 4802718N |
|                 | 9   | N43°22'31.97" W80°21'53.71" | 551448E 4802716N |
|                 | 17  | N43°22'30.97" W80°21'53.63" | 551450E 4802685N |
|                 | 25  | N43°22'30.85" W80°21'55.37" | 551411E 4802681N |
| Hogsback        | 1   | N43°22'23.93" W80°21'12.74" | 552372E 4802475N |
|                 | 8   | N43°22'22.99" W80°21'13.32" | 552359E 4802446N |
|                 | 11  | N43°22'22.44" W80°21'12.84" | 552370E 4802429N |
|                 | 18  | N43°22'23.57" W80°21'12.30" | 552382E 4802464N |

A)



B)

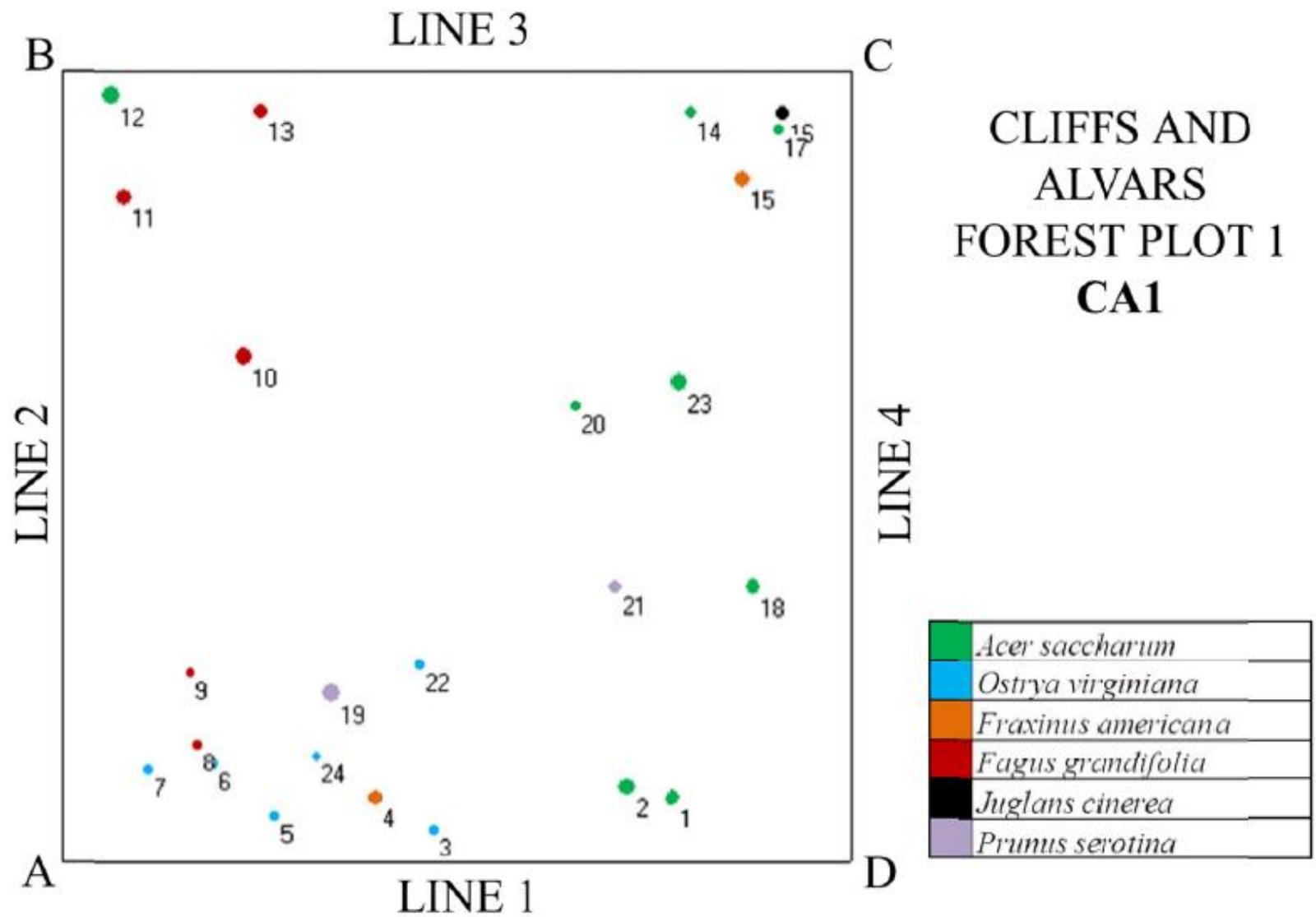


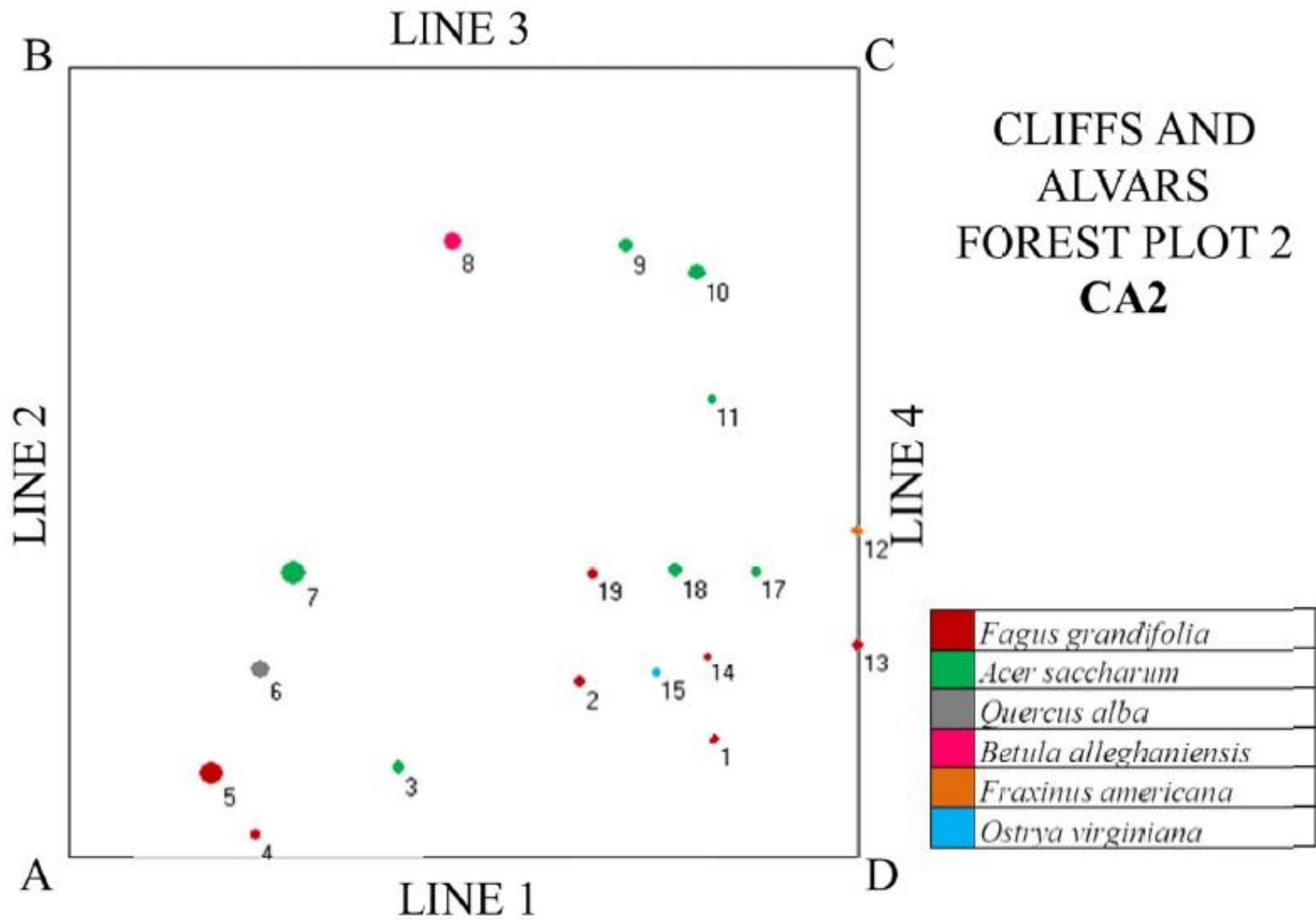
**Figure A.4:** Layout of artificial cover objects (ACOs) on salamander monitoring plots in A) Indian Woods and B) Hogsback.

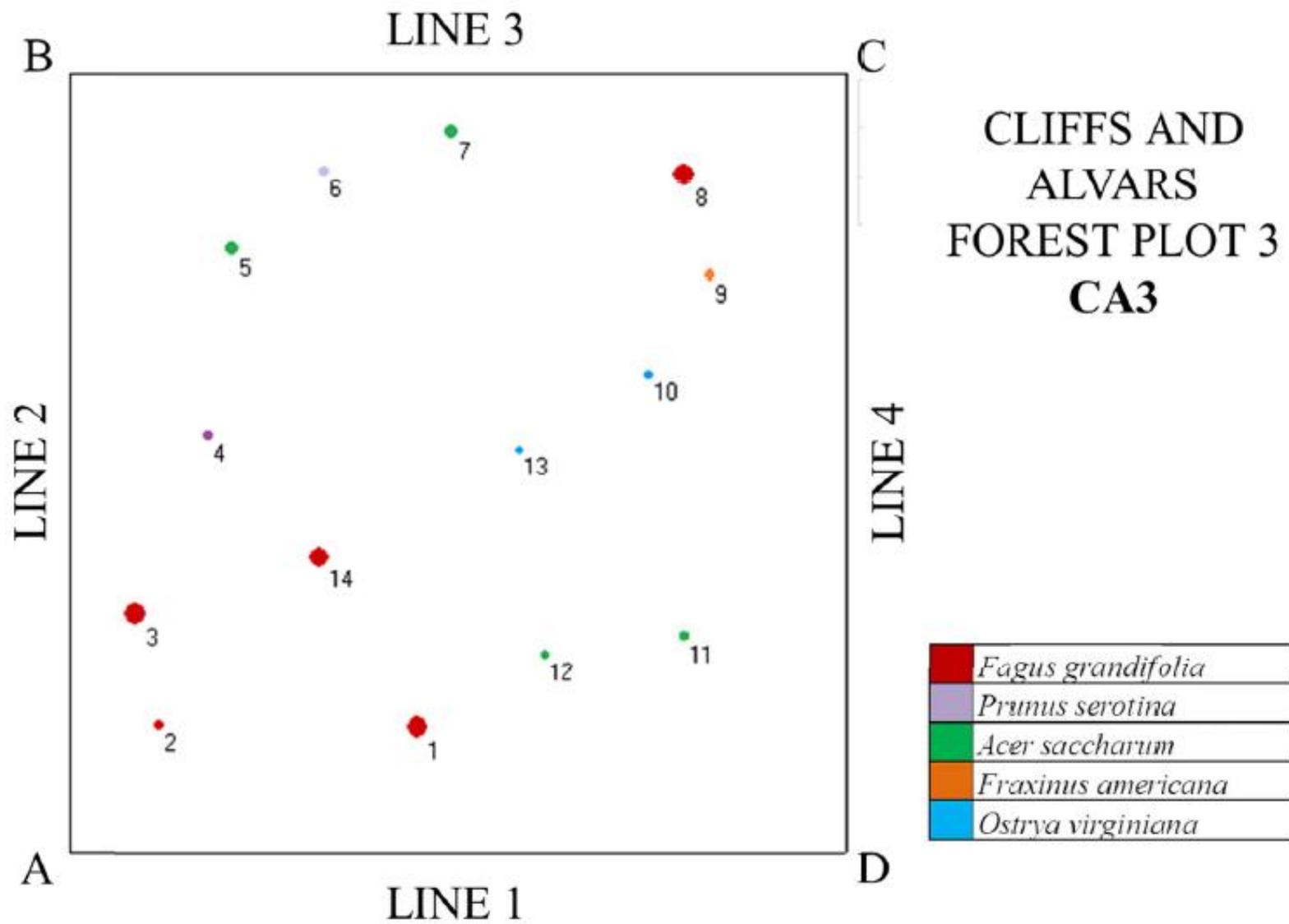
**Table A.2:** GPS coordinates of forest canopy tree biodiversity and health monitoring plots in Cliffs and Alvars, Indian Woods, and the Hogsback (from Robson 2010). The coordinates describe the location of the northwest corner of each plot. The annual soil decay rate monitoring plots are located on all four corners of forest plot one in each stand.

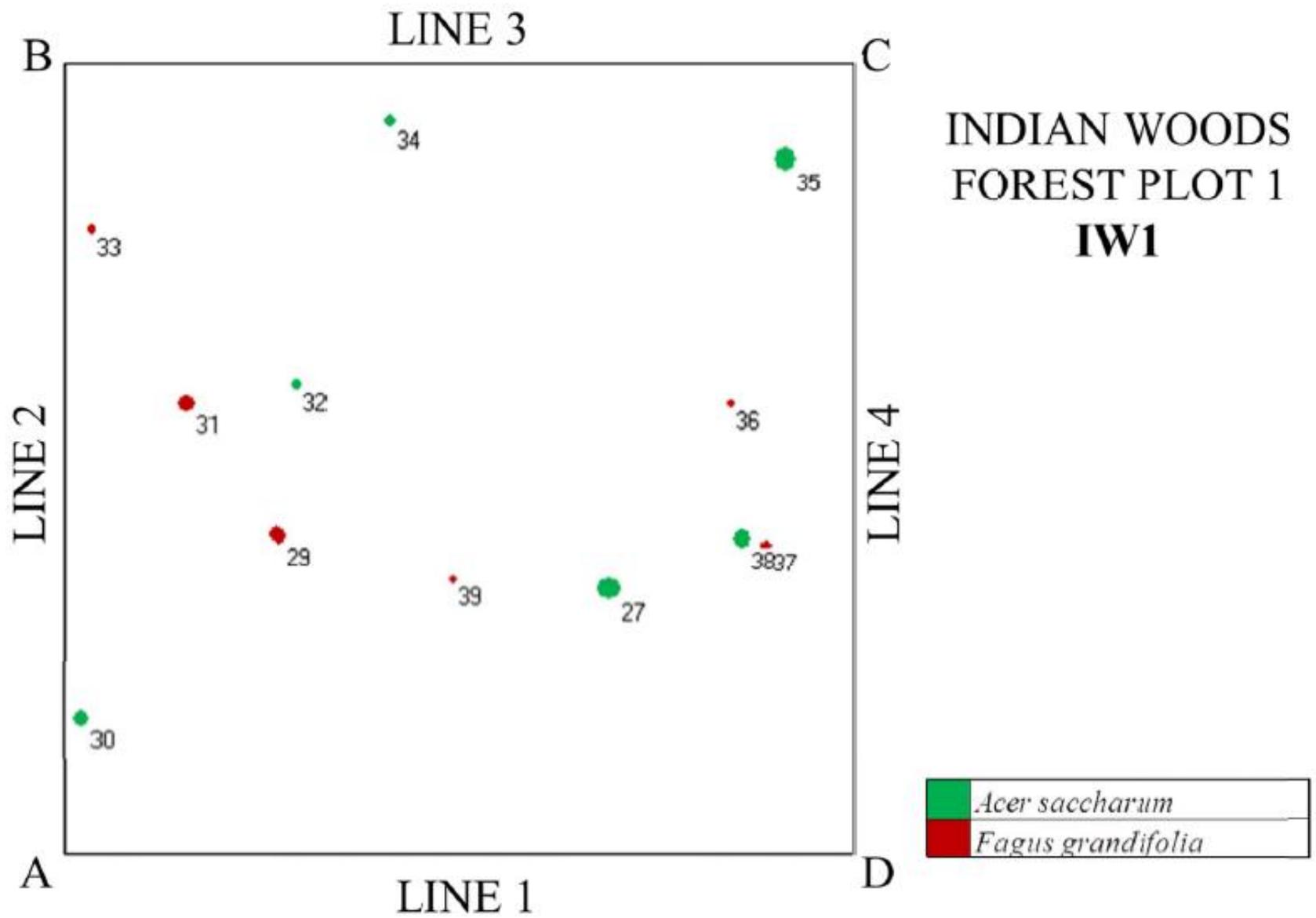
| Monitoring Location | Plot | Latitude and Longitude      | UTM (zone 17T)   |
|---------------------|------|-----------------------------|------------------|
| Cliffs and Alvars   | 1    | N43°22'46.30" W80°21'1.34"  | 552623E 4803167N |
|                     | 2    | N43°22'44.64" W80°21'0.21"  | 552649E 4803116N |
|                     | 3    | N43°22'43.72" W80°20'57.91" | 552701E 4803088N |
| Indian Woods        | 1    | N43°22'27.27" W80°21'51.45" | 551500E 4802571N |
|                     | 2    | N43°22'26.12" W80°21'56.08" | 551396E 4802535N |
|                     | 3    | N43°22'23.62" W80°21'54.78" | 551426E 4802458N |
| Hogsback            | 1    | N43°22'24.18" W80°21'11.10" | 552409E 4802483N |
|                     | 2    | N43°22'23.28" W80°21'12.66" | 552374E 4802455N |
|                     | 3    | N43°22'22.08" W80°21'14.46" | 552334E 4802418N |

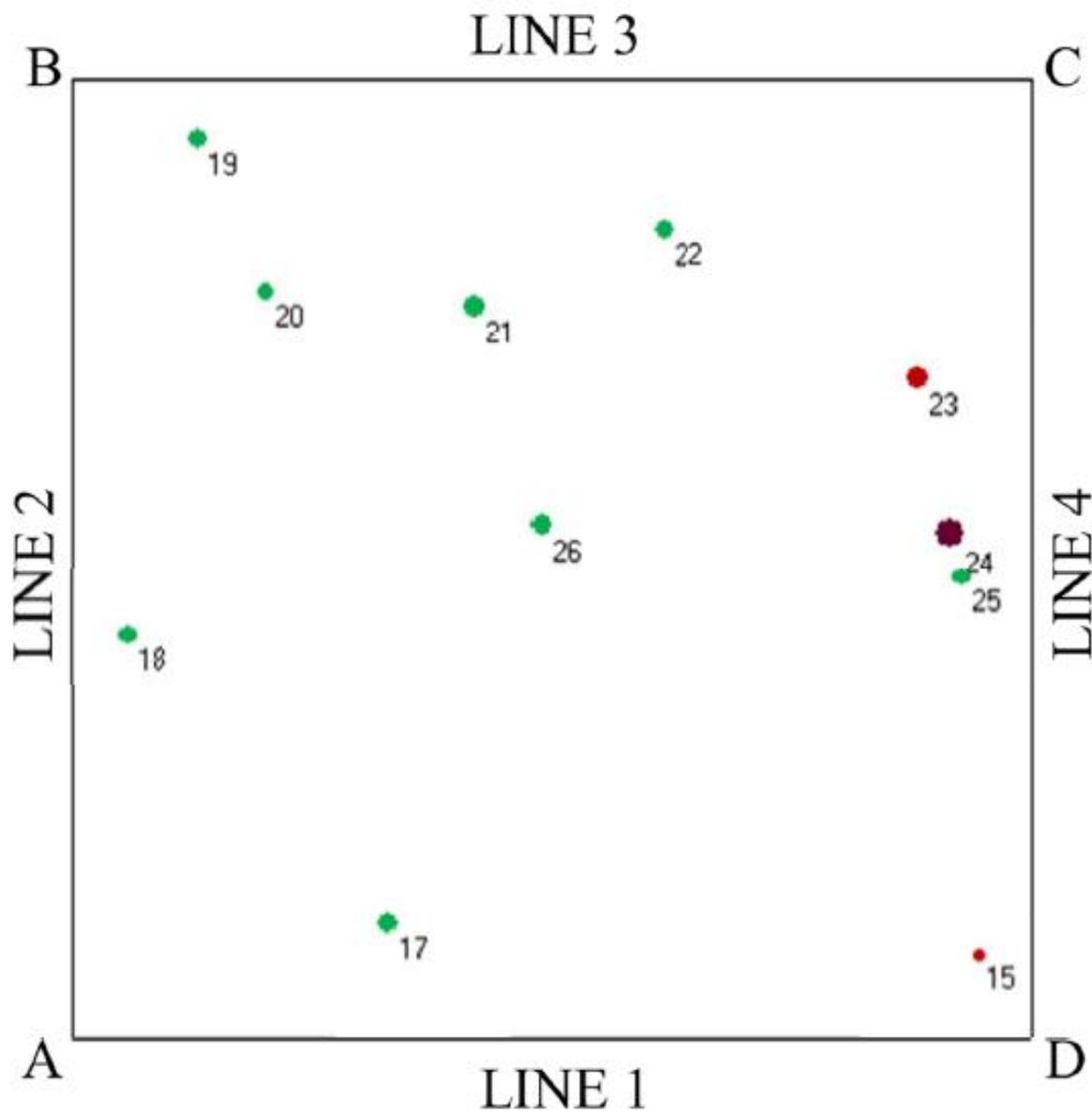
**Figure A.5- Figure A. 13:** Maps of Cliffs and Alvars, Indian Woods, and the Hogsback forest biodiversity monitoring plots showing location of all standing, live trees with a diameter at breast height (dbh) greater than 10.0cm. Sizes of circles are proportional to real tree diameters, colours indicate different species (pages 71-79).

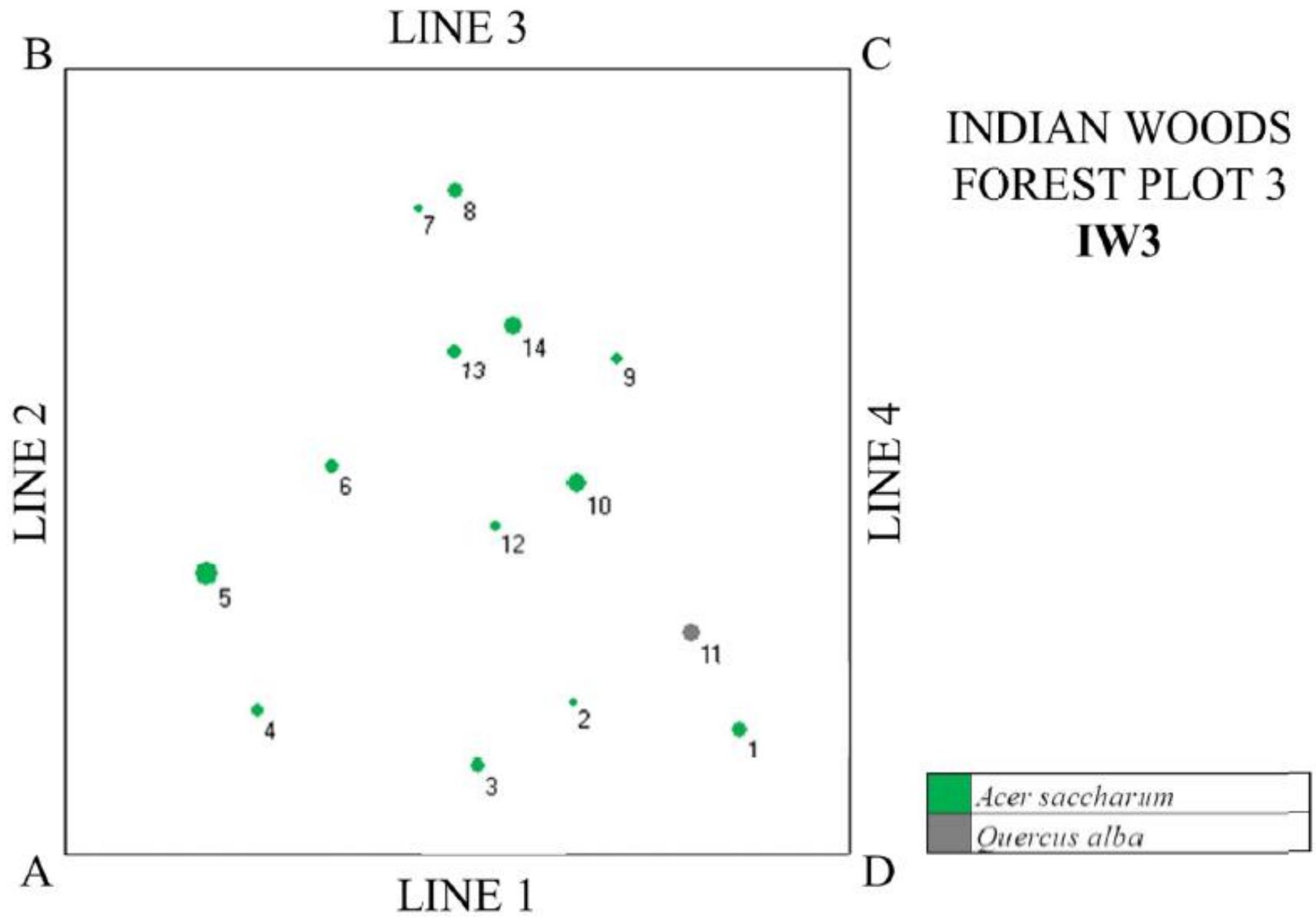


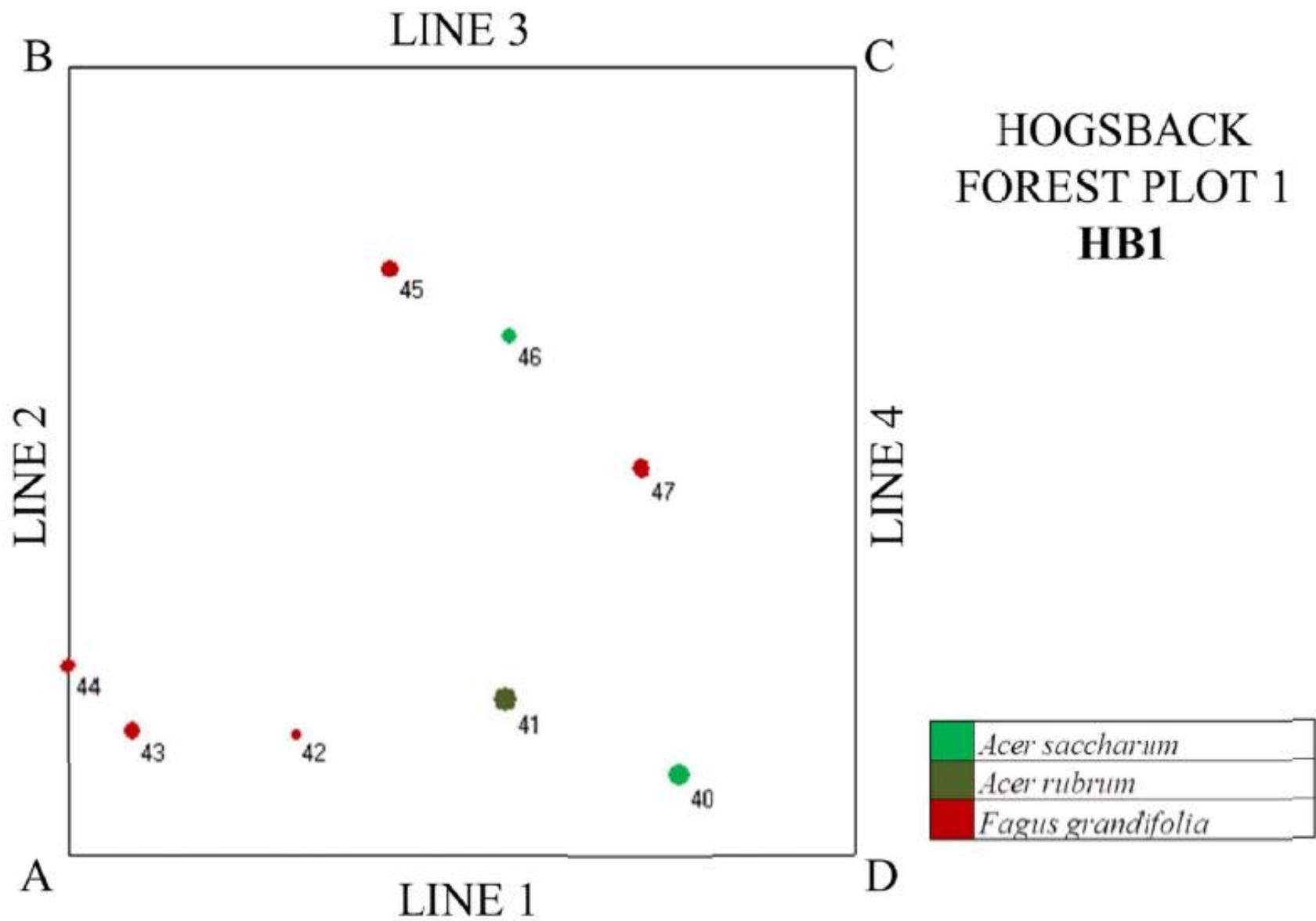


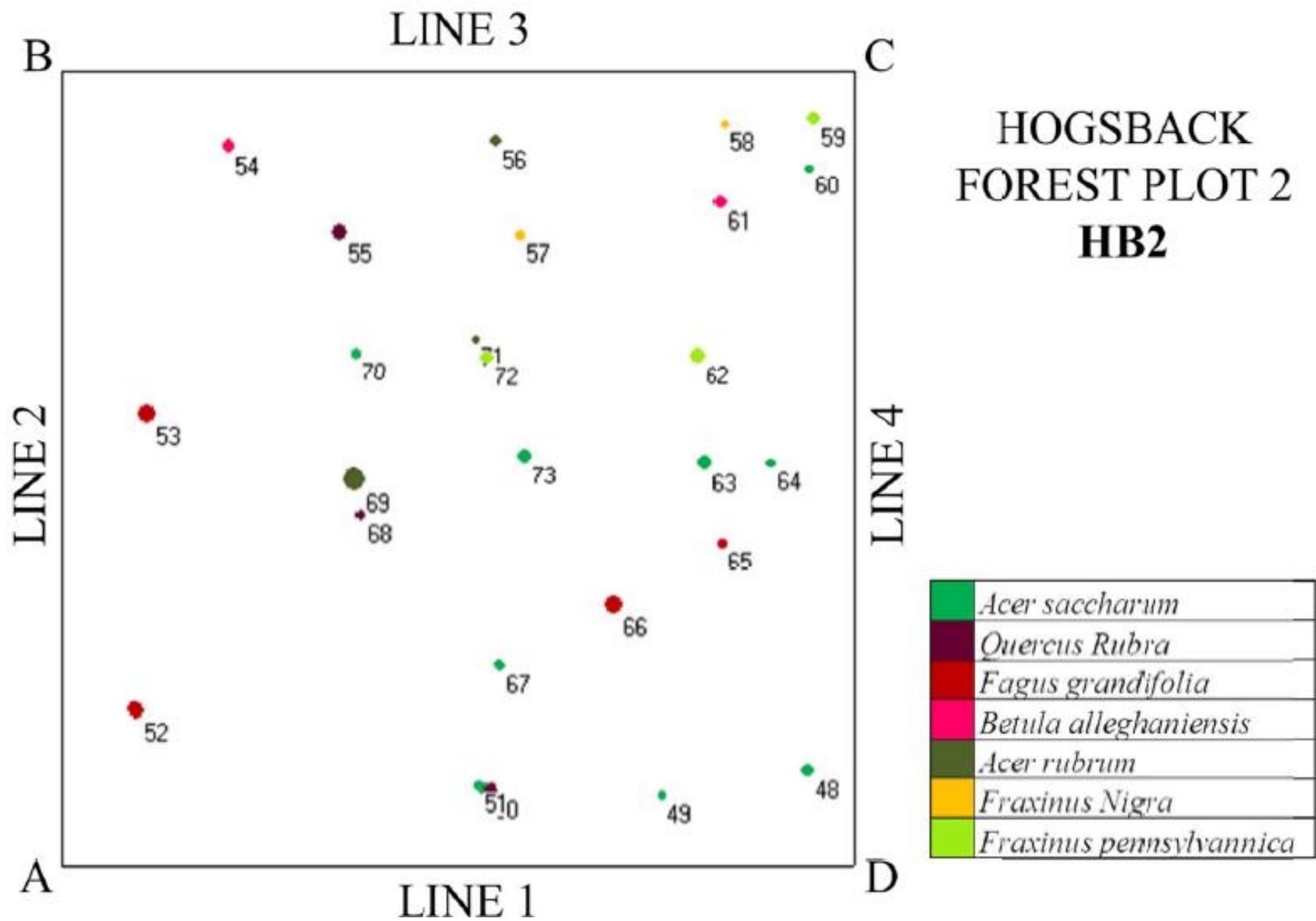


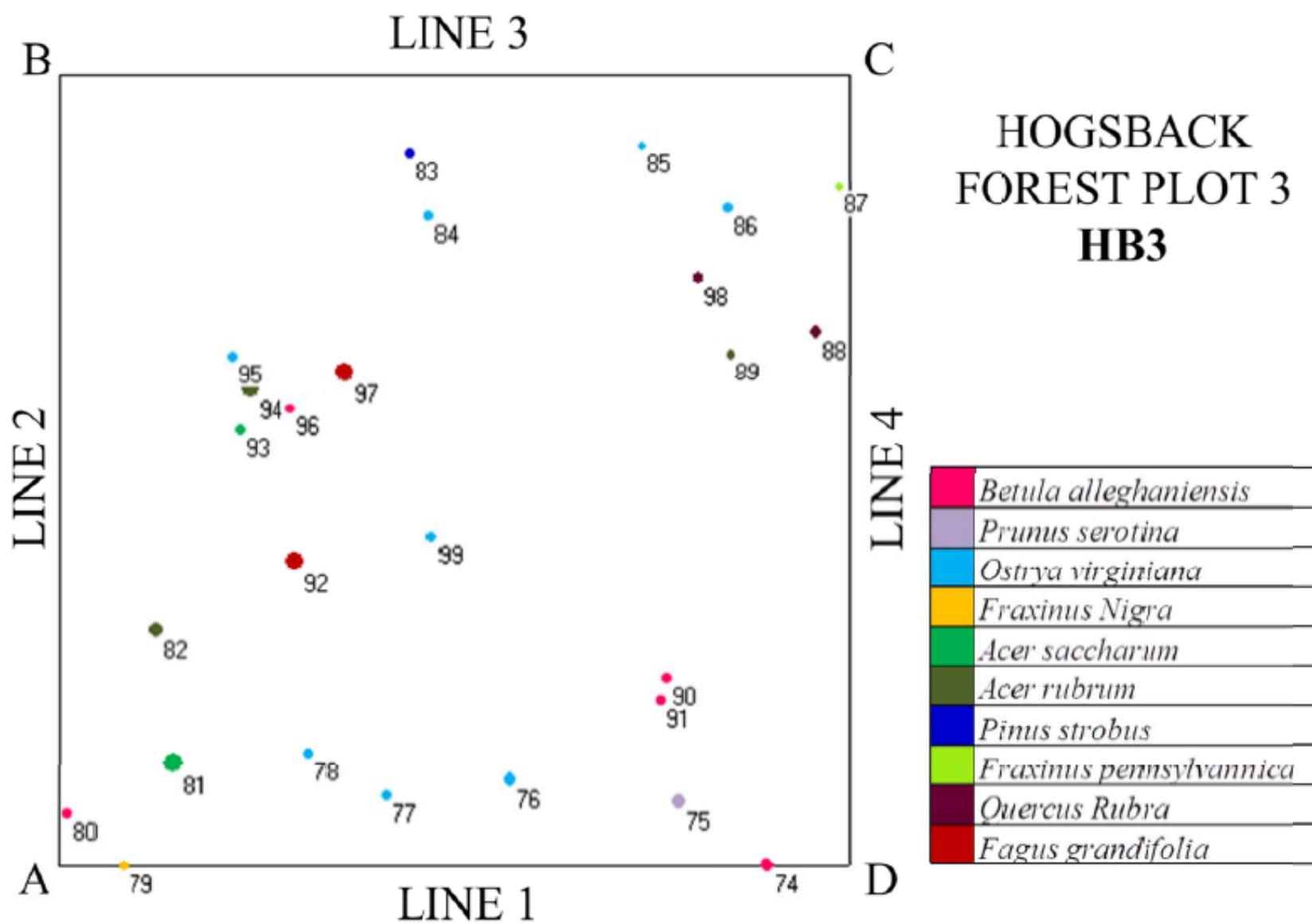












## **APPENDIX B: EQUIPMENT LIST**

### **List B.1:** Salamander monitoring equipment list

- Field data sheets A and B on waterproof paper
- Clipboard
- Pencils
- Nitrile gloves
- Kestral 3000 pocket weather station
- Soil moisture meter (calibrated with screw driver)
- Soil thermometer
- Digital calipers
- Ruler
- Digital pocket scale (with spare batteries)
- Sandwich sized plastic container filled with moist sponges
- Larger plastic container with some moist sponges
- Wash bottle filled with pond water from education pond
- Flagging tape
- Aluminum tags
- Digital camera

### **List B.2:** Soil pH testing equipment list

- 36 re-sealable plastic bags
- Trowel
- Spoon
- Nitrile gloves
- Permanent marker
- Soil pH testing kit (in lab)

### **List B.3:** Forest canopy tree biodiversity monitoring equipment list

- Blank canopy-sample and tree condition field data sheets on waterproof paper
- Past year data sheets & EMAN reference package
- Clipboard
- Pencils
- Flagging tape
- Diameter tape
- Two nylon tape measures (30m)
- Field guide
- Binoculars
- Clinometer
- Pre-labelled tags and steel pigtail stakes

**List B.4:** Soil humus decay rate monitoring equipment list

Installation

- Field data sheet on waterproof paper
- Clipboard
- Pencils
- Nitrile gloves
- Shovel
- Trowel
- Chisel
- Pigtail stakes (12 per plot)
- Tongue depressors (decay sticks), pre-weighed, dried, and labelled
- Pre-prepared mesh bags
- Fishing line

Extraction

- Field data sheet on waterproof paper
- Clipboard
- Pencils
- Nitrile gloves
- Trowel
- Scissors
- Utility knife
- Re-sealable plastic bags
- Permanent marker

Cleaning

- Nitrile gloves
- Scissors
- Two paint brushes (one wet and one dry)
- Paper envelopes

## APPENDIX C: DATA SHEETS AND CODES

**Table C.1:** Beaufort wind codes (Zorn et al. 2004)

| Beaufort Scale | Wind Speed<br>(mph) | Wind Speed<br>(km/h) | Description                                 |
|----------------|---------------------|----------------------|---|
| 0              | 1                   | 1.6                  | Calm. Smoke rises vertically.               |
| 1              | 2                   | 3.2                  | Light. Smoke drifts.                        |
| 2              | 5                   | 8                    | Light breeze. Leaves rustle.                |
| 3              | 10                  | 16                   | Gentle breeze. Lighter branches sway.       |
| 4              | 15                  | 24                   | Moderate breeze. Dust rises. Branches move. |
| 5              | 21                  | 33.6                 | Fresh breeze. Small trees sway.             |
| 6              | 28                  | 44.8                 | Strong breeze. Larger branches move.        |
| 7              | 35                  | 56                   | Moderate gale. Trees move.                  |
| 8              | 42                  | 67.2                 | Fresh gale. Twigs break.                    |
| 9              | 50                  | 80                   | Strong gale. Branches break.                |
| 10             | 59                  | 94.4                 | Whole gale. Trees fall.                     |
| 11             | 69                  | 110.4                | Storm. Violent blasts.                      |
| 12             | 75                  | 120                  | Hurricane. Structures shake.                |

**Table C.2:** Beaufort sky codes (Zorn et al. 2004)

| Sky Code | Description                                |
|----------|--|
| 0        | Clear. No clouds.                          |
| 1        | Partly cloudy. Scattered or broken clouds. |
| 2        | Cloudy (broken) or overcast.               |
| 3        | Sandstorm. dust storm, or blowing snow.    |
| 4        | Fog, thick dust or haze.                   |
| 5        | Drizzle.                                   |
| 6        | Rain.                                      |
| 7        | Snow, or snow rain mixed.                  |
| 8        | Shower(s).                                 |
| 9        | Thunderstorm(s).                           |











## APPENDIX D: SPECIES LISTS

**Table D.1:** Common and scientific names with shorthand abbreviations of all salamander species observed at *rare* Charitable Research Reserve since 2006. The Eastern Red-backed salamander has two colour phases, red- and lead-backed, which are distinguished during sampling.

| Common Name                    | Scientific Name               | Abbreviation |
|--------------------------------|-------------------------------|--------------|
| Yellow-spotted Salamander      | <i>Ambystoma maculatum</i>    | YESA         |
| Blue-spotted Salamander        | <i>Ambystoma laterale</i>     | BLSA         |
| Four-toed Salamander           | <i>Hemidactylium scutatum</i> | FOSA         |
| Eastern Red-backed Salamander* | <i>Plethodon cinereus</i>     | RESA/LESA    |

**Table D.2:** Common and scientific names with shorthand abbreviations of all tree species observed in forest canopy biodiversity monitoring plots at *rare* Charitable Research Reserve since 2009.

| Common Name    | Scientific Name               | Abbreviation |
|----------------|-------------------------------|--------------|
| American Beech | <i>Fagus grandifolia</i>      | FAGUGRAN     |
| Black Ash      | <i>Fraxinus nigra</i>         | FRAXNIGR     |
| Black Cherry   | <i>Prunus serotina</i>        | PRUNSERO     |
| Butternut      | <i>Juglans cinerea</i>        | JUGLCINE     |
| Green Ash      | <i>Fraxinus pennsylvanica</i> | FRAXPENN     |
| Hophornbeam    | <i>Ostrya virginiana</i>      | OSTRVIRG     |
| Red Maple      | <i>Acer rubrum</i>            | ACERRUBR     |
| Red Oak        | <i>Quercus rubra</i>          | QUERRUBR     |
| Sugar Maple    | <i>Acer saccharum</i>         | ACERSACC     |
| White Ash      | <i>Fraxinus americana</i>     | FRAXAMER     |
| White Oak      | <i>Quercus alba</i>           | QUERALBA     |
| White Pine     | <i>Pinus strobus</i>          | PINUSTRO     |
| Yellow Birch   | <i>Betula alleghaniensis</i>  | BETUALLE     |